EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation

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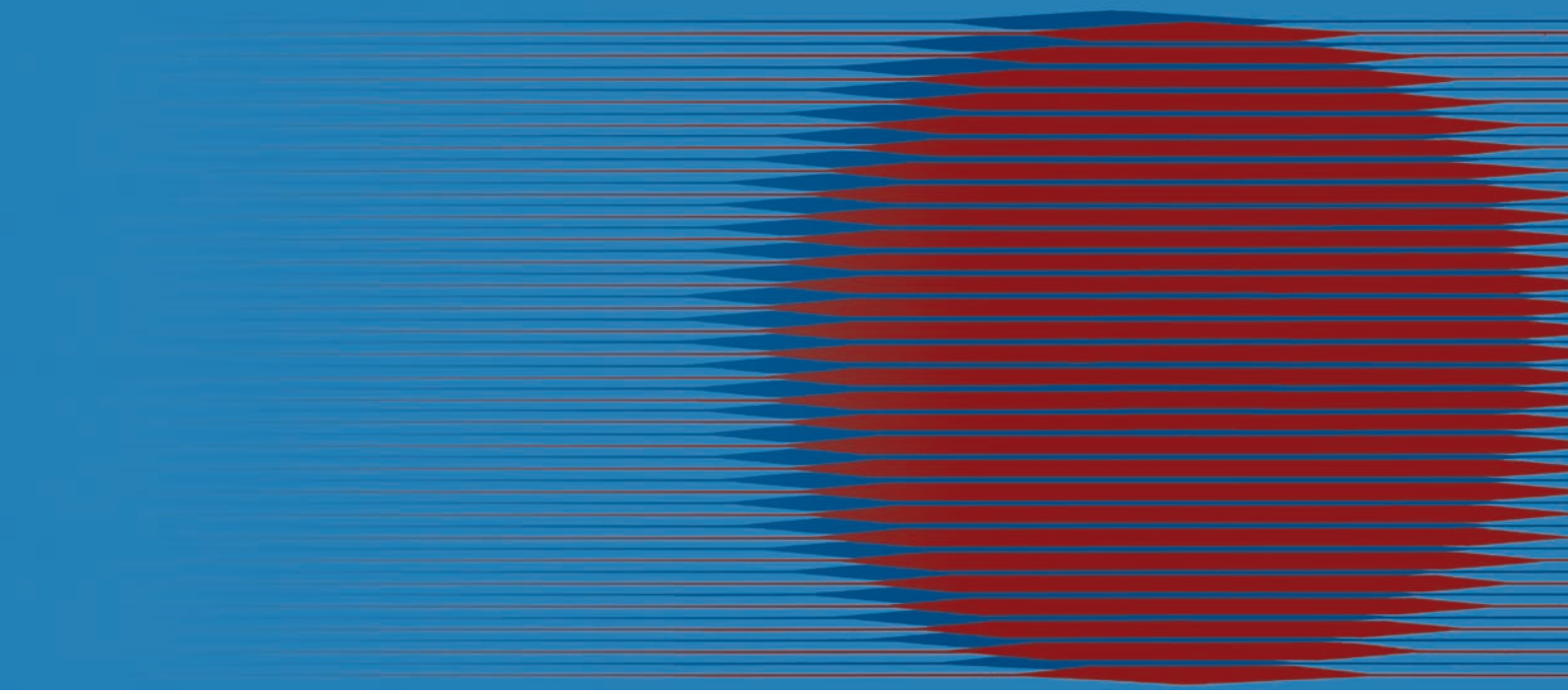
Scientific Annexes C, D and E



UNITED NATIONS

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United Nations Scientific Committee on the
Effects of Atomic Radiation

UNSCEAR 2006
Report to the General Assembly
with Scientific Annexes

VOLUME II
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UNITED NATIONS
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NOTE

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ANNEX C

Non-targeted and delayed effects of exposure to ionizing radiation

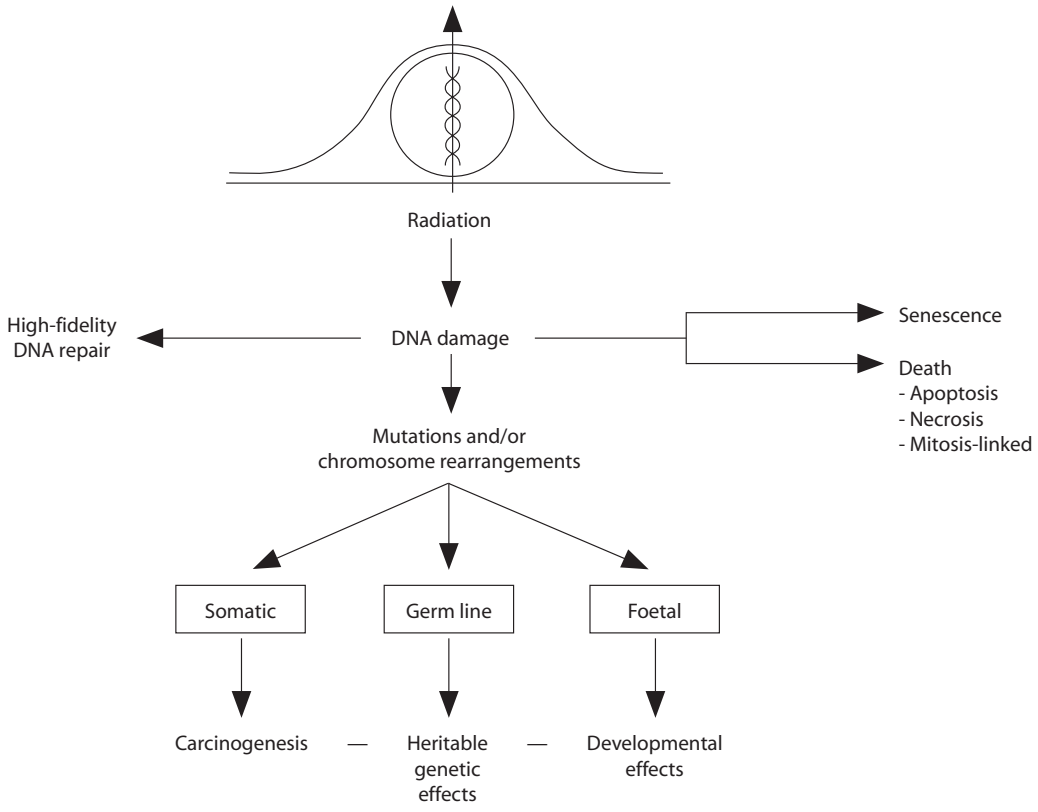
Contents

INTRODUCTION

1. The goal of this annex is to summarize the evidence for non-targeted and delayed effects of exposure to ionizing radiation in vitro and in vivo. Currently, human health risk estimates for effects associated with radiation exposures are based primarily on the view that the detrimental effects of irradiation occur only in irradiated cells. Over the years, a number of non-targeted effects of radiation exposure have been described that challenge this concept. These non-targeted effects include genomic instability occurring in the progeny of an irradiated cell, bystander effects, clastogenic factors produced in plasma from irradiated individuals that can cause chromosome damage when cultured with non-irradiated cells, and heritable effects of parental irradiation that can manifest across generations. This annex considers whether these effects pose new challenges to evaluating risks associated with radiation exposure, understanding radiation-induced carcinogenesis and interpreting epidemiological data on radiation exposure.

2. A central tenet in the radiation sciences has been that the energy from radiation must be deposited in the cell nucleus to elicit a mutagenic and/or clastogenic effect and thus be relevant for its potential to cause damage (figure I). It is implicit in this tenet that the biological consequences of cellular irradiation affect only the irradiated cell and that non-irradiated cells do not share the legacy of the radiation exposure.

Figure I. Prevailing paradigm for the biological effects of cellular exposure to ionizing radiation.
Ionizing radiation deposits energy in the nucleus of the cell. DNA damage is induced, and cellular responses to that damage can affect the fate of the irradiated cell. The damage can be removed and the genetic material restored by high-fidelity DNA repair. DNA repair systems may also eliminate the damage, but error-prone processing can result in gene mutations and clastogenic effects leading to chromosomal rearrangements. Depending upon the cell type, various cellular processes may be initiated that result in carcinogenesis in somatic cells, heritable genetic effects in germ line cells and developmental defects in foetal cells which may or may not be derived from mutational or clastogenic effects. DNA damage might activate cell cycle checkpoint control and cause the damaged cell to go into a protracted senescent state. Alternatively, if the damage is substantial, cell death may occur via a number of cellular pathways.



3. When ionizing radiation is absorbed in biological material, excitations and ionizations occur that are non-randomly distributed along localized tracks. The spatial distribution of these ionization/excitation events produced by different particles varies considerably depending on the quality of radiation. The term "linear energy transfer" (LET) is used to classify radiation quality according to the average energy transferred per unit length of the track. For the purposes of this annex, X- and gamma rays are considered to be low-LET radiation, protons and neutrons are considered to be intermediate LET radiation, and alpha particles and heavy ions are considered to be high-LET radiation.

4. In contrast to the risks associated with exposures to low doses of ionizing radiation (less than about 200 mSv, UNSCEAR 2000 Report [U2]), the risks of cancer after high and moderate doses of radiation are relatively well understood. This understanding is based on data from detailed epidemiological studies of the survivors of the atomic bombings in Japan and other exposed groups, e.g. clinically irradiated populations and those exposed as a result of the Chernobyl accident (UNSCEAR 2000 Report, annex I). However, risks at low doses are generally extrapolated from the high-dose data, applying dose and dose-rate effectiveness factors. Estimating risk is further complicated because environmental exposures are predominantly protracted, low-dose, low-dose-rate exposures, or high-dose-rate exposures delivered in small fractions (see annex A, "Epidemiological studies of radiation and cancer"). This contrasts with the majority of laboratory studies and clinical exposure situations, where exposures are usually acute, high-dose, high-dose-rate exposures. In addition, inherent in many models of radiation risk is that only those cells or tissues actually irradiated are burdened by the legacy of the radiation exposure. A number of non-targeted delayed effects of radiation exposure have been described; the purpose of this annex is to summarize the evidence for these effects and indicate present hypotheses on how they may affect the assessment of health hazards associated with radiation exposure and radiation-induced carcinogenesis.

5. For the purposes of this annex, "non-targeted effects" refers to radiation-induced effects manifesting in cells whose nucleus was not subject to a direct hit by the radiation, i.e. no ionization events due to cellular irradiation were deposited within the volume of that nucleus. In such instances the radiation may have hit the cytoplasm, or neighbouring cells, tissues or organs, or even cells in another culture vessel, and a response is communicated from these irradiated cells to non-irradiated cells to elicit an effect. It must be stressed at this stage that the non-targeted effects of ionizing radiation described in this annex do not imply that the well-documented targeted effects of radiation are irrelevant or unimportant, or that the concept of "dose" needs to be revised. That is not the case. Rather, the goal of this annex is to summarize the literature on non-targeted effects associated with exposure to ionizing radiation and, where possible, to evaluate how such effects may affect risks associated with radiation exposure, the understanding of radiation-induced carcinogenesis, and the mechanistic basis for interpreting epidemiological data on radiation effects.

I. RADIATION-INDUCED GENOMIC INSTABILITY

A. Radiation-induced genomic instability in vitro

6. Genomic instability is an all-embracing term to describe the increased rate of acquisition of alterations in the genome. As compared with the direct effects of radiation, i.e. those effects directly induced as a consequence of energy deposition, radiation-induced instability is observed in cells at delayed times after irradiation and manifests in the progeny of exposed cells multiple generations after the initial insult (figure II). Instability is measured as chromosomal alterations, changes in ploidy, micronucleus formation, gene mutations and amplifications, mini- and microsatellite (short tandem repeat) instabilities and/or decreased plating efficiency (summarized in table 1), and has been the subject of a number of reviews [K17, L14, M11, M12, M14, M48, W12]. These observed delayed effects can persist in unstable clones over time, and in some instances mimic those effects seen in tumour cells. There are likely to be multiple pathways for initiating and perpetuating induced instability [K8, L11], and the relative contributions of the different pathways involved probably depend on the genetic background of the target cell or organism [P3, W1] and on environmental factors (reviewed in references [K4, M32, M33]).

Figure II. Radiation-induced genomic instability.

A single cell survives irradiation and is clonally expanded. During clonal expansion, a number of the progeny of that irradiated cell die (through lethal mutations or delayed reproductive cell death), which results in a persistently reduced plating efficiency in this clone. Alternatively, or as a consequence of the presence of these dead and dying cells, instability events can occur in the progeny of the irradiated cell. These may result in chromosomal rearrangements, aberrations or gaps, micronuclei, mutations, gene amplifications and/or a failure of the cells to correctly separate their chromosomes at mitosis, resulting in aneuploid cells.

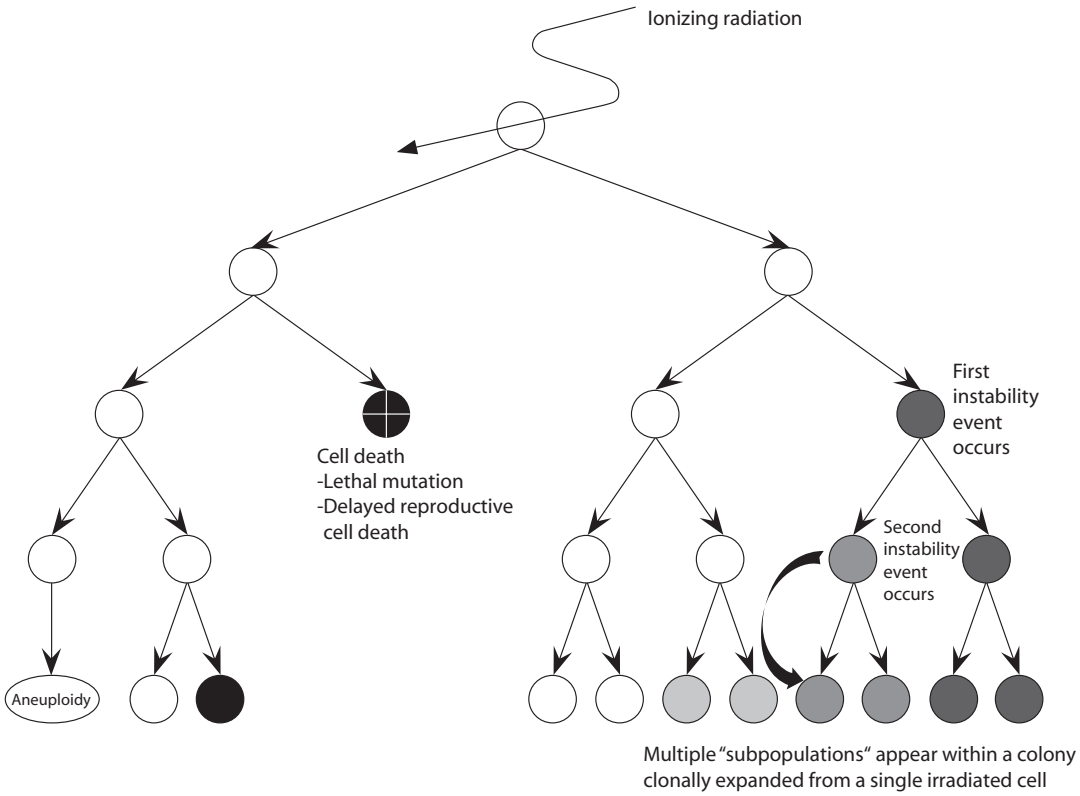


Table 1 In vitro studies of radiation-induced genomic instability

Year	*End point*	*Cell type*	*Radiation type*[a]	*Comments*	*Reference*
1991	Plating efficiency	Chinese hamster ovary cells	X-rays		[C13]
1992	Chromosomal aberrations	Murine haemopoietic stem cells	Alpha particles		[K3]
1992	Mutation frequency	Chinese hamster ovary cells	X-rays		[C12]
1992	Plating efficiency	Chinese hamster ovary cells	X-rays		[C12]
1993	Chromosomal instability	Human skin fibroblasts	Heavy ions: neon, argon	Dose response	[M30]
1993	Chromosomal aberrations	Human lymphocytes	X-rays		[H7]
1993	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays		[M2]
1993	Neoplastic transformation	HeLa × skin fibroblast human hybrid cells	Gamma radiation		[M31]
1993	Plating efficiency	HeLa cells	X-rays	Dose response	[F8]
1993	Plating efficiency	GM10115 human–hamster hybrid cells	X-rays		[M2]
1994	Chromosomal aberrations	HeLa cells	X-rays		[B20]
1994	Chromosomal aberrations	Human haemopoietic stem cells	Alpha particles		[K1]
1994	Giant cell formation	HeLa cells	X-rays		[B20]
1994	Plating efficiency	HeLa cells	X-rays		[B20]
1994	Plating efficiency	Human keratinocyte cells	Gamma radiation; alpha particles		[O5]
1995	Chromosomal aberrations	Human lymphocytes	X-rays		[H8]
1995	Chromosomal aberrations	Murine haemopoietic stem cells	Alpha particles; X-rays		[K2]
1995	Delayed TP53 mutation	Murine epithelial cells	Gamma rays		[S11]
1995	Plating efficiency	Murine haemopoietic stem cells	Alpha particles; X-rays		[K2]
1996	Apoptosis	V79 Chinese hamster ovary cells	X-rays (1–12 Gy)	Dose response up to 3–4 Gy	[J5]
1996	Apoptosis	Human keratinocyte cells; CHOK hamster cells	Gamma rays	Dose response	[L34]
1996	Chromosomal aberrations	Human epithelial cells	X-rays; alpha particles		[D18]
1996	Chromosomal aberrations	TK6 human lymphoblasts	X-rays		[G8]
1996	Chromosomal aberrations	V79 Chinese hamster ovary cells	X-rays (1–12 Gy)	Dose response up to 3–4 Gy	[J5]
1996	Chromosomal aberrations	Human lymphocytes	Alpha particles		[K5]
1996	Micronucleus frequency	V79 Chinese hamster ovary cells	X-rays (1–12 Gy)	Dose response up to 5 Gy	[J5]
1996	Morphological abnormalities	Human keratinocyte cells; CHOK hamster cells	Gamma rays	Dose response	[L34]
1996	Mutation frequency	TK6 human lymphoblastoid cells	X-rays		[G8]
1996	Plating efficiency	Human epithelial cells	X-rays; alpha particles		[D18]
1997	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[L30, L31]
1997	Chromosomal aberrations	V79 Chinese hamster ovary cells	Alpha particles; X-rays	Dose response	[M28]
1997	Chromosomal aberrations	Murine haemopoietic stem cells	Gamma rays		[P9, P15]

Year	*End point*	*Cell type*	*Radiation type*[a]	*Comments*	*Reference*
1997	Micronucleus frequency	V79 Chinese hamster cells	Alpha particles; X-rays	Dose response	[M28]
1997	Mutation frequency	Murine haemopoietic stem cells	X-rays; alpha particles; neutrons		[H12]
1997	Mutation frequency	Chinese hamster ovary cells	X-rays; alpha particles		[L29]
1997	Plating efficiency	V79 Chinese hamster cells	Alpha particles; X-rays	Dose response	[M28]
1998	Chromosomal aberrations	Human fibroblasts	X-rays; neutrons; alpha particles		[K18]
1998	Chromosomal aberrations	Human epithelial cells	Gamma rays; neutrons		[U20]
1998	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays		[D1]
1998	Chromosomal aberrations	Human lymphocytes	Gamma rays		[H9]
1998	Chromosomal aberrations	Murine haemopoietic stem cells	Alpha particles; X-rays		[K18]
1998	Chromosomal aberrations	GM10115 human–hamster hybrid cells	^{125}I		[K9]
1998	Chromosomal aberrations	Human lymphocytes	Gamma rays		[L32]
1998	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[L9]
1998	Chromosomal aberrations	Murine haemopoietic stem cells	Alpha particles		[L20]
1998	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[P14]
1998	Chromosomal aberrations	V79 Chinese hamster ovary cells	X-rays		[T3]
1998	Plating efficiency	GM10115 human–hamster hybrid cells	X-rays		[D1]
1998	Plating efficiency	Murine haemopoietic stem cells	Alpha particles; X-rays		[K18]
1998	Plating efficiency	Murine haemopoietic stem cells	Alpha particles		[L24]
1999	Apoptosis; micronucleus frequency	AGO I522B primary human fibroblasts	X-rays; alpha particles	Dose response	[B22]
1999	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[L6]
1999	Micronucleus frequency	Human SCL-II squamous carcinoma cells	X-rays	Dose response	[K20]
1999	Plating efficiency	AGO 1522B primary human fibroblasts	X-rays; alpha particles	Dose response	[B22]
1999	Plating efficiency	Human keratinocyte cells	Gamma rays; alpha particles		[C14]
1999	Plating efficiency	Human SCL-II squamous carcinoma cells	X-rays	Dose response	[K20]
2000	Chromosomal aberrations	HPV-G and HaCaT human keratinocyte cells	Gamma rays; alpha particles		[M29]
2000	Chromosomal rearrangements	HF19 human fibroblasts		No instability observed	[G25]
2000	Chromosomal aberrations	Human lymphocytes	Alpha particles; X-rays		[A6]
2000	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[L12]
2000	Plating efficiency	HPV-G and HaCaT human keratinocyte cells	Gamma radiation; alpha particles		[M29]
2001	Apoptosis	WTKI human lymphoblastoid cells	^{56}Fe ions; ^{137}Cs gamma rays		[S8]
2001	Chromosomal aberrations	TK6 human lymphoblasts	^{56}Fe ions; gamma rays		[E14]
2001	Chromosomal aberrations	Human lymphocytes	Gamma rays		[B11]

Year	*End point*	*Cell type*	*Radiation type*[a]	*Comments*	*Reference*
2001	Chromosomal aberrations	Human lymphocytes	^{56}Fe ions		[G9]
2001	Chromosomal aberrations	Human lymphocytes	Alpha particles		[K19]
2001	Chromosomal aberrations	GM10115 human–hamster hybrid cells	X-rays; gamma rays; ^{56}Fe ions; neutrons; gold ions	Dose response	[L10]
2001	Chromosomal aberrations	WTK1 human lymphocytes	^{56}Fe ions; ^{137}Cs gamma rays		[S8]
2001	Microsatellite instability; gene amplifications	BEP2D human bronchial epithelial cells	Alpha particles; ^{56}Fe ions		[P16]
2001	Minisatellite instability	4T1 murine mammary adenocarcinoma cells	Gamma rays		[L33]
2001	Mutation frequency	*Saccharomyces cerevisiae*	Gamma rays	Dose response	[B19]
2001	Mutation frequency	TK6 human lymphoblasts	^{56}Fe ions; gamma rays		[E14]
2001	Mutation frequency	Human lymphoid cells	Gamma rays (12 Gy)		[G9]
2001	Mutation frequency	4T1 murine mammary adenocarcinoma cells	Gamma rays (12 Gy)		[L33]
2001	Plating efficiency	*Saccharomyces cerevisiae*	Gamma rays	Dose response	[B19]
2001	Plating efficiency	Human lymphoid cells	^{56}Fe ions		[G9]
2001	Recombination frequency	Saccharomyces cerevisiae	Gamma rays	Dose response	[B19]
2001	Telomere shortening	WTKI human lymphoblastoid cells	^{56}Fe ions; ^{137}Cs gamma rays		[S8]
2002	Apoptosis	Mouse fibroblast clones; V79 Chinese hamster ovary cells	X-rays		[C11]
2002	Chromosomal aberrations	Human fibroblasts	Gamma rays		[B21]
2002	Chromosomal aberrations	Murine haemopoietic stem cells	Gamma rays		[B23]
2002	Chromosomal aberrations	Mouse fibroblast clones; V79 Chinese hamster ovary cells	X-rays		[C11]
2002	Chromosomal aberrations and response to second irradiation	TK6 human lymphoblasts	^{56}Fe ions; gamma rays		[E13]
2002	Mutation frequency	TK6 human lymphoblasts	^{56}Fe ions; gamma rays		[E13]
2003	Chromosomal aberrations	Normal diploid human fibroblasts	Low- and high-LET radiation	No instability observed	[D17]
2003	Chromosomal aberrations	TK6 human lymphoblasts	Gamma rays		[E15]
2003	Global gene expression	Primary human lymphocytes	Gamma rays		[F16]
2003	Chromosomal instability and radiation-induced delayed reproductive death	Haemopoietic stem cells (R-M26/2-1)	Gamma rays	Independent of *TP53* status	[M27]
2003	Delayed lethality and micro-nucleus formation	Human osteoblast cells	Depleted uranium		[M57]
2003	Chromosomal aberrations	TK6 and NH32 human lymphoblasts	Gamma rays	Dose response up to 5 Gy	[S9]
2003	Micronucleus frequency	Human peripheral blood lymphocytes	Gamma rays	Dose response	[J8]
2003	Chromosomal aberrations	Murine haemopoietic stem cells	Gamma rays		[M27]
2003	Delayed *TP53* activation	HTI 080 human fibrosarcoma cells	X-rays; gamma rays		[S24]
2003	Mutation frequency	TK6 human lymphoblasts	^{56}Fe ions; gamma rays		[E15]
2003	Plating efficiency	Murine haemopoietic stem cells	Gamma rays		[M27]

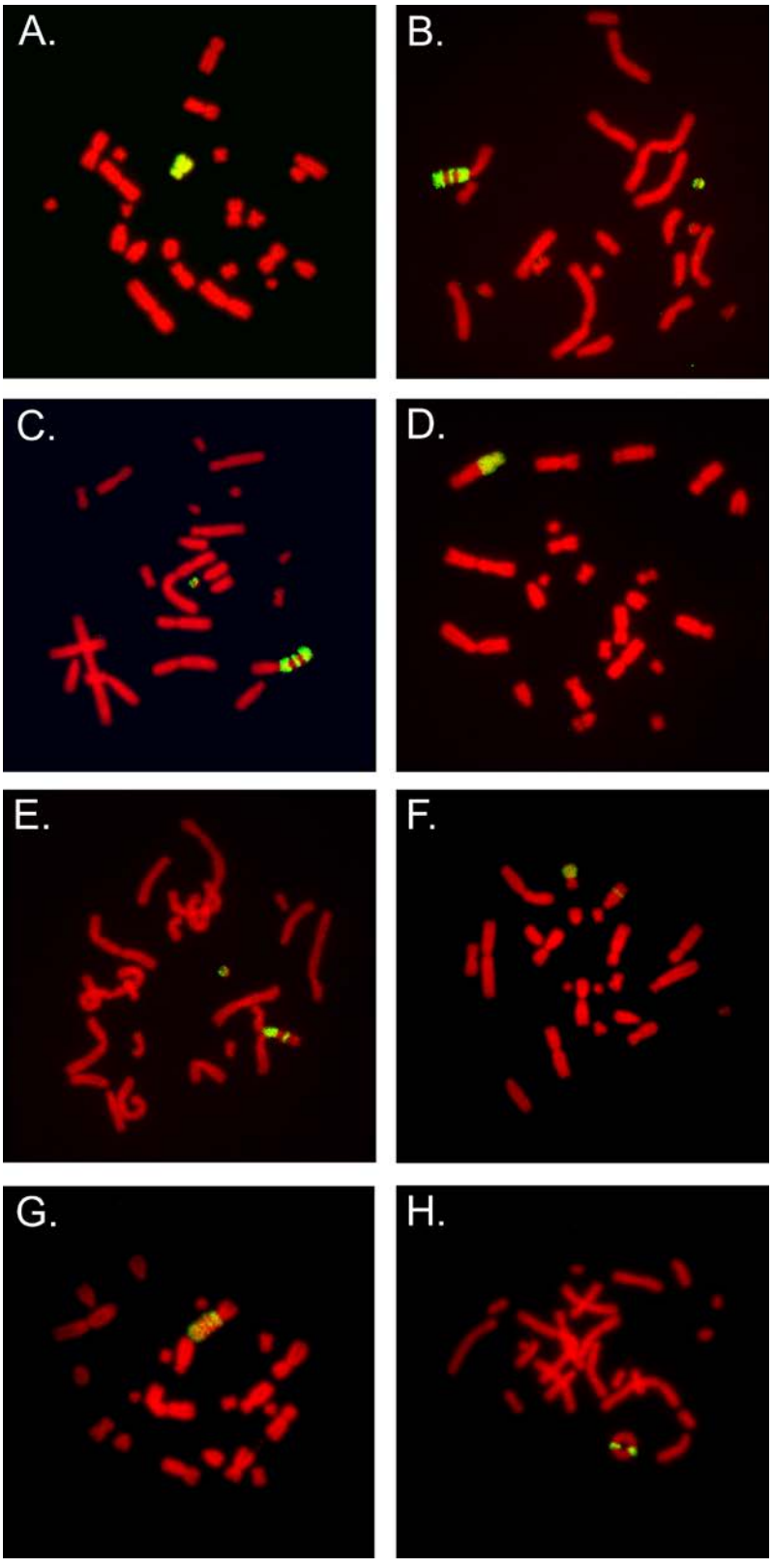
Year	*End point*	*Cell type*	*Radiation type*[a]	*Comments*	*Reference*
2004	DNA damage; comet assay	Chinese hamster ovary cells	X-rays	Low dose	[G23]
2004	GFP-based protein assay for homologous recombination	Human RKO cells	X-rays		[H24]
2004	Cell viability; apoptosis; changes in MAP and ERK signalling	Human lymphoblast cells	X-rays		[R13]
2004	Gene expression analysis	GM10115 human–hamster hybrid cells	X-rays		[S59]
2004	ROS production	Mouse m5S derived cl. 2011-14 cells	X-rays	Cell killing related to time after irradiation	[T6]
2004	Lethal sectoring; division delay	HeLa S3-9IV cells	X-rays; alpha particles		[S47]
2004	Chromosomal aberrations	*Scid* mouse cells	X-rays		[U26]
2004	Mutagenic radicals	Human–hamster hybrid A (L) cells	X-rays	High-LET	[W20]
2005	Chromosomal aberrations	Peripheral blood lymphocytes	Gamma irradiation		[B33]
2005	HPRT frequency; apoptosis; cell survival	Lymphoblastoid TK6 cells			[C20]
2005	H2AX phosphorylation	Human fibroblasts	Si and Fe ions	High-LET	[D26]
2005	Global gene expression profile	Human skin fibroblasts	X-rays	2 cGy and 4 Gy	[D27]
2005	Chromatid and chromosomal aberrations	Haemopoietic stem cells	Gamma rays		[G22]
2005	Micronuclei; chromosomal aberrations	Human peripheral blood lymphocytes	^{60}Co gamma rays	0–4 Gy	[J9]
2005	Genomic patterns of aberrations; radiation-induced mouse lymphomas	Mouse genomic BACs			[M55]
2005	Delayed apoptosis	CGL1 (HeLa × fibroblast) hybrid cells	X-rays	7 Gy	[M56]
2005	Chromosomal rearrangements	TK6 cells and clones with differing TP53 status	Gamma rays	2 Gy	[M54]
2005	Delayed apoptosis	GM10115 human–hamster hybrid cells	X-rays		[N29]
2005	Cell killing	V79 cells	X-rays	Low dose	[S65]
2005	Microarray analysis of isogenic clones to assay for gene expression	Human–hamster hybrid cells	X-rays		[S61]
2005	Gene expression influenced by *TP53* status	TK6; NH32; WTK1	Gamma rays	10 Gy at different times	[T7]

[a] It is not possible to include the range of doses used, as many studies used different doses, dose rates and radiation types within a single study. The reader is referred to the original study for information on the range of doses used.

7. Of the multitude of end points associated with radiation-induced instability, chromosomal changes are the best described. While some investigators describe chromosome gaps [S11, U20] or breaks [K3, L24] as the primary aberrations observed, it is unlikely that these contribute to long-term instability, as gaps have no known phenotype and breaks are generally lethal [C2, C3]. Of more significance are gross chromosomal rearrangements, particularly chromosomal translocations, duplications and partial trisomies [G8, M2, S8], which appear to involve amplification and recombination of large chromosomal regions by a currently unknown mechanism [D1, M16]. An example of chromosomal instability in human–hamster hybrid GM10115 cells clonally expanded from a single cell surviving X-irradiation is presented in figure III.

Figure III. Metaphase spreads from human–hamster hybrid GM10115 cells clonally expanded from a single cell surviving exposure to 5 Gy of X-rays.

A: Metaphase chromosomes from non-irradiated GM10115 cells. Using fluorescence in situ hybridization, the human chromosome 4 in these human–hamster hybrid cells is painted green, and the hamster chromosomes are stained red. B–H: Representative metaphase cells showing the chromosomal rearrangements observed in one colony of cells clonally expanded from a single cell. Radiation-induced instability stimulated the dynamic rearrangement of the genetic material, resulting in multiple subpopulations of cytogenetically rearranged cells within the clonally expanded population. Such cytogenetic rearrangements in GM10115 cells have been used by Morgan and colleagues [K9, L6, L9, L30, L31, M2] as a measure of genomic instability induced by both high- and low-LET radiation.



8. Instability is a frequent event in colonies of surviving cells. Kadhim et al. [K3] reported karyotypic abnormalities in 40–60% of murine stem cells exposed to doses of alpha particles that would produce about one hit per cell. Sabatier and colleagues [S44] observed late passage non-random chromosomal instability in >50% of metaphase cells from human dermal fibroblasts irradiated with a wide range of high-LET radiations (386 to 13,600 keV/μm). Likewise, Limoli et al. [L6] observed that X-rays induced chromosomal instability in ~3% of surviving human–hamster hybrid GM10115 clones per gray of radiation. This increased to ~4% Gy^{-1} after high-LET iron ion exposure [L12, L13]. This observed frequency of instability is grossly in excess of the reported frequency for gene mutations at similar doses. Therefore it is unlikely that mutation in a single gene or gene family is responsible for the unstable phenotype in unstable clones. Instead it is reasonable to suppose that factors contributing to maintaining genomic instability over time include critical pathways in DNA damage and repair [C10, H11, M48, Y3], chromosomal replication [B32], cellular homeostasis [B3, B6, M24, M48] and alterations in gene expression [S59, S60, S61, S62].

9. The high frequency of induced instability observed in different systems raises the intriguing question as to what is really being measured and of the significance of these observations. While the numerous in vitro studies summarized in table 1 have established the occurrence of radiation-induced genomic instability, many of the cell lines used were not "normal" initially, and in many cases they involved tumour-derived cell lines. Dugan and Bedford [D17] have pointed out that instability is sometimes not observed in apparently normal cells after irradiation. However, there are many reports of instability in the progeny of irradiated normal human and murine bone marrow cells [K1, K2] and in cultured human lymphocytes [B11, H7, H8, H9]. Some of this confusion may relate to the role of a functional *TP53* tumour suppressor gene. Both *TP53*-dependent [S9] and *TP53*-independent pathways have been proposed [K5, L8], and Moore et al. [M54] showed that genomic instability might differ both quantitatively and qualitatively as a consequence of altered *TP53* expression. Furthermore, differences in cellular proliferation patterns and susceptibility to mutation between cells and cell lines might also influence the reported results [C19]. Because the majority of reports indicate that irradiated normal primary cells readily demonstrate the instability phenotype (table 1), this implicates ionizing radiation as the causative agent. It also establishes induced instability as a phenotype associated with radiation exposure. As always, however, caution should be exercised when extrapolating from in vitro cell culture systems to the human situation in vivo.

10. When cells or tissues are directly exposed to ionizing radiation, biological effects are generally induced in a dose-dependent manner. One perplexing feature of radiation-induced genomic instability, and of non-targeted effects in general, is the lack of a well-defined dose response profile. Most investigators report that non-targeted effects are independent of dose. However, some investigators have observed a dose response at lower doses that tends to saturate at higher doses, and a few investigators have observed consistent dose-related effects. These data are summarized in table 1 and are reviewed in references [L6, M32]. Furthermore, radiation-induced instability appears independent of dose rate, although this has not been extensively investigated to date [L6]. In addition, there does not appear to be a significant LET effect for radiation-induced genomic instability, with both high- and low-LET radiations being effective [H2, K34, L12].

11. Many of the genomic changes described under the title of induced instability are changes of the same type as observed in human tumours. Radiation-induced cancers have no known molecular signature, and continued investigation aimed at understanding the processes and pathways by which radiation induces genomic alterations in the progeny of irradiated cells should provide insights into the mechanisms of radiation-induced transformation and carcinogenesis [M62]. Kennedy et al. [K10] demonstrated replication dependence of radiation-induced transformation of C3H 10T½ cells. A similar replication dependence was also reported for radiation-induced mammary cancers in rats, in which expression of epigenetic initiation required replication of irradiated mammary stem cells in the tissue microenvironment [K7].

12. While the multiple phenotypes associated with radiation-induced genomic instability are relatively well characterized, the molecular, biochemical and cellular events that initiate and perpetuate instability remain unknown. Directly induced DNA damage, e.g. induced DNA double-strand breaks, is probably not causative [M13]. Instead, deficiencies in cellular responses to DNA damage [C10, Y3], changes in gene expression [B6] or perturbations in cellular homeostasis [B3] are more likely to be involved, and provide a rational explanation as to why the unstable phenotype can persist. In the GM10115 cell system, clones of unstable cells continue to show the dynamic production of novel chromosomal rearrangements for over four years post-irradiation [N1]. Attempts to define the target for induced instability indicate that, while the nucleus may be the ultimate target [B6, K9], there is evidence for a persistent increase in reactive oxygen species (ROS) in cultures of cells showing radiation-induced genomic instability. A role for enhanced oxidative stress in perpetuating the unstable phenotype was first described by Clutton et al. [C5], and was later confirmed in studies by Limoli et al. [L7, L9, L10] and Redpath and Gutierrez [R3]. A persistent induction of ROS has also been shown to cause delayed reinduction of *TP53* in normal human fibroblasts [R6]. A study by Roy et al. [R5] revealed that hypoxia (2% oxygen) significantly reduced X-ray-induced delayed effects, specifically cell death, giant cell formation and chromosomal aberrations, compared with cells cultured under their "normal" 20% oxygen conditions. The role of ROS in radiation-induced genomic instability has been reviewed in detail by Mikhailov and colleagues [B45, M48]. It should be noted that oxygen tension in normal tissue shows a typical Gaussian distribution of values with a median between 40 and 60 mm Hg, and no values below 10 mm Hg [A13]. Tumours, on the other hand, invariably

show a distribution with much lower oxygen tension [B44]. As will be described later, a role for ROS in non-targeted radiation-induced bystander effects has also been described, suggesting a potential commonality in processes involved in these delayed effects of exposure to ionizing radiation.

13. It is well known that most mammalian cells do not divide indefinitely in vitro or in vivo, owing to a process termed replicative senescence. In human cells, replicative senescence can be caused by telomere shortening, but murine cells senesce despite having long, stable telomeres. Parrinello et al. [P19] showed that the phenotypes of senescent human fibroblasts and mouse embryonic fibroblasts (MEFs) differ under standard culture conditions that include 20% oxygen. The MEFs did not senesce in physiological (3%) oxygen levels, but underwent a spontaneous event that allowed indefinite proliferation in 20% oxygen. The proliferation and cytogenetic profiles of DNA repair-deficient MEFs suggested that DNA damage limits MEF proliferation in 20% oxygen. Indeed, MEFs accumulated more DNA damage in 20% oxygen than in 3% oxygen, and more damage than human fibroblasts in 20% oxygen. These results identify oxygen sensitivity as a critical difference between mouse and human cells, explaining their proliferative differences in culture, and possibly their different rates of cancer induction and ageing. Furthermore, they may contribute to explaining some of the differences between mouse and human studies described later in this annex.

14. There is emerging evidence implicating a role for extranuclear and even extracellular events in initiating and perpetuating radiation-induced chromosomal instability. Kadhim et al. [K3] analysed chromosomal instability in murine haemopoietic stem cells following alpha particle irradiation. Many of the surviving cells were those that were not traversed by an alpha particle during irradiation. Expanding these studies, Wright and colleagues used a protective metal grid to shield regions of the cell culture flask and lethally irradiated the non-shielded regions of the flask. They then cultured the non-irradiated, shielded cells and examined the clonal progeny for induced chromosomal instability. A high frequency of instability was observed in the progeny of cells that were not directly hit by radiation [L24]. Clearly, induced instability has an extracellular component, and signals from irradiated cells can stimulate chromosomal rearrangements in non-targeted cells within the radiation environment (reviewed in reference [M10]). These observations have implications for the fate of cells surviving radiation exposure in that some of these surviving cells may develop genomic instability. These observations also indicate that even cells outside the radiation field can manifest phenotypes similar to those of irradiated cells.

B. Induced genomic instability after in vivo irradiation followed by in vitro analysis

15. Weissenborn and Streffer were the first to describe induction of genomic instability after irradiation in vivo followed by analysis in vitro. They reported structural and numerical chromosomal anomalies as well as micronuclei at the first, second and third mitosis after in vivo irradiation of one- or two-cell mouse embryos with X-rays or neutrons [W6, W7]. These observations were extended by Ullrich and Davis [U18], who irradiated inbred BALB/c mice and at varying intervals after irradiation removed and cultured the mammary glands in vitro. Cytogenetic analysis indicated that instability could develop and persist in situ in a mature, fully differentiated tissue after in vivo irradiation. Furthermore, there was a dose-dependent increase in the frequency of delayed aberrations at low doses (0.1–1 Gy) that reached a plateau at higher doses [U18].

16. Cellular studies on radiation-induced murine mammary cancer demonstrated strain-dependent differences in susceptibility, presumably resulting from differences in sensitivity to neoplastic initiation [U17]. Similar strain susceptibility is apparent for in vivo irradiation followed by in vitro analysis of induced instability. Mammary cells from BALB/c mice are more susceptible to radiation-induced genomic instability than those from C57BL/6 or F_1 hybrid crosses of C57BL/6 and BALB/c mice [P9, U20]. Studies of DNA repair in the radiosensitive BALB/c mouse revealed inefficient end-joining of gamma-ray-induced double-strand breaks in DNA. This is apparently due to reduced expression of the DNA-PKcs protein and lowered DNA-PK activity in these mice. This may impair the animals' ability to appropriately respond to induced damage and may thus account for the increased instability [O4, Y3]. Most DNA repair processes have evolved to prevent genomic instability induced by endogenous lesions [L49] and induced DNA damage (for a comprehensive discussion, see the BEIR VII [C23] and French Academies [T8] reports).

C. Induced genomic instability after in vitro irradiation followed by in vivo analysis

17. Conversely, instability induced in vitro can be transmitted in vivo following transplantation of irradiated cells into recipient animals. Paquette and Little [P3] irradiated C3H 10T½ cells and cultured half in cell culture in vitro; the other half was transplanted into syngeneic and non-immunosuppressed C3H mice. Interestingly, a higher frequency of minisatellite instability was observed in those irradiated cells injected into mice than those cultured in vitro. Watson et al. [W3] reported the induction and long-term persistence of chromosomal instability after murine bone marrow cells were irradiated in vitro and then transplanted into female CBA/H mice that had received 10 Gy of X-irradiation less than two hours before to eradicate the host bone marrow. These studies were later extended to demonstrate that instability induced by X-ray or neutron irradiation in vitro can be transmitted in vivo [W4]. A recent analysis of a series of radiation-induced sarcomas [G10] showed a prevalence (53%) of somatic *TP53* mutations, which was significantly higher than that for sporadic sarcomas (16.8%).

The mutations were inactivating and associated with the loss of the other *TP53* allele. This loss of heterozygosity was due to the loss of a large fragment of the chromosome or of the whole chromosome, probably indicating a more general chromosomal instability similar to that previously described [L1].

18. Watson et al. [W2] have provided convincing evidence that the induction of genomic instability following in vitro irradiation and in vivo expression can result from a non-targeted bystander-like effect. That is, rather than resulting from the direct effect of radiation exposure being passed on to the progeny of that irradiated cell, instability might also result from soluble signals being passed from irradiated cells to non-irradiated cells. When non-irradiated cells were mixed with cells irradiated with 0.5 Gy of neutrons at 0.04 Gy/min and then transplanted into recipient CBA/H mice, instability was observed in the non-irradiated cell population [W2]. An elegantly conceived chromosomal marker system allowed the investigators to distinguish between the irradiated and non-irradiated transplanted cells and cells derived from the host mouse. Irradiated and non-irradiated cells were distinguished by using marrow from CBA/H mice (40XY cells) and the congenic CBA/H strain (40XY6T6 cells) homozygous for the stable T6 reciprocal translocation between chromosomes 14 and 15. Using this system, unambiguous evidence for non-clonal chromosomal aberrations was observed in clonal populations derived in vitro from neutron-irradiated bone marrow cells. Furthermore, after transplantation with neutron-irradiated cells, translocations and deletions were observed for a period of 3–13 months. Significantly, there was also a higher frequency of unstable aberrations in the bone marrow of the recipient mouse. These results implicate an in vivo bystander-like mechanism in the induction of chromosomal instability, and suggest that the instability observed in the non-irradiated cells is not an artefact of clonal selection. This result was confirmed by Xue et al. [X2], who injected nude mice with a mixture of human colon LS174T adenocarcinoma cells and LS174T-cells prelabelled with lethal doses of DNA-incorporated 5-[125I]iodo-2'-deoxyuridine (^{125}IUdR). A distinct inhibitory effect on the growth of the unlabelled LS174T tumour cells was observed. Because ^{125}IUdR is incorporated into DNA, almost all the electrons emitted during radioactive decay have a subcellular range of <0.5 μm. This led the authors to conclude that the inhibitory result was due to a bystander effect generated in vivo by factors present within and/or released by the ^{125}IUdR-labelled cells. However, it is also possible that debris and breakdown products from the heavily irradiated cells might affect bystander cells, and these non-labelled cells might even incorporate ^{125}I released from dying cells.

19. Currently the mechanisms underlying the induction and persistence of instability are not understood. The induction of chromosomal aberrations in vivo by a bystander-like mechanism might provide insights into the mechanisms as well as link instability to bystander effects. Bystander effects can be mediated by cell-to-cell gap junction communication and secretion of soluble factors. These secreted factors [S39] might include extracellular cytokine-like factors [L2, N8] that are able to increase intracellular levels of ROS in non-irradiated cells [L23, M10, M16]. Lorimore et al. recently proposed a potential mechanism for these in vivo radiation-induced bystander effects [L22]. They found persistent macrophage activation combined with neutrophil infiltration following 4 Gy whole-body irradiation of mice. The inflammatory nature of the observed responses may provide a mechanism for the long-term production of genetic damage by a bystander effect, ultimately contributing to radiation-induced instability and potentially leukaemogenesis. This will be discussed in more detail in the section on radiation-induced genomic instability and bystander effects.

D. Radiation-induced genomic instability in vivo

20. In reviewing the literature on in vivo non-targeted effects of ionizing radiation, it becomes obvious when considering the mouse studies that many of the observed effects are highly dependent upon the mouse strain used and the sex of the animal studied [M58]. Consequently, there has been an effort throughout this annex to identify the mouse strain used when comparing conflicting data. It is also apparent that even the same mouse strain can vary significantly when bred in different colonies in different laboratories. Differences due to sex might also exist, but not all of the studies provide adequate details on the sex of the animals used. While much has been learned from animal models [F7], caution should be exercised when extrapolating from the animal studies to the human situation.

21. The reports of radiation-induced genomic instability in vivo are summarized in table 2, which also lists the end point used to assay instability, the model system, the type of radiation used and whether or not genomic instability was observed. In this section the methods of analysing instability in vivo will be highlighted along with potential areas of conflict and associated caveats.

22. Nowell [N19] first proposed that genomic instability might be a driving force in tumorigenesis and a hallmark of many cancers [C7, L3]. There is accumulating evidence suggesting that instability may represent a critical step in the genesis of certain radiation-induced cancers [L14, S3, U20]. Implicit in this annex is the hypothesis that radiation-induced genomic instability provides relevant underlying mechanistic contributions to some radiation-induced cancers. While the precise relationship between radiation-induced genomic instability and radiation carcinogenesis remains to be determined, understanding the mechanisms of induced instability might provide valuable insights into health risks associated with radiation exposure and the carcinogenesis process in general.

Table 2 In vivo studies of radiation-induced genomic instability (RIGI) and transgenerational effects

Year	*End point*	*Cellular system*	*Radiation type*	*RIGI*[a]	*Reference*
1976	Graft versus host reactions; non-specific bystander activity	(PVGc × Wistar) F1 hybrids; PVGc spleen cells	X-rays	+	[J10]
1979	Chromosomal aberrations	Haemopoietic cells in atomic bombing survivors	Neutrons; gamma rays	–	[K21]
1979	Tumour induction	Mouse localized exposures	X-rays; neutrons	+	[U19]
1980	Tumour induction	Mouse localized exposures	X-rays; neutrons	+	[U16]
1982	Foetal deaths, malformations in F1 mice	Mouse whole-body irradiation	X-rays	+	[K13]
1982	Tumour induction in F1	Mouse whole-body irradiation	X-rays	+	[N17]
1984	Foetal deaths, malformations in F1 mice	Mouse whole-body irradiation	X-rays	+	[K12]
1985	Tumour induction	Mouse whole-body irradiation	X-rays	+	[C6]
1988	Chromosomal aberrations	Skin fibroblasts (mouse zygotes irradiated)	X-rays; neutrons	+	[P1]
1988	Chromosomal aberrations	Mouse embryo	X-rays; neutrons	+	[W14]
1988	Congenital abnormalities	Skin fibroblasts (mouse zygotes irradiated)	X-rays; neutrons	+	[P1]
1988	Micronucleus frequency	Mouse embryo	X-rays	+	[W14]
1989	Cell proliferation in F1 and F2 generations	Mouse whole-body irradiation	Gamma rays	+	[O2]
1989	Chromosomal aberrations	Mouse zygotes	X-rays	+	[P17]
1989	Chromosomal aberrations	Mouse embryo	X-rays	+	[W6]
1989	Micronucleus frequency	Mouse zygotes	X-rays	+	[P17]
1990	Cancer prevalence	Children of nuclear plant workers	X-rays; gamma rays	+	[G2]
1990	Foetal deaths, malformations	Mouse embryos	X-rays	+	[M26]
1991	Chromosomal aberrations	Blood lymphocytes of uranium miners after whole-body irradiation	Alpha particles	–	[M34]
1991	Sister chromatid exchanges	Blood lymphocytes of uranium miners after whole-body irradiation	Alpha particles	–	[M34]
1993	Chromosomal aberrations	Skin fibroblasts in atomic bombing survivors	Neutrons; gamma rays	–	[H13]
1993	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[D10]
1994	Cancer prevalence	Children of nuclear plant workers	X-rays; gamma rays	–	[D6]
1994	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[S1]
1994	Minisatellite instability	C3H 10T½ murine cells (irradiated in vitro, then injected into mice)	X-rays	+	[P3]
1995	Cancer prevalence	Progeny of cancer patients who had undergone radiation therapy	X-rays	–	[H4]
1995	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[F2]
1995	Tumour induction in F1	Mouse whole-body irradiation	X-rays	–	[C4]
1996	Chromosomal aberrations	Murine bone marrow (in vitro irradiation)	Alpha particles	+	[W3]
1996	Minisatellite instability	Chernobyl survivors after whole-body irradiation	Gamma rays	+	[D11]
1996	Minisatellite instability	Haemopoietic cells in atomic bombing survivors	Neutrons; gamma rays	+/–	[S5]

Year	*End point*	*Cellular system*	*Radiation type*	*RIGI*[a]	*Reference*
1996	Neoplastic transformation	Epithelial cells of whole-body-irradiated mice	X-rays	+	[U17]
1997	Cancer prevalence	Children of nuclear plant workers	X-rays; gamma rays	–	[D7]
1997	Cell proliferation in F1 and F2 generations	Mouse whole-body irradiation	Gamma rays	+	[W10]
1997	Minisatellite instability	Chernobyl survivors after whole-body irradiation	Gamma rays	+	[D12]
1997	Mutation frequencies in F1	Mice after whole-body irradiation	Gamma rays	+	[L25]
1998	Chromosomal aberrations	Human lymphocytes after whole-body irradiation	Gamma rays	–	[S2]
1998	Chromosomal aberrations	Lymphocytes from uranium miners	Alpha particles (radon)	+/–	[S25]
1998	Chromosomal aberrations	Blood lymphocytes from plutonium workers	Gamma rays	–	[W9]
1998	Micronucleus frequency	Rat lung cells after partial-volume irradiation	Gamma rays	+	[K11]
1998	Micronucleus frequency	Lymphocytes from uranium miners	Alpha particles (radon)	+	[S25]
1998	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[D15]
1998	Tumour induction in F1	Mouse whole-body irradiation	Gamma rays	+	[L20]
1999	Chromosomal aberrations	Lymphocytes of Chernobyl survivors after whole-body irradiation	Gamma rays	+	[G3]
1999	Chromosomal aberrations	Lymphocytes of Chernobyl recovery operations workers and workers from the nuclear power plant	Gamma rays	+/–	[L36]
1999	Chromosomal aberrations	Haemopoietic cells in atomic bombing survivors	Neutrons; gamma rays	–	[N6]
1999	Chromosomal aberrations	Mouse epithelial cells after whole-body irradiation	X-rays	+	[U18]
1999	Chromosomal aberrations	Mouse bone marrow cells after whole-body irradiation	X-rays	+	[X1]
1999	Prenatal mortality; developmental and skeletal defects in F2 mice	Mouse zygotes	X-rays	+	[P7]
1999	Sister chromatid exchanges	Lymphocytes of Chernobyl recovery operations workers and workers from the nuclear power plant	Gamma rays	+	[L36]
1999	Minisatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	+/–	[L18]
2000	Chromosomal aberrations	Foetal haemopoietic cells of mice after foetal irradiation	Gamma rays	+	[D3]
2000	Chromosomal aberrations	C57BL/6 mice after whole-body irradiation	Gamma rays	+	[S20]
2000	Chromosomal aberrations	Blood lymphocytes of patients after whole-body irradiation for radiotherapy	X-rays	–	[T2]
2000	Chromosomal aberrations	Mixture of irradiated and non-irradiated murine bone marrow cells	Neutrons	+	[W2]
2000	Micronucleus frequency	CBA mice after prenatal irradiation	Gamma rays	–	[A1]
2000	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[D16]
2000	Tumour induction in F1	Pregnant mice	Gamma rays	+	[U21]
2000	Minisatellite instability	Sperm from three seminoma patients	X-rays	–	[M5]

Year	*End point*	*Cellular system*	*Radiation type*	*RIGI*[a]	*Reference*
2001	Chromosomal aberrations	Mouse bone marrow cells after whole-body irradiation	Alpha particles (^{224}Ra); X-rays	–	[B12]
2001	Chromosomal aberrations	Mouse bone marrow cells after whole-body irradiation	X-rays	+	[M1]
2001	Chromosomal aberrations	Blood lymphocytes of uranium miners after whole-body irradiation	Alpha particles	–	[M35]
2001	Chromosomal aberrations	Blood lymphocytes of cancer patients after whole-body irradiation	Gamma rays	–	[V1]
2001	Chromosomal aberrations	Murine bone marrow; irradiated cells or whole-body irradiation	X-rays; alpha particles; neutrons	+	[W4]
2001	Chromosomal aberrations	Blood lymphocytes from plutonium workers	Gamma rays	–	[W8]
2001	Ductal dysplasia	Epithelial cells of ATM$^{+/-}$ mice	X-rays	+	[W15]
2001	Minisatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	–	[L17]
2001	Minisatellite instability	Haemopoietic cells in atomic bombing survivors	Neutrons; gamma rays	+/–	[N7]
2001	Minisatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	+	[W5]
2001	Mutation frequencies in F1	Mice after whole-body irradiation	Gamma rays	+	[N14]
2001	Signal kinase activity in F3	Mouse whole-body irradiation	Gamma rays	+	[B4]
2001	Sister chromatid exchanges	Blood lymphocytes of uranium miners after whole-body irradiation	Alpha particles	–	[M35]
2002	APRT, HPRT mutation frequency	Mouse T-lymphocytes after whole-body irradiation	X-rays	+	[L35]
2002	Cancer prevalence	Progeny of radiation workers	X-rays; gamma rays	+	[D20]
2002	Cell proliferation in F1 and F2	Mouse whole-body irradiation	Gamma rays	+	[B5]
2002	Chromosomal aberrations	Mouse T-lymphocytes after whole-body irradiation	X-rays	+	[L35]
2002	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[B2]
2002	Microsatellite instability in F0, F1 and F2 generations	Mice after whole-body irradiation	Neutrons; X-rays; gamma rays	+	[D9]
2002	Minisatellite instability	Chernobyl survivors after whole-body irradiation	Gamma rays	+	[D19]
2002	Minisatellite instability	Children living in Semipalatinsk	^{131}I, ^{90}Sr and ^{137}Cs	+	[D8]
2002	Mutation frequencies in F1	Mice after whole-body irradiation	Gamma rays	+	[S26]
2002	Signal kinase activity in F3	Mouse whole-body irradiation	Gamma rays	+	[V2]
2003	ROS content; state of DNA structure	Bone marrow cells of male mice after whole-body irradiation	X-rays (1.5 Gy)	+	[M58]
2003	Minisatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	–	[K37]
2004	DNA double-strand breaks; morula and gastrula formation	*Oryzias latipes* F3 embryos derived from male founders	Gamma rays	+	[A18]
2004	Epigenetic global genomic DNA methylation changes	Male and female mouse whole-body irradiation	Low-dose X-rays	+	[K30]
2004	Latency of cancer risk	Cohorts of underground miners exposed to radon	Radon	–	[L41]

Year	*End point*	*Cellular system*	*Radiation type*	*RIGI*[a]	*Reference*
2004	Chromosomal aberrations; transformation frequency; cell killing; DNA damage	Mouse embryonic fibroblasts from mice deficient in Hsp70.1 and Hsp70.3		+	[H26]
2004	Epigenetic global genomic DNA methylation changes	Male and female mouse whole-body irradiation	High-dose X-rays (5 Gy)	+	[P21]
2004	Somatic mutation assay	F1 and F2 progeny of *Oryzias latipes*	Gamma rays	+	[S42]
2004	Destruction of haematopoietic progenitor and stem cells in F1	Mice after sublethal irradiation		+	[Z8]
2004	Chromosomal aberrations; tumour incidence; blood counts	Pregnant mice; mice foetal liver and spleen cells	1 Gy irradiation	+	[U22]
2004	Apoptosis; cell proliferation and differentiation	Mouse limb bud cells	0.3 and 5 Gy	+	[W21]
2004	Micronuclei; number of ovules; male fertility	Pea plant seedlings for two generations	Gamma rays	+	[Z8]
2004	Mini- and microsatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	–	[S55]
2005	Chromosomal aberrations	Pregnant Swiss albino mice	Gamma rays (1–1.5 Gy)	+	[U23]
2005	Initiation of intestinal adenoma	Apc mice	X-irradiation	–	[E20]
2005	Locus-specific mutations	*Oryzias latipes* spermatogonial stem cells	Gamma rays (0.03 cGy/min and 95 cGy/min)	+	[S43]
2005	Chromosomal instability	Congenic haematopoietic cells into irradiated mouse host	Gamma rays	+	[L47]
2005	Chromatid aberrations; chromosomal radiosensitivity	Prostrate patients with prostatic hyperplasia	Ionizing radiation	+	[H25]
2005	Clonogenic survival; apoptosis	Bladder explants from C57BL6 and CBA/Ca mice after whole-body irradiation	Low-dose irradiation (0.5 Gy)	+	[H25]
2005	Chromosomal aberrations	Clonal T-cells from atomic bombing survivors	Gamma rays	–	[K29]
2005	Chromosomal instability	Haemopoietic stem cells	Gamma rays	+	[L47]
2005	Tumour formation	Female B6C3 F1 mice	Gamma rays (1.9 Gy)	+	[S66]
2005	Microsatellite instability	Children of Chernobyl recovery operations workers	Gamma rays	–	[F15]

[a] +: genomic instability was observed; –: genomic instability was not detected.

1. Mouse models for radiation-induced genomic instability in vivo

23. As described above, transmissible genomic instability has been observed after irradiation in vivo followed by culture in vitro and vice versa. The picture following irradiation in vivo and subsequent expression of instability in vivo is less clear and more controversial. Following irradiation of bone marrow cells from 12 week-old CBA/H mice with 0.5 Gy of alpha particles, Watson et al. [W3] observed a constant frequency (10–13.4%) of cells with stable chromosomal aberrations for up to 17½ months. This increased to 49.8% at 24 months in pooled samples from three CBA/H mice. They noted significant variation between individuals, with a few animals exhibiting little or no induced instability despite CBA/H being an inbred mouse strain. In contrast, protracted whole-body gamma irradiation of prenatal CBA-Ca mice at either 44, 99 or 265 mGy/day (to a total dose of 0.7, 1.6 or 4.2 Gy) did not induce damage in erythroid stem cells that could be detected as persistent or delayed chromosomal aberrations as measured by micronucleated erythrocytes at 35 days after irradiation [A1]. Bouffler et al. [B12] also failed to find evidence of transmissible chromosomal instability 50 or 100 days after in vivo exposure of CBA/H mice either to alpha particles from the bone-seeking radionuclide ^{224}Ra or to X-rays. Likewise, chromosomal instability was not detected in peripheral blood lymphocytes from C57BL/6 mice for up to 30 days after whole-body gamma radiation [S21] or up to 21 months [S20]. These last results are consistent with in vitro studies indicating that induced instability was not observed in C57BL/6 mice [P9, W1]. Furthermore, no instability was reported in bone marrow

cells from Swiss mice up to 100 days after exposure to 3 Gy of X-rays [X1]. These results were initially presumed to indicate that the Swiss mouse strain, like the C57BL/6 mouse strain, is refractory to radiation-induced instability. However, this does not appear to be the case. When Swiss albino mice were exposed to 0.25–1.5 Gy of gamma radiation on day 14 or 17 of gestation, significant dose-dependent increases in chromosomal aberrations, micronuclei and/or changes in ploidy were observed in the bone marrow at 12 months of age [D3]. The investigators concluded that radiation-induced genomic instability in the foetal haemopoietic cells of the mouse persisted post-natally [D3]. These data are summarized in table 2.

24. Although not designed to specifically investigate radiation-induced genomic instability, a number of studies have examined the persistence of cytogenetic rearrangements in animals at delayed times after irradiation. Hande et al. [H18, H19, H20, H21] used female Swiss mice to study the induction and persistence of dicentrics and translocations in splenocytes up to 112 days after exposure to 2 Gy of whole-body X irradiation. The frequencies of dicentrics decreased exponentially with time, while the frequencies of translocations were constant in the period 0–7 days and then decreased linearly or exponentially. No new chromosomal rearrangements were observed, suggesting that there was no delayed cytogenetic instability in these animals. Similar studies using other mouse models have reported similar results [T5].

25. In attempting to reconcile the apparently conflicting results described above, Bouffler et al. [B12] have noted the sensitivity of mouse bone marrow cells to perturbations through transplantation and culture, and emphasized the need for sound control experiments to be performed concurrently. For instance, some of the radiation-induced transmissible chromosomal instability reported by Watson et al. [W3] could be attributed to the low background frequency of aberrations observed in the control repopulating cells. This is in contrast to the higher background described by Bouffler et al. [B12]. It is also possible that the disparate literature on radiation-induced genomic instability in vivo reflects the inherent variability between the inbred mouse strains used and differences due to sex within the animal strains used.

26. To investigate the in vivo non-targeted effects of low-LET radiation, Lorimore et al. [L47] used the same congenic sex-mismatch bone marrow transplantation protocol as used by Watson et al. [W2] to repopulate the haemopoietic system from a mixture of gamma-irradiated and non-irradiated haemopoietic stem cells such that host-, irradiated donor- and non-irradiated donor-derived cells could be distinguished. Chromosomal instability in the progeny of irradiated haemopoietic stem cells accompanied by a reduction in their contribution to the repopulated haemopoietic system was observed and is consistent with a delayed genomic instability phenotype being expressed in vivo. However, chromosomal instability was also shown in the progeny of the non-irradiated haemopoietic stem cells, implicating a bystander-like mechanism. Studies of the influence of irradiated recipient stromal microenvironment and experiments replacing irradiated cells with irradiated cell-conditioned medium revealed the source of the in vivo bystander effect to be the descendants of irradiated cells rather than the irradiated cells themselves. Lorimore et al. [L47] speculated that it is possible that a radiation-induced genomic instability phenotype in vivo need not necessarily be a reflection of intrinsically unstable cells but the response to ongoing production of inflammatory-type damaging signals [L22] as a long-term unexpected consequence of the initial radiation.

27. While the literature is replete with apparently contradictory reports of radiation-induced instability in mouse model systems, these results clearly indicate that genetic factors can play a major role in the instability phenotype and that analysis of radiation-induced genomic instability in vivo is significantly more complicated than in vitro. Critical analysis of radiation-induced genomic instability in vivo is not a trivial undertaking. In any animal model there is likely to be some inherent genomic instability that complicates the selection of appropriate control populations. Such experiments generally involve inbred strains of mice, and even in radiation-sensitive populations only a small percentage, generally <50%, will exhibit an instability phenotype. Furthermore, extrapolating such results to other mouse strains or outbred populations is difficult at best. Until a careful study involving sound and relevant controls as well as statistically relevant numbers of animals exposed to a homogeneous quality of radiation is carried out, the induction of radiation-induced genomic instability in vivo will remain controversial.

2. Human studies

28. Radiation therapy has improved over recent years, and many of the cancer patients treated with radiation are surviving longer than did those in the past. Second cancers occurring in the irradiated field have been reported in some of these patients, suggesting a direct role of the radiation exposure [B37, B38]. Data on second cancers occurring in children irradiated for cancer indicate that some genetic predisposition to cancer may also predispose them to radiotherapy-related second cancers [D22, E19, F14]. Nevertheless, it is still difficult to identify the radiation-induced lesions initiating the second malignancy. At the time of diagnosis, multiple genomic alterations are present in the tumours, and the majority are likely to represent secondary events occurring during tumour evolution and subsequent selection. This underscores that caution must be applied to analysis of radiation-induced genomic instability and its role in human carcinogenesis. The subsequent discussion in this section highlights the controversies and contradictions inherent in the human studies. To this end it is reasonable to expect that analysis of normal, healthy populations of individuals would not provide evidence of instability regardless of the individuals' radiation history. Indeed, the majority of studies investigating instability in radiation-exposed populations have analysed samples from normal, healthy individuals and did not find evidence of instability [T2, T4]. It is also reasonable

to expect that analysis of instability in individuals manifesting phenotypic effects of radiation exposure, e.g. cancer or leukaemia, might well show evidence of induced instability. Once again, limited studies indicate that this is the case [N6, N7]. Whether or not the observed instability is a direct or non-targeted effect of radiation exposure, or a secondary selective effect of disease evolution, cannot be definitively determined at present. Furthermore, this question is unlikely to be resolved in the foreseeable future. This caveat should be kept in mind in the following discussion.

29. As has been described utilizing the mouse as a model system, both induction and lack of induction of transmissible radiation-induced genomic instability have been reported in humans, and once again genetic factors appear to play a role in the observed instability [K4]. Induced chromosomal instability has been described in long-term cultures of human lymphocytes following irradiation and culture in vitro [H9]. Using the same lymphocyte culture protocol, chromosomal instability was reported in blood samples from individuals exposed during the radiation accident in Estonia in 1994 [S2]. Radiation exposure was variable, protracted and not precisely determined. Furthermore, blood samples were taken well after radiation exposure. No dose response was apparent, and contrary to previous studies from the Lambert laboratory, chromosomal instability was also observed in long-term cultures from non-exposed controls [S2]. In contrast, cytogenetic analysis of 18 individuals who had received between 35 and 80 Gy of fractionated radiation therapy for different cancers showed no increase in aberrant cell types as a function of time after completing therapy. Thus no cytogenetic evidence that fractionated radiotherapy induced a persistent or late-manifesting state of genomic instability was found [T2]. It should be stressed that the majority of patients treated for different malignancies received localized, partial-body irradiation with emphasis on minimizing damage to normal tissue. Consequently, different proportions of bone marrow stem cell populations and peripheral blood lymphocytes would have been exposed to the radiation. It is likely that more cells than the number actually analysed (<200 per patient), would have to be interrogated before evidence of persistent transmissible chromosomal instability would be observed in these individuals, if it indeed existed [T2].

30. The availability of cultured lymphocyte preparations from radiation workers with internal deposits of plutonium has provided the opportunity to examine whether protracted irradiation of bone marrow cells had induced a transmissible genomic instability in descendant cells in the peripheral blood [W8]. Bone marrow dose calculations provided individual cumulative estimates at the time of sampling ranging up to 1.8 Sv. Chromosome analysis revealed no significant differences, either in comparisons between the total group of plutonium workers and controls for comparable periods or when the comparisons were restricted to a group of plutonium workers with initial bone marrow plutonium doses of greater than 0.25 Sv. There was therefore no evidence from this study for the induction of persistent transmissible genomic instability in the bone marrow of radiation workers with internal deposits of plutonium [W8]. Likewise, clonally expanded T-cell lymphocyte populations did not demonstrate increased chromosomal instability using either G-band analysis or multicolour fluorescence in situ hybridization [K29].

31. The long-term effect of radiation exposure on uranium miners employed by the Wismut uranium mining company in the former German Democratic Republic was investigated by scoring the frequency and percentage of micronuclei with and without a centromere. Kryscio et al. [K38] reported that genomic instability had occurred in the lymphocytes of miners, especially those with cancer.

32. A number of investigators have studied the alpha radiation risks in patients who received injections of Thorotrast, an X-ray contrast medium used in Europe, Japan and the United States from the late 1920s to 1955. Thorotrast was composed of thorium dioxide and contained ^{232}Th, a naturally occurring radionuclide. Because the physical half-life of ^{232}Th is 14 billion years and Thorotrast is not appreciably eliminated from the body, the tissues in which it was deposited are irradiated by alpha rays for the entire lifetime of the subject. The major causes of death among the Thorotrast patients are liver cancer, liver cirrhosis, leukaemia and other cancers. Mutation analyses of the *TP53* gene and loss of heterozygosity (LOH) studies at the 17p locus were performed by Ishikawa et al. [I2] to characterize the genetic changes in Thorotrast-induced liver tumours. LOH was not frequent; most mutations were transitions, suggesting that genetic changes in Thorotrast-induced cancers were mainly delayed mutations and not the result of the direct effects of radiation.

33. Likewise Iwamoto et al. [I3] analysed mutations in *TP53* from 20 Thorotrast recipients who developed cancer, mostly of hepatic bile duct and blood vessel origin. Of the 20 cases, 19 had *TP53* point mutations. Moreover, the accompanying non-tumour tissues from these patients also had *TP53* mutations, albeit at lower frequency. The distribution pattern of the point mutations was significantly different between the non-tumour and tumour tissues, with most mutations in malignant tissues located in the highly conserved domains of the *TP53* gene. These results support the idea that *TP53* mutations are important in the genesis of Thorotrast-induced tumours but that these point mutations are a secondary outcome of genomic instability induced by the irradiation. A similar result was reported by Kamikawa et al. [K24], who investigated mutations of the *RAS* and the *TP53* genes in archival sections of liver cancers induced by Thorotrast. These investigators were unable to rule out the possibility that genetic insults occurred indirectly in the proliferating cells adjacent to the necrosis rather than being a direct effect of alpha particles.

34. Wada et al. [W17] also investigated genetic changes in the *TP53* gene in 19 autopsy cases of liver malignancies. LOH at the 17p13 locus and mutations in *TP53* were analysed. A number of cases were informative: four cases

showed LOH and eight contained mutations. The direct action of alpha particles was thought to result in relatively large deletions, such as those detected by LOH. Therefore the low frequency of such changes (27%) compared with point mutations (47%) suggests that the genetic changes in the *TP53* gene in the liver tumours related to Thorotrast were not caused mainly by direct actions of alpha particles but rather by indirect effects that may have been due to cycles of necrosis and regeneration. This study was recently expanded to compare Thorotrast-induced liver cancers to those not associated with Thorotrast exposure. LOH at 37 loci was investigated. Liu et al. [L46] found frequent LOH at microsatellite markers D4S1538, D16S2624 and D17S1303 to be common to all the subtypes of liver cancer, independent of the specific carcinogenic agent. In contrast, LOH at marker D4S1652 was generally not observed in Thorotrast-induced cancers. LOH analysis revealed that Thorotrast-induced cancers share some LOH features with cancers not induced by Thorotrast, and Liu and colleagues concluded that induced LOH is not simply due to direct insult to DNA by alpha particles, but can occur through complex mechanisms, including bystander effects [L46]. Such a conclusion is reasonable given the analysis of Goto et al. [G18], who used imaging plate autoradiography to examine the microdistribution of alpha particles in pathological sections of tissues from Thorotrast patients. They found that the amount of thorium deposited in tumour tissue was correlated with that in non-tumour tissue, and that Thorotrast deposition was not associated with DNA damage determined by histochemistry. Goto et al. [G18] concluded that radioactive thorium always migrates in macrophages within the deposited organs, and that the organs are evenly exposed to alpha particles.

35. In an evaluation of Thorotrast-induced genomic instability, Liu et al. [L45] analysed microsatellite instability in Thorotrast-induced liver cancers. The frequency of microsatellite instability cases was 62.5% in Thorotrast-induced cancers, whereas it was 22.7% in non-Thorotrast induced cancers. Liu and colleagues suggested that microsatellite instability induced by exposure to Thorotrast mainly reflects clonal expansion of cancer cells and is partly due to inactivation of the DNA mismatch repair gene hMLH1 by hypermethylation. A recent finding also suggests that methylation changes in DNA can be associated with radiation-induced genomic instability [K35].

36. Littlefield et al. [L16] examined the cumulative genetic damage in a 72-year-old man who was treated with a 32 mL bolus of Thorotrast during cerebral angiography performed more than 40 years earlier. Peripheral T-lymphocytes were cultured to quantify the frequencies and cellular distributions of asymmetrical and symmetrical types of chromosomal aberrations. Assays of glycophorin A (GPA) mutations in red blood cells were also performed. Their results revealed that approximately 30% of the lymphocytes in this patient contained one or more chromosomal aberrations, the majority of which were of the “stable” type. About one third of the lymphocytes with chromosome damage carried multiple aberrations, suggesting that significant numbers of stem cells survived exposures to alpha particle radiation that induced complex genomic alterations. Increased frequencies of GPA mutations were observed, demonstrating that genomic damage was also induced in erythroid progenitors. Despite the relatively severe burden of somatic cell damage induced by 40 years of internal alpha particle irradiation, the patient remained free of any serious illness. Furthermore, these results provided no in vivo evidence for the continued expression of genomic instability. A similar observation was reported by Hande et al. [H3], using a fluorescence in situ hybridization technique that made possible the detection of intrachromosomal rearrangements and deletions. They described the quantification of stable intrachromosomal aberrations in lymphocytes of healthy former nuclear weapons workers who were exposed to plutonium. Even many years after occupational exposure, more than half the blood cells of the healthy plutonium workers contained large (>6 Mb) intrachromosomal rearrangements. The yield of these aberrations was highly correlated with plutonium dose to the bone marrow. It is significant that, despite the relatively high frequency of intrachromosomal aberrations, there was no evidence of transmissible chromosomal instability and no obvious detrimental health consequences in the populations sampled.

37. Nevertheless, a role for radiation-induced genomic instability has been described for solid tumours developing after radiotherapy for bilateral retinoblastoma [L1]. Genome alterations of second tumours (five osteosarcomas, one malignant peripheral sheath nerve tumour, one leiomyosarcoma) occurring in the field of irradiation of seven patients treated for bilateral retinoblastoma were studied. Because of a germ line mutation in the retinoblastoma gene (RB1), these patients were predisposed to develop radiation-induced tumours. In all radiation-induced tumours analysed, the normal RB1 allele was lost, whereas the germ line mutated allele was retained and the two *TP53* alleles were inactivated. A comparison of these tumours with the non-radiation-induced tumours led Lefevre et al. [L1] to conclude that this loss was due to the radiation-induced chromosomal instability rather than a direct effect of ionizing radiation. A similar observation was reported by Ryabchenko and colleagues [R12] after analysis of chromosomal aberrations in peripheral lymphocytes taken from Hodgkin’s disease patients after prolonged (up to 31 years) remission periods. The mean frequency and patterns of aberrations in remission patients were significantly different from comparison groups (healthy donors and primary Hodgkin’s disease patients). New cancer cases were diagnosed in a number of the remission patients, leading the investigators to suggest that the tumorigenic potential of radiochemotherapy is mediated via induction of genomic instability in exposed cells. Long after the therapy, the instability may become an initiating event in the development of new malignancies in affected tissues, whereas the instability induced in haemopoietic stem cells may reveal itself in peripheral lymphocytes derived from previously exposed precursor cells. The caveat, of course, is that these individuals were cancer patients and may have been inherently predisposed to second cancers.

E. Genomic instability and radiation-induced leukaemia

1. Mouse models

38. Plumb et al. [P8] have reviewed the relationship between radiation-induced genomic instability and radiation-induced leukaemia. They presented evidence that genomic instability plays a role during radiation leukaemogenesis. However, with the exception of a high incidence of non-clonal chromatid-type cytogenetic aberrations in neutron-induced acute myeloid leukaemia in mice [B26], the genetic lesions described (including non-clonal chromosomal aberrations, LOH and minisatellite/microsatellite mutations) were similar to those detected in de novo leukaemias and cancers. This damage was not transmissible and the authors interpreted these observations as evidence of apoptosis or other cell death. Nevertheless, this radiation-induced damage in vivo was indistinguishable from de novo multistage leukaemogenesis. Thus it is not yet possible to define a type of genomic instability in radiation-induced leukaemias in mice that demonstrates a specific characteristic of immediate or delayed effects of the initiating exposure to ionizing radiation. Evidence for radiation-induced genomic instability in mouse leukaemia and haemopoietic stem cells led MacDonald et al. [M1] to conclude that the induced instability contributed significantly to the induced leukaemia. A similar conclusion was reached by Ban et al. [B1], who suggested that loss of *TP53* function triggers the tumorigenic process leading to stem cell leukaemia through the induction of chromosomal instability. These authors also pointed out that the aetiology of stem cell leukaemia is likely to differ from that of myeloid leukaemia, because different results were observed in acute myeloid leukaemia (AML) [B1].

39. Interestingly, however, susceptibility to radiation-induced leukaemia is genetically separable from sensitivity to radiation-induced genomic instability [B13]. A series of matings, backcrosses and intercrosses between CBA/H mice susceptible to radiation-induced acute myeloid leukaemia and radiation-resistant C57BL/6 mice was carried out, and acute myeloid leukaemia and thymic lymphoma susceptibility was analysed. No simple genetic relationship between susceptibility to radiation-induced leukaemia and the sensitivity of the haemopoietic stem cells to induced instability was found.

2. Human studies

40. Cytogenetic analysis of leukaemia patients among the survivors of the atomic bombings in Japan revealed that patients exposed to >2 Gy exhibited a higher incidence of chromosomal aberrations and more complex chromosomal rearrangements than did patients exposed to lower radiation doses or unexposed patients [T1]. A more recent cytogenetic analysis of the heavily exposed patients with acute myelocytic leukaemia or myelodysplastic syndrome in the cohort of bombing survivors indicated persistent chromosomal instability [N6]. These cytogenetic observations are supported by studies demonstrating high frequencies of microsatellite instability in those bombing survivors with acute myelocytic leukaemia and a history of high exposure [N7]. These investigators concluded that this persistent instability might strongly influence the development of leukaemia in humans exposed to ionizing radiation. This study stimulated two letters to the editor of the journal in which it was published. The first, by Little [L40], claimed that although there was evidence that the microsatellite instability rate was higher in the AML cases among the bombing survivors than in the control group, the evidence that this higher rate was related to the radiation dose these cases received was weak. Little went on to show that the number of loci for which microsatellite mutation data were not detectable was higher in the bombing survivor cases than in the control group. The second letter, by Cox and Edwards [C15], raised the issue of the statistical strength of the dose-related association between expression of genomic instability in AML and the probability of causation by radiation. In response to these letters, Plumb [P18] pointed out that, from a biological perspective, the striking similarities in the leukaemias that arose in the bombing survivors and in therapy patients indicated that a significant proportion of the AML cases among the bombing survivors described by Nakanishi et al. [N6, N7] could indeed have been induced by the radiation. Reconciling mathematical models [L40] and statistical analysis [C15] with the biological observations will always be difficult, however, particularly with the small number of AMLs observed in this unique population.

41. Mazurik and colleagues [M63] investigated molecular, biochemical and cytogenetic parameters in blood samples from 17 radiation accident victims who between 1.7 and 43.8 years previously had suffered acute radiation sickness ranging in severity from grade I to grade IV. All patients showed ~25–30% reduction in oxidative status and increased levels of both stable and unstable chromosomal aberrations that correlated with the severity of the acute radiation sickness. These data were interpreted as evidence for delayed genomic instability in these radiation exposed individuals.

F. Role of telomeres and telomerase in radiation-induced genomic instability

42. Telomeres are specialized DNA–protein complexes at the ends of linear chromosomes. They are composed of a repetitive DNA sequence and associated proteins. Telomere alterations, caused by replication-mediated shortening, direct damage or defective telomere-associated proteins, can generate chromosomal instability, which can be observed in senescence and during the immortalization process (reviewed in reference [M47]). Telomeres are essential for proper maintenance of chromosomes and may play a role in ageing and cancer [G13, G14]. Telomere length can be maintained by telomerase [B25], or by the alternative mechanism of telomere lengthening which is telomerase-independent [M7, S50]. Telomere length abnormalities observed in

radiosensitive cells suggest that, in some human cells, short telomeres might correlate with radiation sensitivity [M49]. This may or may not be the case in mouse cells, as both hypersensitivity [G19] and the absence of an effect have been described [M49]. To complicate the matter further, long but dysfunctional telomeres have been found to correlate with chromosomal radiosensitivity in a mouse AML cell line 7926 [F13].

43. Another role for telomerase appears to be the de novo formation of telomeres, or chromosome healing to stabilize broken chromosomes [M41, S35]. Telomere loss results in sister chromatid fusion and prolonged breakage–fusion–bridge cycles leading to extensive DNA amplification and large deletions (reviewed in reference [M37]). Significantly, the loss of a single telomere can result in the instability of multiple chromosomes [S46] and generate many of the types of cytogenetic rearrangements commonly associated with human cancer. Telomere dysfunction can also trigger extensive DNA fragmentation and the evolution of complex chromosomal abnormalities in tumours in mice [A10] and humans [G11, M37]. This also appears to involve repeated cycles of dicentric chromosome formation, anaphase bridging, subsequent breakage and refusion events [G12]. In addition, telomere dysfunction can result in increased mutation rates and genomic instability (reviewed in references [F9, M37]).

44. It is noteworthy that exposure to ionizing radiation can induce telomerase activity both in vitro [H23, N27] and in vivo [H22]. This appears to be *TP53*-dependent [N26] and has been suggested as a measure for monitoring the radiocurability of tumour cells [S49]. Radiation-induced telomerase activation depends on dose rate, is not related to cell cycle redistribution or to the induction of cell death, and is likely to be the consequence of specific regulatory responses to ionizing radiation [P20].

45. Telomere repeat-like sequences are also seen as discrete bands at distinct intrachromosomal sites in a number of vertebrate species [M42]. These interstitial telomere sites probably represent ancestral telomere fusion events or amplification of the repeat sequences in ancestral karyotypes as latent telomeres [L39, M42]. There is compelling evidence that some of these interstitial telomere sites may be hot spots for both spontaneous [A11, H15] and radiation-induced chromosome damage [A9, B27, S36, S57, S58]. While it is clear that interstitial telomere sequences can influence the radiation sensitivity of chromosomes, many of these studies were performed in hamster cells where the interstitial telomeres are located at or near pericentromeric heterochromatin. Analyses of radiation sensitivity in a naturally occurring short interstitial telomere in a human chromosome (2q31, [A16]) and of a transfected 800bp telomeric repeat in human chromosome 4q indicate that human interstitial telomere sequences might not be prone to spontaneous [D24] or radiation-induced [D25] breakage.

46. The involvement of telomeric repeats in radiation-induced chromosomal instability was first described by Sabatier et al. [S44] in high-LET-irradiated human fibroblasts. They demonstrated that instability acquired by human chromosomes recurrently involved telomeric associations. This was not due to drastic telomere shortening [S45]. A role for recombination involving interstitial telomere-like repeat sequences in inducing chromosomal instability was later demonstrated by Day et al. [D1] in an in vitro Chinese human–hamster hybrid model system. In this instance it was apparent that rearrangements involving unstable chromosomes occurred preferentially at the sites of interstitial telomere sequences rather than at the true terminal telomeres as observed by Sabatier [S45]. This difference is likely to be due to the telomeres on transformed hamster chromosomes being very small relative to human telomeres [S56]. Ojima et al. [O6] have reported that telomeres are destabilized several generations after X-irradiation in normal human fibroblasts. Their data suggest X-irradiation might not affect telomeres directly, but rather by inducing a delayed instability.

47. Initially it was thought that an interstitial telomere-like repeat sequence on chromosome 2 played an important role in the deletions in somatic haemopoietic cells that characterized the earliest phases of radiation-induced AML in the CBA/H mouse [B28]. Subsequent detailed molecular analysis of break points suggested that, while telomere sequences were located close to regions frequently involved, break points were not exactly coincident with telomere sequences [F10]. The regions of frequent breakage appeared to have properties expected of matrix/scaffold attachment regions [F10]. At this stage one can only speculate on whether telomeric changes are a cause, an effect or a combination of both in contributing to genomic instability independent of radiation exposure.

G. Conclusions

48. Radiation-induced genomic instability is now well established in a number of normal and transformed cell lines in vitro. Instability can manifest as multiple different end points and can result from both targeted and non-targeted events. The results of studies of induced instability in vivo are more complex and contradictory. In mice, observation of the instability phenotype appears to be dependent upon the mouse strain used and the power of the investigation in terms of the numbers of animals investigated. The evidence for induced instability in exposed human populations is controversial, with both positive and negative effects being reported. It is possible that radiation-induced genomic instability provides the impetus for those genomic alterations associated with radiation carcinogenesis. This view should be tempered by the high frequency with which instability is observed both in vitro and in vivo, and the general lack of a dose–response curve. Overall, a specific role for induced instability in the genesis of radiation-induced cancers has yet to be definitively demonstrated.

II. BYSTANDER EFFECTS AND RADIATION EXPOSURE

49. There has been a resurgence of interest in radiation-induced bystander effects, largely because of the development of single-cell charged-particle irradiators. The term "bystander effect" was adopted from the gene therapy literature, where it usually refers to the killing of several subpopulations of tumour cells by targeting only one "type" of cell within a heterogeneous population [F6]. For the purposes of this annex, the definition proposed by Djordjevic will be used [D5]. That is, "bystander effect" describes the ability of cells affected by an agent to convey manifestations of damage to other cells not directly targeted by the agent or not necessarily susceptible to it per se. Thus radiation-induced bystander effects are effects manifesting in cells that were non-irradiated neighbours of irradiated cells or that received factors secreted or shed by irradiated cells. It is implicit in this review that in vitro bystander effects are the result of a signal generated by an irradiated cell interacting with a non-irradiated cell [C22] and are not the result of radiation-induced changes in the culture medium [Z2], or due to experimental variables such as the cell culture environment [M50]. This underscores the critical role of adequate and appropriate controls. A historical perspective describing key events in the study of radiation-induced bystander effects has been presented by Mothersill and Seymour [M45].

A. Bystander effects in vitro

50. Radiation-induced bystander effects in vitro embrace a number of different non-targeted experimental effects, some of which are likely to be detrimental to the cell, whereas others are not. Different effects are observed in different cell types, and depend on the cell type producing the bystander signal after irradiation and the cell type receiving the bystander signal (summarized in table 3). Consequently, no rigid rules can be applied to the multitude of responses occurring in cells not targeted by radiation. For convenience, bystander effects have been divided into four, not necessarily mutually exclusive, subcategories.

Table 3 In vitro studies of the bystander effect

Year	*Origin of bystander effect*	*End point*	*Cell type*	*Radiation type*	*Reference*
1992	Low-fluence alpha particle irradiation	Sister chromatid exchanges	Human fibroblasts and epithelial cells	Alpha particles	[N4]
1996	Low-fluence alpha particle irradiation	Sister chromatid exchanges	Human lung fibroblasts	Alpha particles	[D2]
1997	Medium transfer	Sister chromatid exchanges	Human lung fibroblasts	Alpha particles	[L2]
1997	Medium transfer	Clonogenic survival	Human epithelial cells; human fibroblasts	Gamma rays	[M18]
1997	Medium transfer	Clonogenic survival	Human keratinocytes	Gamma rays	[S12]
1997	Microbeam irradiation	Si- mutants induced	A_L human–hamster hybrid cells	Alpha particles	[H14]
1998	Low-fluence alpha particle irradiation	p53, p21, MDM2, CDC2, RAD5 1 protein levels	Human fibroblasts	Alpha particles	[A3]
1998	Low-fluence alpha particle irradiation	Plating efficiency; chromosomal aberrations	Murine bone marrow cells	Alpha particles	[L24]
1998	Medium transfer	Clonogenic survival	Human keratinocytes	Gamma rays	[M22]
1998	Microbeam irradiation	Micronucleus frequency; apoptosis	Human fibroblasts	Alpha particles	[P10]
1999	Cytoplasmic irradiation by microbeam	Clonogenic survival; CD59 mutation frequency	A_L human–hamster hybrid cells	Alpha particles	[W13]
1999	Low-fluence alpha particle irradiation	HPRT mutations	Chinese hamster ovary cells	Alpha particles	[N5]

Year	*Origin of bystander effect*	*End point*	*Cell type*	*Radiation type*	*Reference*
2000	Cytoplasmic irradiation by microbeam	Clonogenic survival; CD59 mutation frequency	A_L human–hamster hybrid cells	Alpha particles	[Z1]
2000	Low-fluence alpha particle irradiation	GI checkpoint	Human fibroblasts	Alpha particles	[A8]
2000	Medium transfer	Clonogenic survival; AP-endonuclease; *TP53*; ROS	Human lung fibroblasts (HFL-I)	Alpha particles	[I10]
2000	Medium transfer	Clonogenic survival; intracellular calcium levels; mitochondrial membrane potential; ROS levels	Human keratinocytes	Gamma rays	[L28]
2000	Medium transfer	Clonogenic survival	Human keratinocytes	Gamma rays	[S13]
2001	Co-culture	Clonogenic survival	V79 Chinese hamster cells (3-D tissue culture model)	^{3}H beta particles	[B24]
2001	Co-culture	p53, HSP72 protein levels	A-172 human glioblastoma cells	X-rays	[M3]
2001	Co-culture	Enhanced plating efficiency; micronucleus frequency	Human salivary gland cells	X-rays or carbon beam	[S30]
2001	Low-fluence alpha particle irradiation	Changes in gene expression; induction of DNA damage	Human fibroblasts and epithelial cells	Alpha particles	[A4]
2001	Medium transfer	Clonogenic survival; apoptosis; transformation frequency	CGLI human HeLa × skin fibroblast hybrid cells	X-rays	[L5]
2001	Medium transfer	Clonogenic survival; intracellular calcium levels; mitochondrial membrane potential; ROS levels	Human keratinocytes	Gamma rays	[L38]
2001	Medium transfer	Clonogenic survival	Human urothelium cells	Gamma rays	[M39]
2001	Microbeam irradiation	Micronucleus frequency	Primary human fibroblasts	Alpha particles	[B9]
2001	Microbeam irradiation	Transformation frequency	C3H 10T½ murine fibroblasts	Alpha particles	[S6]
2001	Microbeam irradiation	Clonogenic survival	C3H I0T½ murine fibroblasts	Alpha particles	[A7]
2002	Co-culture	Enhanced plating efficiency and proliferation	Human salivary gland cells	Carbon beam	[S15]
2002	Co-culture on double Mylar dishes	Clonogenic survival; CD59 mutation frequency	A_L human–hamster hybrid cells	Alpha particles	[Z4]
2002	Low-fluence alpha particle irradiation	Micronucleus frequency	Human fibroblasts	Alpha particles	[L43]
2002	Low-fluence alpha particle irradiation	HPRT mutations; sister chromatid exchanges	Human fibroblasts and epithelial cells	Alpha particles	[L37]
2002	Low-fluence alpha particle irradiation	Chromosomal aberrations	Chinese hamster ovary cells	Alpha particles	[N3]
2002	Low-fluence alpha particle irradiation	HPRT mutations; sister chromatid exchanges	Chinese hamster ovary cells	Alpha particles	[N21]
2002	Medium transfer	Clonogenic survival; AP-endonuclease; *TP53*; ROS	Human lung fibroblasts (HFL-I)	Alpha particles	[I1, I5]
2002	Medium transfer	Clonogenic survival; intracellular calcium levels; mitochondrial membrane potential; ROS levels	Human keratinocytes	Gamma rays	[L26, L27]
2002	Medium transfer	Clonogenic survival	Thirteen cell lines: human epithelial carcinoma cells, SW48 human colon carcinoma cells	Gamma rays	[M21, M38]
2002	Microbeam irradiation	Micronucleus frequency; apoptosis	Sections of human and porcine ureter	Alpha particles	[B7]

Year	*Origin of bystander effect*	*End point*	*Cell type*	*Radiation type*	*Reference*
2002	Microbeam irradiation	Clonogenic survival	V79 Chinese hamster cells	Alpha particles	[S27]
2003	Co-culture	Micronucleus frequency	Human fibroblasts	Carbon-ion beam: beta particles	[S28]
2003	Co-culture	Enhanced plating efficiency and proliferation	Human salivary gland cells	Carbon beams	[S29]
2003	Low-fluence alpha particle irradiation	HPRT mutations	Chinese hamster ovary cells	Alpha particles	[N20]
2003	Medium transfer	Clonogenic survival	Human keratinocytes	Gamma rays	[M27]
2003	Microbeam irradiation	Micronucleus frequency; apoptosis	Sections of human and porcine ureter	Alpha particles	[B8]
2003	Medium transfer	Apoptosis; micronuclei	Human–hamster hybrid cells (GM10115; Fe10-3; LS12)	X-rays	[N1, N2]
2003	Carbon ion beam	Micronuclei; gap junctions; ROS	Human fibroblasts	Carbon ion beam	[S28]
2003	Helium ion microbeam	Micronuclei; nitric oxide	T98G cell nuclei from human glioblastoma	Helium ion beam	[S16]
2003	Microbeam irradiation	A_L cell mutagenic assay	A_L cells	Charged particle microbeam; alpha particles; X-rays	[Z6]
2004	Medium transfer	RPA expression	Primary human fibroblasts	Gamma irradiation	[B42]
2004	Medium transfer	Micronucleus frequency	Chinese hamster ovary cells	Ultrasoft X-ray microprobe	[K31]
2004	Medium transfer	Transformation frequency	HeLa × skin fibroblast hybrid cells	Low-dose X-rays	[K32]
2004	Co-culture	Cell proliferation using ^{3}H-TdR incorporation	Antigen presenting cells (J774A.1) and T-lymphocytes (EL-4)	Low-dose irradiation	[L44]
2004	Chronic and acute irradiation	iNOS accumulation	WTP53 cells	Chronic gamma rays; acute X-rays	[M59]
2004	Co-culture and medium transfer	Oncogenic transformation frequency	C3H 10T½ cells	High- and low-dose X-rays	[M51]
2004	Cell–cell contact during irradiation	Oncogenic transformation frequency	C3H 10T½ cells	X-rays	[M43]
2004	Co-culture and medium transfer	Cloning efficiency; cell numbers	Repair-deficient human cell lines	Gamma rays	[M44]
2004	Co-culture	Chromosomal instability	Human fibroblast BJ1-htert	Alpha irradiation	[P22]
2004	Co-culture	Apoptosis; necrosis	LY (L5178Y) suspension cells; human salivary gland cells	Carbon ions; X-rays	[S31]
2004	Co-culture	Micronuclei; nitric oxide	Glioma cells; primary human fibroblasts	He ion particles	[S32]
2004	Co-culture	A_L cell mutagenic assay	A_L cells	Low-dose X-rays	[Z10]
2004	Microbeam irradiation	Micronuclei and cell cycle delays	Human fibroblasts	90 keV/μm alpha particles	[P6]
2005	Co-culture	Transposition of chromosomal loci	Human lymphocytes	X-rays (10 cGy)	[T4]
2005	Medium transfer	Micronucleus frequency; apoptosis		X-rays	[K33]
2005	Medium transfer	Mutation and deletion in mitochondrial DNA	Human keratinocytes (HPV-G)	Gamma irradiation	[M60]
2005	Medium transfer	Micronucleus frequency; HPRT mutation frequency	GM10115 human–hamster hybrid cells	X-rays	[N30]
2005	Co-culture	Sister chromatid exchanges and chromosomal aberrations	Hamster cell lines (V3 and irs3)	Low-fluence alpha particles	[N25]

Year	*Origin of bystander effect*	*End point*	*Cell type*	*Radiation type*	*Reference*
2005	Co-culture	Micronuclei; nitric oxide; ROS	Glioma cells (T98G) and fibroblasts (AG01522)	Helium particles	[S23]
2005	Medium transfer	Micronuclei; induction of $p21^{waf1}$ protein; gamma H2AX foci; ROS; clonogenic survival	Human fibroblasts	X-rays	[Y5]
2005	Bystander assay	COX-2 signalling involving mitogen-activated protein kinases		Charged particle beam	[Z5]
2005	Co-culture	Micronuclei; apoptosis	Mouse embryonic stem cells	Alpha particles	[Z7]
2006	Apoptosis from bystander effect	Iodine incorporation for apoptosis; gap junctions; connexin-43 expression	Non-small-cell lung cancer	Ionizing radiation	[Z9]

1. Bystander effects after cytoplasmic irradiation

51. The most convincing demonstration of the bystander effect has come from studies using charged-particle microbeams [F3, F4, F5, R1]. The microbeam is capable of putting an exact number of particles through a specific subcellular compartment of a defined number of cells in a particular radiation environment.

52. Using the microbeam at the Radiological Research Accelerator Facility of Columbia University in the United States [R1], Wu et al. [W13] targeted and irradiated the cytoplasm of human–hamster (A_L) cells. They observed a significant increase in mutations at the CD59 (S1) nuclear gene locus while causing minimal cytotoxicity. Cytoplasmic irradiation with a single alpha particle doubled the spontaneous mutation frequency, while a two- to threefold increase was observed after four cytoplasmic traversals. The mutation spectrum was similar to the spontaneous, non-irradiated mutation spectrum, but different from that observed after targeted nuclear irradiation. The addition of the free radical scavenger dimethyl sulphoxide or the intracellular glutathione inhibitor buthionine-S-R-sulfoximine indicated that the mutagenicity of cytoplasmic irradiation depended on the generation of ROS (figure IV). Shao et al. [S32] also used a charged-particle microbeam, at the Gray Cancer Institute in the United Kingdom, to target individual glioma cells cultured alone or in co-culture with primary human fibroblasts. They found that even when only a single cell within the glioma cell population was precisely traversed through its cytoplasm with one helium ion, bystander responses were induced in the neighbouring non-irradiated glioma cells or fibroblasts. Significantly, the yield of bystander-induced micronuclei was similar when the cytoplasm or nucleus of a cell was targeted, indicating that direct DNA damage is not required for switching on cell-signalling mechanisms after low-dose irradiation. Two important conclusions can be reached from these experiments. First, because bystander effects are observed after cytoplasmic irradiation, the target for genetic effects of radiation is larger than the nucleus. Secondly, cytoplasmic traversal by alpha particles may be more deleterious in the long term than nuclear traversal. This is because, as the number of nuclear traversals increases, the probability of cell killing increases, whereas after cytoplasmic irradiation the increased mutagenicity occurs where there is negligible killing of the irradiated cells [W13].

2. Bystander effects after low fluences of alpha particle irradiation

53. It is implicit in the evaluation of bystander effects that bystander cells were not hit by the radiation but received signals from an irradiated cell that generated a response in the bystander cell. Broad-beam irradiation with low fluences of alpha particles does not traverse every cell in the radiation environment, and data suggest that a sizeable portion of the damage observed after exposure to low fluences of alpha particles results from responses occurring in cells that were not actually traversed by an alpha particle.

54. Nagasawa and Little [N4] observed small increases in sister chromatid exchange frequency in ~30% of cells analysed, even though <1% of the cell nuclei were actually traversed by an alpha particle. This observation was later confirmed and extended by Lehnert and co-workers [D2, L2]. The induced sister chromatid exchanges could be inhibited by superoxide dismutase, once again indicating a role for ROS [L2, N8, N9]. The alpha-particle-induced increase in ROS appears to be temporally linked to enhanced production of tumour necrosis factor alpha and interleukin 1, which in turn operate in an autocrine manner to up-regulate interleukin 8 [N9]. Low fluences of alpha particles can also increase mutation yield [N5] and cause accumulation of the tumour suppressor protein *TP53* in a higher percentage of the exposed population than calculated to receive a nuclear traversal by one or more alpha particles [H5]. Whether or not mutation induction or induced gene expression is mediated by ROS is uncertain, but whatever the mechanism, it involves gap-junction-mediated intercellular communication in the transmission of damage signals from irradiated to non-irradiated cells (figure V[A]). By examining changes in gene expression after low-fluence alpha particle irradiation, Azzam et al. [A3, A4] demonstrated the involvement of connexin-43-mediated intercellular communication in the transmission

of damage signals to non-irradiated cells. In gap-junction-competent cells, induction of $p21^{Waf1}$ protein far exceeded the fraction of cells whose nuclei had been traversed, and correlated with the induction of micronuclei as a measure of DNA damage as well as with increased Ser-15 phosphorylation of *TP53* (reviewed in reference [A5]).

Figure IV. Induced S1 mutations in human–hamster A_L cells per 10^5 survivors.

AL cells were irradiated with four alpha particles through the nucleus (1) or the cytoplasm (2). Dimethyl sulphoxide (8%) from 10 minutes before until 10 minutes after cytoplasmic irradiation with four alpha particles significantly suppressed mutation yield (3). Treatment with d-dimethyl sulphoxide alone did not increase mutation frequency (4). In contrast, pretreatment of A_L cells with a 10 μM dose of buthionine-S-R-sulfoximine for 18 hours, which reduced the intracellular glutathione content to <5% of control levels, increased the mutagenicity of cytoplasmic irradiation with four alpha particles by four- to fivefold (5). Treatment with buthionine-S-R-sulfoximine alone had no significant effect on mutation frequency (6). These data strongly implicate ROS as being the mediator of the mutagenic response of cytoplasmic irradiation (adapted from reference [W13]). The insert represents a schematic of the irradiation protocol.

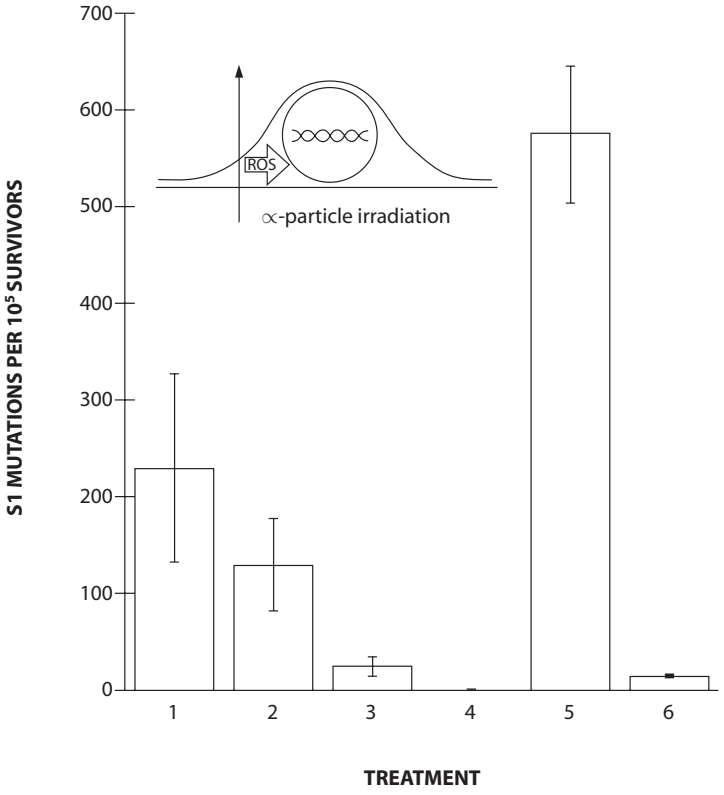
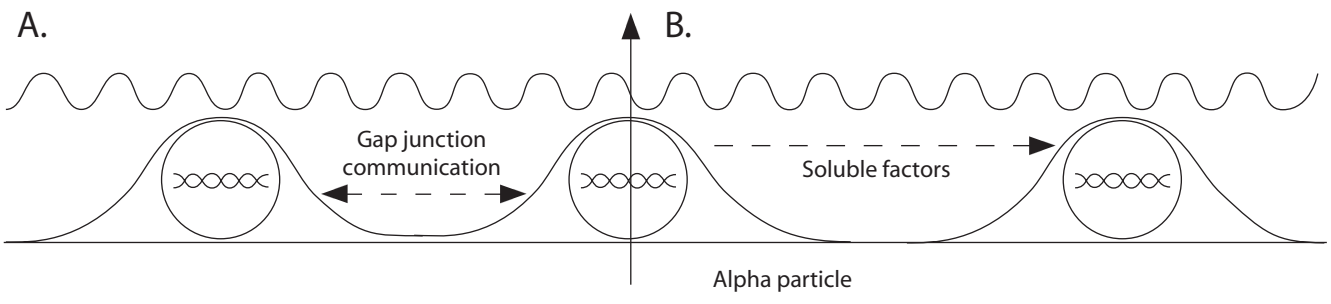


Figure V. Bystander effects are those effects occurring in cells that were not directly subjected to the deposition of energy by radiation but were in contact with irradiated cells or received a signal from an irradiated cell.

A cell is irradiated through the nucleus with an alpha particle (vertical arrow). This irradiated cell then communicates a signal to a non-irradiated bystander cell by intercellular cell-to-cell gap junction communication (A) or the transmission of soluble factors from the irradiated cell to the non-irradiated cell via the cell medium (B).

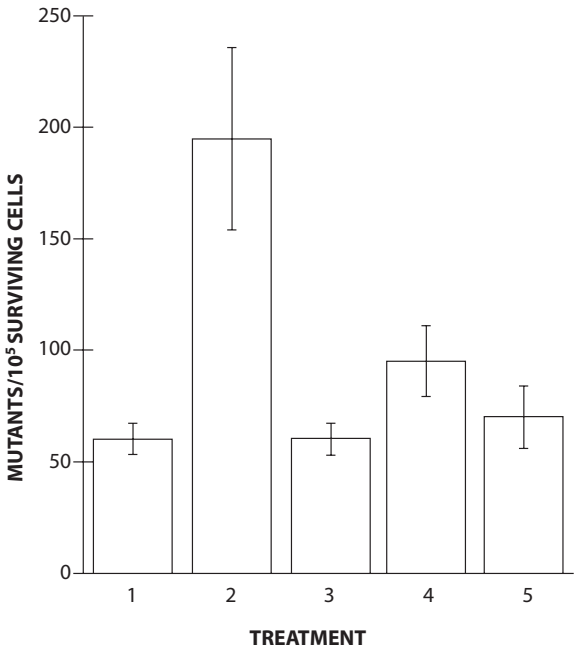


55. A bystander effect for chromosomal aberrations induced by low fluences of alpha particles in wild-type and repair-deficient (xrs-5) Chinese hamster ovary cells has been described [N3]. Gross chromosomal aberrations are generally associated with DNA double-strand breaks [M15], suggesting that the factors responsible for the bystander effect are also ultimately capable of cleaving the double helix. As an explanation of their data, Nagasawa and Little [N3] proposed that the relatively small bystander effect in wild-type cells was due to proficient double-strand break repair as compared with the significantly enhanced bystander effect, manifesting as dramatic increases in aberration yields in repair-deficient xrs-5 cells. This study was expanded to examine potential bystander effects for sister chromatid exchanges and chromosomal aberrations in hamster cell lines deficient in either DNA-PKcs (V3 cells, deficient in non-homologous DNA end joining) or RAD51C (irs3 cells, deficient in homologous recombination). Cells were irradiated with very low fluences of alpha particles such that <1% of the nuclei were traversed by an alpha particle. Wild-type cells showed a prominent bystander response for sister chromatid exchange induction; an even greater effect was observed in V3 cells. On the other hand, no significant induction of sister chromatid exchanges was observed in the irs3 RAD51C-deficient bystander cells. In contrast, a marked bystander effect for chromosomal aberrations occurred in V3 cells, and the induction of chromosomal aberrations in irs3 bystander cells was minimal and similar to that of wild-type cells. On the basis of these findings, Nagasawa et al. [N25] hypothesized that homologous recombination is essential for the induction of sister chromatid exchange in bystander cells; but homologous recombination is unable to repair the damage induced in non-homologous end-joining-deficient bystander cells that leads to either sister chromatid exchange or chromosomal aberrations.

3. Bystander effects after irradiation with a charged-particle microbeam

56. Using human–hamster hybrid AL cells, Zhou et al. [Z1] located all cells in the radiation environment and exposed 20% of these to 20 alpha particles using the Columbia University microbeam. This dose of radiation allowed less than 1% of the irradiated cells to survive. They then assayed surviving cells for mutations in the target human chromosome and found a mutation frequency four times that of background (figure VI). Since the irradiated cells were exposed to lethal doses of radiation, these mutations must have arisen in non-exposed bystander cells. Furthermore, the mutation spectrum observed in bystander cells was significantly different from the spontaneous spectrum and from that observed after cytoplasmic irradiation, suggesting that different mutagenic mechanisms were involved in the two processes [Z1].

Figure VI. CD59 mutants per 10^5 surviving A_L cells.
(1) Non-irradiated cells. (2) Observed mutation yield when 20% of A_L cells were exposed to a lethal dose of 20 alpha particles per cell. (All cells irradiated with 20 alpha particles should have been killed.) (3) Expected mutation yield when 20% of A_L cells were exposed to a lethal dose of 20 alpha particles per cell. (4) Effect of two hours of pretreatment and 72 hours of post-treatment with 40 μM Lindane when 20% of A_L cells were exposed to a lethal dose of 20 alpha particles per cell. (5) Effect of 40 μM Lindane alone on A_L cells (adapted from reference [Z1]).



57. Unlike cytoplasmic irradiation [W13] or the bystander effect reported by Lehnert et al. after low fluences of alpha particle irradiation [L2, N8, N9], the bystander effect described by Zhou et al. [Z1] was not modulated by addition of the free radical scavenger dimethyl sulphoxide. Instead, when A_L cells were treated with the cell-to-cell gap junction communication inhibitor Lindane, the bystander effect was significantly reduced but not eliminated (figure VI).

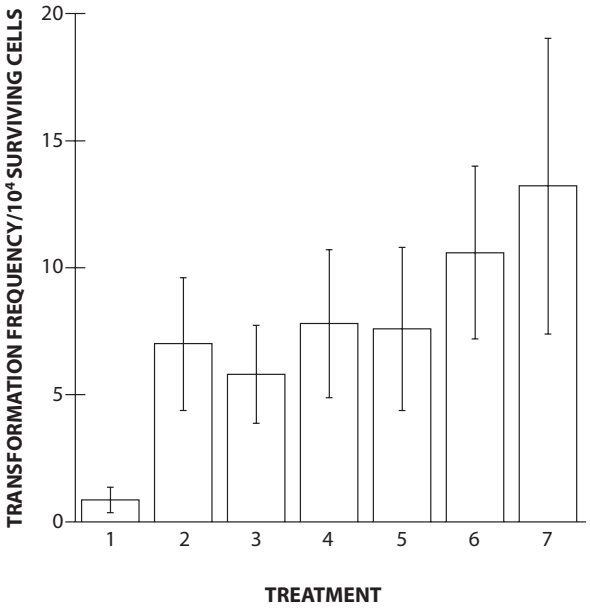
58. In a subsequent study, Zhou et al. [Z3] used the Columbia University microbeam to deliver exactly one alpha particle through the nuclei of 5%, 10%, 20% or 100% of the A_L cells. Their results indicated that the frequencies of induced mutations and chromosomal changes in populations where known fractions of nuclei were hit were consistent with non-hit cells contributing significantly to the response. When 10% of a confluent mammalian cell population was irradiated with a single alpha particle, the mutation yield was similar to that observed when 100% of the cells were irradiated. This effect was significantly reduced in cells pretreated with octanol, which inhibits gap-junction-mediated intercellular communication, or in cells carrying a dominant negative connexin-43 vector. These results indicate that a single alpha particle can induce genomic damage in cells that were not irradiated. Since a cell cannot receive a

lower dose of radiation after exposure to alpha particles than a single traversal, these data suggest that at the very lowest radiation dose of alpha particles, i.e. a single particle, the genotoxic risk for high-LET radiation may be underestimated. It should be noted that traversal of a single alpha particle results in a dose of 0.074–0.17 Gy, depending upon the nuclear cross-sections of a given cell type and assuming an average LET of 90 keV/μm [R1].

59. The precision of intracellular irradiation and the high throughput available at the microbeam facility at Columbia University have also enabled studies of oncogenic transformation to be carried out. Sawant et al. [S6] utilized sparsely populated monolayers of mouse C3H 10T½ cells and irradiated the nucleus of every cell, or every tenth cell at random, with either two or four alpha particles. The yield of transformed foci was determined morphologically, and the frequency of transformation was similar whether 100% or only 10% of the cells were irradiated (figure VII).

Figure VII. Transformation frequency in C3H 10T½ cells in vitro.

(1) Non-irradiated controls. (2) 10% of the cells received two alpha particles. (3) 100% of the cells received two alpha particles. (4) 10% of the cells received four alpha particles. (5) 100% of the cells received four alpha particles. (6) 10% of the cells received eight alpha particles. (7) 100% of the cells received eight alpha particles (adapted from reference [S6]).

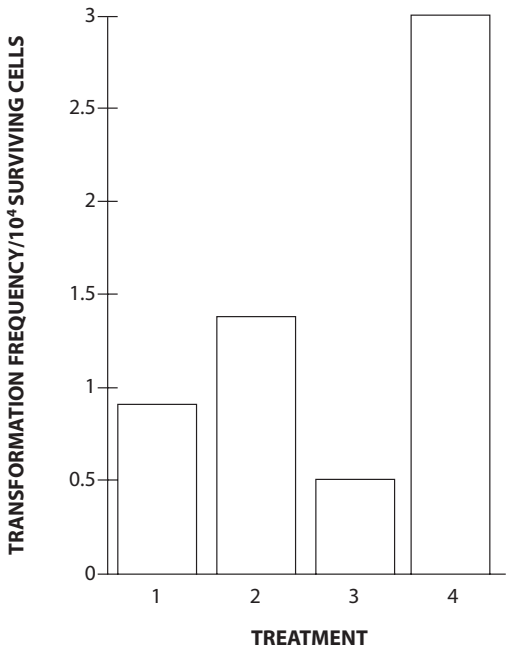


Furthermore, Lindane (the cell-to-cell communication gap junction inhibitor) reduced but did not eliminate this bystander effect. In subsequent studies from the Columbia University group, a role for secreted factors or factors released from irradiated cells into the culture medium has also been identified. However, it is clear from studies of bystander-induced cell killing and oncogenic transformation that cell-to-cell gap junction communication is the most important mediator of the bystander effect in C3H 10T½ cells after microbeam irradiation [M43].

60. Related studies using microbeam-generated alpha particles have important implications for evaluating potential hazards associated with radiation exposure [M6]. One alpha particle per nucleus, the lowest possible dose of high-LET radiation, effectively elicits a bystander response in different cell types [B9, Z3]. By comparing the biological effectiveness of exposure to exactly one alpha particle per cell with that expected from normal broad-beam irradiation similar to that used in the experiments described above (using a mean of one particle per cell), it is clear that exactly one particle per cell is less biologically effective than a mean of one particle per cell. Miller et al. [M6] found that the transformation frequency after irradiation with exactly one alpha particle per nucleus did not differ significantly from that observed in non-irradiated cells (figure VIII). This suggested that the increased transformation frequency observed after broad-beam alpha irradiation, where a mean of one alpha particle traversal per cell results in a Poisson distribution of particles per cell, is a consequence of the minority of cells subjected to multiple (≥2) alpha particle traversals.

Figure VIII. Transformation frequency in C3H 10T½ cells in vitro.

(1) Non-irradiated controls for the microbeam experiment. (2) Cells receiving exactly one alpha particle per nucleus from the Columbia University microbeam. (3) Non-irradiated controls for the broad-beam experiment. (4) Cells receiving a mean of one alpha particle per nucleus after broad-beam irradiation. Note that the transformation frequency in cells receiving exactly one alpha particle per nucleus is only slightly different from that in the non-irradiated controls (adapted from reference [M6]).



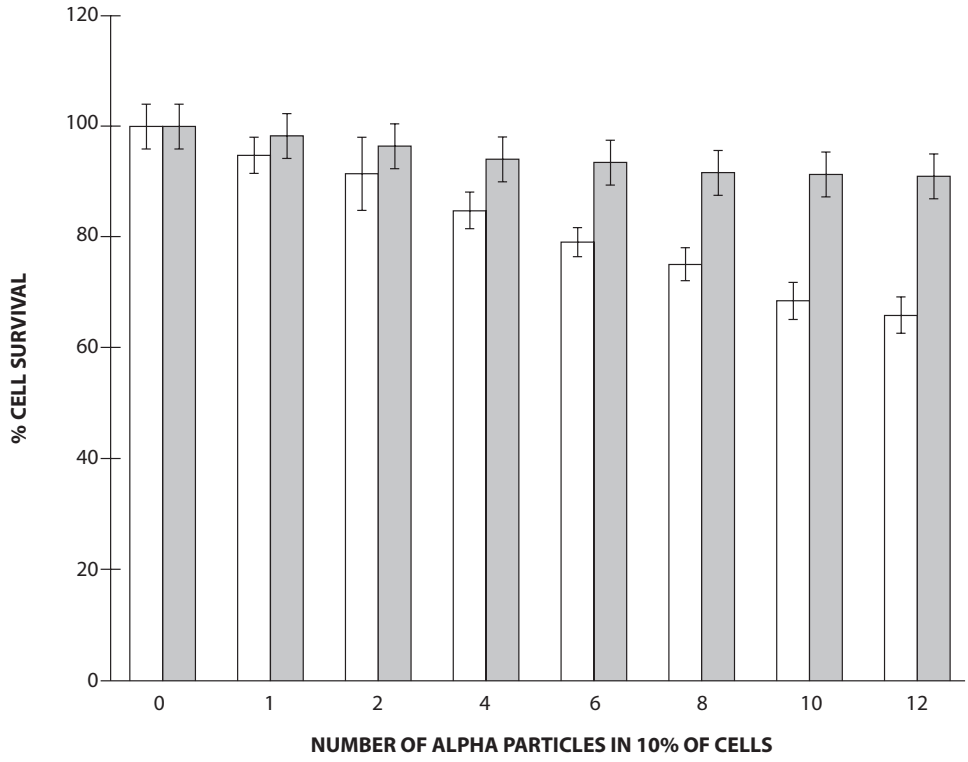
61. An important advantage of using low fluences of alpha particles or a charged-particle irradiator is that it represents environmentally relevant exposure conditions, where most cells in a tissue would not be traversed by an alpha particle. It should be stressed, however, that to irradiate cells in vitro with a high-energy alpha particle, target cells must be grown as monolayers on a Mylar substrate. Under these culture conditions, cells by necessity are more flattened and more elongated that would reasonably be expected in a three-dimensional in vivo situation. Consequently, the energy deposited per traversal is much less biologically effective. To address this issue, three-dimensional model tissue culture systems are being developed. Any evaluation of potential hazards associated with these types of high-LET radiation must consider these unique cell culture conditions.

62. There is also a microbeam at the Gray Cancer Institute, where ^{3}He ions with an LET of 100 keV/μm have been used for targeted cellular irradiation [F5]. In addition, the Gray Cancer Institute microbeam has recently been adapted so that the effects of ultrasoft X-rays can also be investigated [F3]. Using this microbeam, evidence for a bystander effect in primary human fibroblasts, as measured by micronucleus formation and cell killing, revealed significantly more damage than expected on the basis of direct effects of radiation [B7, B8, B9, P11, P12]. The bystander effects observed using the Gray Cancer Institute microbeam can occur when cells are considerable distances apart from one another and not in contact, thus eliminating potential cell-to-cell gap junction communication [B9, P10]. This indicates that radiation-induced soluble factors may be released into the culture medium and affect non-irradiated cells (figure V[B]), and suggests that there are at least two different types of bystander effect, those effects mediated by cell-to-cell gap junction communication and those induced by secreted soluble factors (figure V). Alternatively, there are at least two mechanisms for the same biological effect. Intriguing data from Sawant et al. [S7] indicated that, as the number of alpha particles through a fixed (10%) number of cells increases, more bystander-induced cell killing occurs (figure IX). These observations suggested that increasing induced damage in 10% of the cells in the culture produced more cell-to-cell gap junction communication and/or increased secretion of cytotoxic substances into the culture environment. A strategy combining medium transfer experiments and gap junction inhibitor studies would enable investigators to determine the relative contributions of these bystander-mediated cytotoxic effects and ultimately this would aid in identification of the secreted factors.

63. Interestingly, some novel insights into the mechanisms of radiation-induced bystander effects in vitro have been revealed using the single-cell microbeam at the Gray Cancer Institute. When human glioblastoma T98G cell nuclei were individually irradiated with an exact number of helium ions, it was found that, when only one cell in a population of approximately 1,200 cells was targeted, cellular damage

Figure IX. Bystander effect for cell survival.

Ten per cent of the cells, selected randomly, were exposed to increasing numbers of alpha particles from 2 to 12, and the percentage of cell survival was determined by reduction in plating efficiency. Open bars: experimentally determined survival. Filled bars: expected survival if only irradiated cells were killed (adapted from reference [S7]).



measured as induced micronuclei was increased by 20% [S16]. When the percentage of cells individually targeted was increased from 1% to 20%, the yield of micronuclei in the population greatly exceeded that predicted on the basis of the yield when all of the cells were targeted assuming no bystander effect. However, when 2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO), a nitric-oxide-specific scavenger, was present in the culture medium, the micronucleus yields decreased to the predicted values. This indicates that nitric oxide contributed to the bystander effect. Moreover, the medium harvested from cells targeted with helium ions showed a cytotoxic effect by inducing micronuclei in non-irradiated T98G cells, and this bystander response was also inhibited by c-PTIO treatment. The induction of micronuclei in the population could also be decreased by c-PTIO treatment when 100% of cells were individually targeted by one or two helium ions, indicating a complex interaction of direct irradiation and bystander signals. A role for nitric oxide in the medium-mediated bystander effect has now been described in a number of studies [S15, S16, S31, S32]. Furthermore, the secretion of nitric oxide as a bystander effect has been linked to the induction of radioresistance in recipient cells [M3, M4]. However, it is not clear whether nitric-oxide-mediated bystander effects occur in conjunction with, or independent of, increased levels of ROS, and how this might affect the characterization of subsequent radiation sensitivity.

64. Microbeam experiments using high-Z elements (460 MeV ^{40}Ar, 1,260 keV/µm and 260 MeV ^{20}Ne, 380 keV/µm) have also been performed at the Japan Atomic Energy Research Institute [S33]. Confluent normal human fibroblasts were targeted and the induced micronuclei evaluated after replating the cells. Even when only a single cell was hit, a 1.4-fold increase in the frequency of micronuclei was observed, indicative of a bystander effect. The observed increase in micronucleus frequency saturated when four cells were targeted and could be suppressed when dimethyl sulphoxide (a scavenger of ROS) or PMA (an inhibitor of gap junction communication) was present at the time of irradiation. Thus a role for nitric oxide, ROS and cell-to-cell gap junctions has been invoked in bystander responses.

65. It is likely, however, that there are multiple bystander pathways. By using the Columbia University charged particle beam in conjunction with a strip dish design, Zhou et al. [Z5] show that the cyclooxygenase-2 (COX-2, also known as prostaglandin endoperoxide synthase-2) signalling cascade plays an essential role in the bystander process. Treatment of bystander cells with NS-398, which suppresses COX-2 activity, significantly reduced the bystander effect. This provided evidence that the COX-2-related pathway, which is essential in mediating cellular inflammatory response, is a critical signalling link for the bystander phenomenon in this assay system. Furthermore, any signalling pathway likely to be involved is complicated by the genotype of the exposed organism. For example, Zhu et al. [Z7] examined the ability of mouse embryonic stem cells differing in the status of the DNA repair gene, Rad9, to express a bystander effect after exposure to alpha particles. All populations, when confluent, demonstrated a dose-independent bystander effect with respect to cell killing and apoptosis. Minimal alpha particle induction of micronuclei in bystander cells was observed, except for the Rad9$^{-/-}$ mutant, where a significant increase above background was detected. Therefore the Rad9 null mutation selectively sensitizes mouse embryonic stem cells to spontaneous and high-LET-radiation-induced bystander apoptosis and micronucleus formation, but it has much less impact on cell killing by direct or bystander alpha particle exposure.

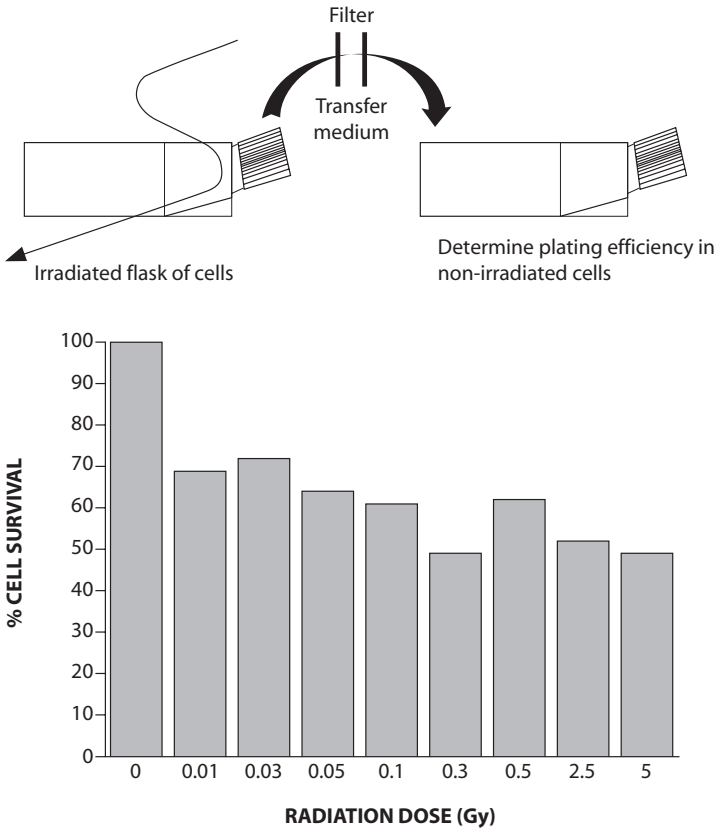
66. At present there is no detailed information on potential bystander effects occurring after cellular exposure to low-LET radiation in non-irradiated cells subsequently cultured in the same physical proximity. This is because the development of focused low-LET radiation sources has lagged well behind that of high-LET charged particle microbeams, owing to practical and technical complications. Gamma rays interact with matter in a number of ways, including photoelectric absorption, Compton scattering and pair production. Since the electrons will undergo scattering as they exit the vacuum system, it is not practical at present to irradiate single cells or subcellular structures, as is possible with the charged particle microbeams described above. However, by using pulsed electron beams, a low-LET microbeam can be constructed that will mimic many important aspects of the interaction of gamma rays with cells. Such devices are currently available at the University of Maryland [S37] and Texas A&M University [W16] in the United States. Consequently, it is anticipated that in the foreseeable future many of the studies that have been performed with high-LET alpha particles or intermediate-LET ultrasoft X-rays can be replicated using these new low-LET microbeams. In addition, the ability to experimentally vary the LET distribution by changing the incident beam energy will allow investigation of the relative biological importance of various parts of the energetic electrons' track and hence evaluate how this might modulate bystander responses [S38].

4. Bystander effects after transfer of medium from irradiated cells

67. Mothersill and Seymour [M18] demonstrated a significant reduction in plating efficiency in non-irradiated cultures of human epithelial cells (but not fibroblasts) that received culture medium from irradiated cultures. These observations indicated that irradiated cells secreted a toxic substance, a "bystander factor", into the culture medium that can kill non-irradiated cells (figure X). Medium irradiated in the absence of cells had no effect on survival when transferred to non-irradiated cells. Not all cells are capable of producing the toxic bystander factor, nor are all cells capable of receiving and responding to the secreted signal [M17, M18, M22, M25]. The effect was dependent on the cell number at the time of irradiation, could be observed as early as 30 minutes post-irradiation, and was still effective when medium transfer occurred 60 hours after irradiation.

Figure X. Bystander effects in an immortalized human keratinocyte cell line as demonstrated by medium transfer experiments.

Upper portion: schematic of the experimental protocol. A flask of cells is irradiated, and as a function of time post-irradiation, medium is removed from the irradiated cells, filtered and transferred to non-irradiated cells. Clonogenic survival is then investigated in these non-irradiated cells. Bystander effects are indicated by a reduction in plating efficiency in the non-irradiated cells that were cultured in medium from the irradiated cells. Lower portion: plating efficiency expressed as percentage of survival after transfer of medium from cells exposed to increasing doses of ionizing radiation (adapted from reference [S13]).



68. Whether this is the same bystander phenomenon as described above for bystander effects after low fluences of alpha particles or irradiation with a charged particle microbeam has yet to be determined. If it is not an identical phenomenon, it is likely to be similar to those bystander effects defined above that involve the secretion of soluble factors [M43, S64, Z2]. However, one caveat in these studies should be mentioned. When A_L cells were plated on either one or both sides of double Mylar dishes before irradiation, and one side (with or without cells) was irradiated with alpha particles, different effects on different cellular end points were observed for both survival and mutation [Z2]. When the side with cells was irradiated, the surviving fraction among the non-irradiated cells was significantly lower than that of the controls after 48 hours co-culture. However, such a change was not detected after 1 hour co-culture or when medium alone was irradiated. Furthermore, co-cultivation with irradiated cells had no significant effect on the spontaneous mutagenic yield of non-irradiated cells collected from the other half of the double Mylar dishes. These results suggested that the irradiated cells released certain cytotoxic factors into the culture medium that killed the non-irradiated cells. Importantly, such factors had little effect on mutation induction, indicating that different bystander end points may involve different mechanisms with different cell types. This is supported by the study by Wang and Coderre [W18], who used a co-culture system to examine bystander effects transmitted through the medium from the directly targeted cells to tumour cells growing on an insert well beyond the range of the alpha particles. Alpha particle doses of 0.1–6.0 Gy to the targeted cells on the Mylar membrane, followed by a 2 hour co-incubation of the cells on the insert in the irradiated medium above the irradiated cells, all caused an approximately 50% increase in micronucleus formation in the non-targeted co-cultured cells. Addition of the radical scavenger dimethyl sulphoxide to the medium during the irradiation and the 2 hour post-irradiation incubation period completely blocked the bystander effect, whereas addition of a nitric oxide scavenger had no effect.

69. Medium transfer experiments demonstrated that irradiation can lead to secretion of a factor or factors by irradiated cells that can reduce cloning efficiency, predominantly

by stimulating apoptosis [L26, L27, L28, S12], increasing neoplastic transformation [L5] or inducing genomic instability [S12] in non-irradiated cells. The first detectable effect on recipient cells after transfer of medium containing the bystander factor from irradiated cells was a rapid calcium pulse (1–2 minutes) followed 30–120 minutes later by changes in mitochondrial membrane permeability and the induction of ROS [L28, L38, M24]. Cell-to-cell contact during irradiation was not required to induce killing of bystander cells, but medium from cell cultures irradiated at high densities resulted in the greatest amount of bystander-induced cell death [M22]. Furthermore, the use of apoptosis inhibitors or medium from lactate dehydrogenase or glucose-6-phosphate dehydrogenase mutant cells reduced or prevented the bystander effect [M24]. Treatment with the antioxidants L-lactate and L-deprenyl prevented bystander-factor-associated cell killing [M24], suggesting that energy/redox metabolism may be involved in the medium-mediated bystander response.

70. Mothersill and co-workers [M44] showed that repair-deficient human cell lines produced a moderate to severe amount of bystander-induced cell death after medium transfer to autologous cells or to a reporter cell line. Normal "repair-proficient" lines have much less severe, or no, bystander-induced effects on cloning efficiency. These results are in agreement with the observations of Little and colleagues [L42, N3]. Mothersill et al. [M44] interpreted these data as supporting the hypothesis that bystander effects play a protective role in biological systems by terminating division in cells containing DNA damage. Thus the repair-deficient cells, irrespective of the actual repair defect, may respond to the occurrence of DNA damage in the population by removing large numbers of cells from the proliferating pool. It should be noted that repair-deficient cell lines tend to show an increased frequency of induced genomic instability, again suggesting a commonality in the mechanism of radiation-induced genomic instability and bystander effects.

71. The majority of medium transfer experiments reported have utilized low-LET radiation to induce the bystander effect and have come primarily from a single laboratory (reviewed in references [M19, M20]). A fascinating observation from these studies, but one that is difficult to reconcile with those of other studies, concerns the radiation doses required to elicit a bystander response. In human keratinocytes, low-LET ^{60}Co gamma ray doses of 0.01–0.5 Gy reduced clonogenic survival after transfer of irradiated medium. This was entirely due to bystander effects. The magnitude of cell killing was relatively constant and appeared to saturate at doses in the range 0.03–0.05 Gy [S13]. At doses of greater than 0.5 Gy, cell killing was the result of the direct effects of radiation as well as the dose-independent bystander effect [S13]. These observations are difficult to explain in terms of the classical cell survival curve, where there is very little if any cell killing observed at radiation doses of a few milligrays in directly irradiated cells.

72. In contrast to the cell-killing effects reported by Mothersill and Seymour after medium transfer from gamma-irradiated cultures, Iyer and Lehnert [I4] have observed quite different cellular responses in human fibroblast cells cultured in supernatants from alpha-irradiated fibroblasts. They observed decreases in basal levels of *TP53* and CDKN1AQ in non-irradiated cells, rather than increases as described by others (e.g. [A4, H5]). These decreases were accompanied by increases in proliferating cell nuclear antigen and CDC2, apparently mediated by TGF-beta 1 and the induction of intracellular ROS [N8]. In contrast to the detrimental effects on cell well-being characteristic of bystander effects so far described, Iyer and Lehnert [I4] showed that their decreased *TP53*/CDKN1A bystander effect correlated with enhanced cell proliferation.

73. Attempting to reconcile these conflicting results raises a number of questions. While the quality of radiation and the cell types under investigation are different, these studies highlight the family of responses characterizing the bystander effect. Mechanistically, factors transferred via cell-to-cell gap junction communication or secreted into the culture medium may interact with those non-irradiated cells in an antagonistic manner, ultimately killing the non-irradiated cells (reviewed in reference [S39]), but the reasons why cultured cells should secrete cytotoxic factors and why no such dramatic reduction in plating efficiency is observed when populations of cells are irradiated with low doses of low-LET radiation are not immediately obvious. Likewise, the pro-mitogenic response reported by Lehnert et al. [L2, L48] after medium transfer is contradictory to those direct effects observed in irradiated cells where irradiation can inhibit cell growth. Cell proliferation following irradiation appears contrary to the long-term well-being of the cell, tissue, organ or organism, whereas cell death would be likely to protect against the possibility of detrimental mutations, chromosomal rearrangements, and so on. Clearly bystander effects can modify cellular responses to radiation, and it remains to be determined whether these effects characterized in non-irradiated cells in vitro have a major role in the response of irradiated cells in vitro or in irradiated and non-irradiated cells in vivo.

B. Bystander effects in vivo

74. Bystander effects have been observed predominantly by using single-cell in vitro systems that do not have realistic multicellular morphology. Given that the bystander phenomenon must involve cell-to-cell interactions, the relevance of such single-cell in vitro studies is questionable. However, Belyakov et al. [B36] have described bystander responses in a three-dimensional, normal human tissue system. While not a true in vivo assay, this model skin system does provide some semblance of multicellular interactions. End points were induction of micronucleated and apoptotic cells. Non-irradiated cells up to 1 mm distant from irradiated cells showed a significant enhancement in the effect over background levels, with an average increase in effect of 1.7-fold for micronuclei and 2.8-fold for apoptosis. The surprisingly long range of bystander signals in a human

tissue model system suggests that bystander responses may be important in extrapolating potential radiation effects from epidemiologically relevant doses down to very low doses (<200 mGy), where non-hit bystander cells would likely predominate [M36].

75. Compared with the number of in vitro studies on bystander effects, there are relatively few studies on bystander effects in vivo, and these are summarized in table 4. Many of these studies appear to have been performed not to look specifically at non-targeted effects of radiation but for other purposes, so that critically evaluating bystander effects in vivo is premature at present. It should be mentioned at this stage that investigators are beginning to move from single-cell systems to multicellular systems using primary explant techniques [B8, B36] as well as three-dimensional model systems [P11]. Consequently, it is anticipated that as more information from these model systems becomes available and more focused studies in vivo are undertaken, it will be possible to critically re-evaluate bystander effects in vivo.

Table 4 In vivo studies of the bystander effect

End point	*Cellular system*	*Radiation type*	*Reference*
Chromosomal aberrations	Chinese hamster ovary and liver	$^{239}PuO_2$ particles; alpha particles	[B16, B17, M40]
	Mixture of irradiated and non-irradiated mice bone marrow cells	Neutrons	[W2]
Micronucleus frequency	Rat lung	Gamma rays	[K11, K22]
Calcium mobilization; alkaline phosphatase levels; embryonic development	Rat incisor, thyroid and abdomen	X-rays	[C8, H1]
Regenerative capacity	Earthworm	X-rays	[M8]
Macrophage activation; respiratory burst; NO activation; neutrophil infiltration	*TP53*$^{-/-}$ mice	Gamma rays	[L22]
Growth of tumour	Mixture of human colon LS174T adenocarcinoma cells and ^{125}I-labelled LS174T cells	^{125}I beta particles	[X2]
	C57BL/6 mice	Gamma rays	[C1]

76. Despite the caveats outlined above, there are studies indicating a bystander effect in vivo. Chinese hamsters were injected with different sized particles of the internally deposited alpha emitter plutonium. The radioactive particles concentrate in the liver and produce chronic low-dose radiation exposure, with the dose and dose rate being highest to cells located closest to the largest particles. However, analysis of induced chromosome damage in these livers revealed increased cytogenetic damage that was not directly related to the local dose distribution [B17]. These observations were interpreted as indicating that all the cells in the liver were at the same risk of induced chromosome damage despite only a small fraction of the total liver being exposed to the radiation. The cumulative incidence of liver cancer as a function of time after plutonium injection and total dose was also determined. Neither the time of tumour onset nor the tumour incidence varied with particle size, indicating that the number of cells hit by alpha particles was not a factor in tumour induction in irradiated livers [B16].

77. These two studies suggest that radiation-induced genetic damage and ultimately tumour induction are related to the total dose to the organ, i.e. the whole liver, rather than the dose to individual cells or the number of cells traversed by an alpha particle [B16, B17]. Furthermore, these data raise the intriguing possibility that the target for induced bystander effects may actually be limited to the specific organ irradiated and that adjacent non-irradiated organs are not targets for bystander effects. It is certainly not unexpected that multicellular organs function in response to genotoxic stress in a coordinated fashion [G24], and recently Barcellos-Hoff and Brooks have hypothesized that multicellular responses through extracellular signalling are integral components of predicting cancer risk after radiation exposure [B3]. To this end, there is evidence from in vivo studies of radiation-induced genomic instability that delayed instability has a significant bystander component [W2, X2]. Nevertheless, a recent report of the International Commission on Radiological Protection (ICRP) [I11] suggests that early initiating cellular and molecular events are the major determinants of risk at low doses, rather than cell-, tissue- and host-modifying factors.

78. The evidence for in vivo bystander effects has been reviewed in detail [B43, K25] and shows that these effects probably involve a genetic component [M61]. In addition to damage directly induced by the deposition of energy in the irradiated cell, consideration must now be given to

these indirect effects of radiation, and a model quantifying these considerations has been proposed [B14]. An irradiated cell can send out a signal and induce a response in a cell whose nucleus was not hit by radiation. Thus a detrimental bystander effect, e.g. chromosomal aberrations, in essence "modifies" the biological effectiveness of a given radiation dose by increasing the number of cells that experience adverse effects over that directly exposed to the radiation. Significantly, these bystander effects appear to be limited to the organ irradiated, i.e. are organ-specific [B43]. Thus, at the present state of our knowledge, it is reasonable to assume that any bystander effect induced in vivo is accounted for in models of organ risk evaluation. As a result, it is unlikely that the resurgence of interest in these non-targeted radiation effects will substantially alter risk estimates as discussed in detail in the BEIR VII report [C23]. Nevertheless, it cannot be excluded that increasing the knowledge basis for in vivo bystander effects at low doses and low dose rates in specific organs may affect current organ risk estimates.

III. RELATIONSHIP BETWEEN RADIATION-INDUCED GENOMIC INSTABILITY AND BYSTANDER EFFECTS

79. The evidence for effects occurring in cells that themselves were not irradiated but are the progeny of irradiated cells (radiation-induced genomic instability), and non-targeted cellular effects usually associated with direct exposure to ionizing radiation occurring in non-irradiated cells (bystander effects), has been reviewed in the previous paragraphs. Many of the end points associated with these two phenomena are the same: induced chromosomal rearrangements, micronuclei, increased mutation, increased transformation and cell killing. So what is the relationship, if any, between induced instability and bystander effects? Chromosomal instability in haemopoietic cells can be induced by an indirect, non-targeted bystander type of mechanism [K3, L24]. Persistently increased levels of intracellular ROS have been reported in chromosomally unstable cells [C5, L7, L8, L9, L10, R3, R6], and provide a plausible mechanism for perpetuating instability over time (reviewed in references [M10, M16]). Considerable experimental support for this hypothesis comes from Wright and co-workers [L22, W2, W4], and this has recently been reviewed in detail [L23]. The resultant intercellular signalling cascades, cytokine production, nitric oxide production and persistent free radicals all have the potential to mediate both instability and bystander effects (reviewed in references [L23, M10]).

80. To critically evaluate the hypothesis that chromosomally unstable GM10115 clones perpetuate instability by secreting a bystander-like factor into the culture medium, thus driving the delayed production of chromosomal rearrangements, Nagar et al. took medium from a chromosomally unstable clone of GM10115 human–hamster hybrid cells, filtered it and cultured non-irradiated GM10115 cells in this medium. None of the non-irradiated GM10115 cells were able to survive and form colonies in medium from the unstable clone. Nagar et al. called this novel effect, by which cells cultured in medium from chromosomally unstable GM10115 cells die, the death-inducing effect [N1]. The unstable clones showing the death-inducing effect also showed increased numbers of apoptotic cells and elevated levels of intracellular ROS [N2], either or both of which might contribute factors to the culture medium responsible for the death-inducing effect. Furthermore, Nagar et al. [N1] have interpreted this observation as indicating that unstable clones of cells do secrete factors that, while generally not toxic to the unstable clone, most likely contribute to the perpetuation of the unstable phenotype. It should be stressed that the death-inducing effect is separate from the bystander effect observed after transferring medium from irradiated cells. Nagar et al. did not observe a reduction in plating efficiency when medium from irradiated GM10115 cells was transferred to non-irradiated GM10115 cells. This indicates that GM10115 cells either do not secrete a cytotoxic bystander factor or are not susceptible to a bystander factor, and that the death-inducing effect is not the same as the bystander effect described by Mothersill and co-workers. Likewise, chromosomal instability was not detected in GM10115 cells after transferring medium from irradiated cells as described by Mothersill and Seymour [M18, M21] for induced bystander effects.

81. It should be noted that Mothersill et al. [M44] have also reported that they do not find a bystander response in some Chinese hamster cell lines after medium transfer. This implies that, like the human–hamster hybrid GM10115 cell line, CHOK1 hamster cells may be deficient in producing a bystander signal or in responding to that signal. It is interesting that when medium from irradiated CHOK1 cells was added to either non-irradiated CHOK1 cells or repair-deficient XR1 hamster cells, the plating efficiency actually increased rather than decreased [M44]. However, significantly increased bystander effects after cellular exposure to low fluences of alpha particles have been described in repair-deficient Chinese hamster cells compared with wild-type hamster cells [N3, N20, N25].

82. Evidence increasingly suggests that induced instability and bystander effects are linked (reviewed in references [L23, M10, M16]), and it is likely, given the commonality of the end points observed, that both phenomena could be manifestations of the same non-targeted processes [M10, M16]. Furthermore, a significant contribution from bystander-like factors could help explain the high frequency of radiation-induced instability reported, for example, in references [K3, L6, M2].

83. The Committee continues to hold the view that mechanistic information is important for its recommendations on radiation-induced health effects at doses of below ~200 mSv and for risk assessment for individuals. The latter aspect is important because epidemiology always refers to populations, and genomic instability, for example, varies among individuals.

A. Relationship between radiation hypersensitivity at low doses and bystander effects

84. To date, investigation of the relationship between radiation hypersensitivity at low doses and bystander effects has been limited to a single study. Joiner et al. [J4] described radiation hypersensitivity at radiation doses where

the in vitro bystander effect would be expected to predominate. Interestingly, an investigation into the relationship between radiation-induced low-dose hypersensitivity and the bystander effect indicated a weak inverse correlation between these two low-dose phenomena. Specifically, those cells exhibiting a large bystander effect did not show radiation hypersensitivity [M23]. Should these results be confirmed, they would suggest that, at very low radiation doses, bystander effects might dominate the overall cellular response. Furthermore, such bystander effects at doses of a few milligrays could have some bearing on the apparent elimination of damaged cells and the absence of repair of radiation-induced DNA double-strand breaks in the very low dose range (1.2 mGy) in X-irradiated normal human fibroblasts as reported by Rothkamm and Lobrich [R14].

B. Relationship between radiation adaptive response and bystander effects

85. The radiation adaptive response refers to the phenomenon by which cells irradiated with a sublethal dose of ionizing radiation (an "adaptive" dose of a few centigrays) become less susceptible to subsequent exposure to high doses of radiation (a "challenge" dose of several grays). There is a vast literature on "adaptive responses", and this section is not intended to be an exhaustive literature review. Instead, the goal is to provide examples of the main evidence for low doses of radiation protecting against a subsequent high-dose radiation challenge. The adaptive response to radiation was first described as a reduction in chromosomal aberration frequency in stimulated human lymphocytes [O7]. Subsequent reported adaptive responses include reduction of cell killing [I6], micronucleus formation and sister chromatid exchange [I7, I8], mutation [K26, R11, U25] and transformation [A17, R8]. An adaptive response has also been described after clinical [M53], environmental [G16] or occupational [B34] exposures to radiation. The mechanism for this radioadaptation is thought to be that low radiation doses enhance DNA repair ability and antioxidant activity, resulting in more proficient cellular responses to the subsequent challenge [G21, I9, S48].

86. Reports of the adaptive response to radiation are conflicting, however, because radioadaptation is not consistently a robust effect in all cell systems [A14, B35, B40, H16]. The variation among different studies could be related to a number of factors, including cell type [R7], cell culture conditions, cell cycle effects, types of radiation used, doses and dose rates, as well as time interval between irradiations [S54]. Current uncertainties in interpreting experimental data from both adaptive response and bystander investigations do not permit any firm conclusions regarding the relationships between these two low-dose phenomena to be reached at present.

87. Nevertheless, radiation-induced bystander effects can be considered a competing phenomenon with respect to an adaptive response [S7]. Using the Columbia University charged particle microbeam and the A_L cell mutagenic assay, Zhou et al. [Z6] showed that pretreatment of cells with a low dose of X-rays four hours before alpha particle irradiation significantly decreased this bystander mutagenic response. Furthermore, bystander cells showed an increase in sensitivity after a subsequent challenging dose of X-rays. Using the same irradiation system, Mitchell et al. [M51] found that an adaptive dose of 2 cGy of X-rays cancelled out the majority of the bystander effect produced by alpha particles. For oncogenic transformation, but not cell survival, radioadaptation could occur in non-irradiated cells via a transmissible signal.

C. Conclusions

88. Ionizing-radiation-induced bystander effects are those effects occurring in cells that were not traversed by radiation but were induced by signals from irradiated cells. Mechanistically, the signal is passed from cell to cell by gap junction communication or is secreted into the culture medium where it can be transferred to non-irradiated cells. Both positive effects for the cell (e.g. increased cell proliferation or an induced radioprotective adaptive response) and negative effects (e.g. cytogenetic damage or cytotoxic bystander responses) have been described. Bystander effects induced by high-LET radiation have been described in a number of different cell types in studies using either low radiation fluences of alpha particles or charged particle microbeams. A bystander effect induced by low-LET radiation is less well established and to date has only been demonstrated after medium transfer experiments. Experimental verification in different laboratories and the use of newly developed low-LET microbeams will extend these observations. To date, low-LET bystander effects appear to be a low-dose phenomenon, and reconciling low-dose bystander cytotoxicity with the lack of directly induced cell killing at these same doses is perplexing.

IV. ABSCOPAL EFFECTS OF RADIATION

A. Review

89. An abscopal effect may be defined as a significant tissue response to irradiation in tissues definitively separate from the region exposed to radiation. The response must be measurable, and the distance separating the responding tissues and the portal(s) of irradiation must be great enough to rule out any possible effect of scattered radiation [N15]. Originally described by Mole [M52] in 1953, the word abscopal comes from the Latin ab (position away from) and scopus (mark or target). The mechanism of the abscopal effect is unknown, although a variety of underlying biological events can be hypothesized, including a possible role for the immune system [M46, U24].

90. An example illustrating radiation-induced abscopal effects is the study of early DNA damage induced in rat lung cells following single-dose, partial-volume (lung base and lung apex) irradiation [K11]. When the lungs were removed at 16–18 hours after whole-lung irradiation, an average of ~0.85 micronuclei per binucleate cell were observed in the irradiated animals, compared with 0.02 micronuclei per binucleate cell in the lungs from control animals. When only the lung base was irradiated, the frequency of micronuclei in cells from the irradiated field was 0.85. However, non-irradiated cells from the out-of-field lung apex also showed a significant increase in the frequency of micronuclei, 0.43 per binucleate cell, significantly higher than the non-irradiated control value. Cells from the lungs of rats injected with superoxide dismutase within one hour prior to irradiation of the lung base and processed 16–18 hours after irradiation showed a reduction in the number of micronuclei in the shielded lung apex, indicating the potential involvement of oxygen radicals [K11].

91. Ohba et al. [O3] described the case of a 76-year-old Japanese man with hepatocellular carcinoma that regressed after radiotherapy for thoracic vertebral bone metastasis. Serum levels of tumour necrosis factor alpha increased after radiotherapy, and the investigators suggested that such abscopal-related regression might be associated with host immune response, involving cytokines such as tumour necrosis factor alpha. To understand the potential mechanisms, Camphausen et al. [C1] examined whether the abscopal effect was mediated through *TP53*. Non-tumour-bearing legs of C57BL/6 (wild-type *TP53*) and *TP53* null B6.129S2-Trp53(tm1Tyj) mice were irradiated to determine whether an abscopal effect could be observed against Lewis lung carcinoma and T241 (fibrosarcoma) implanted at a distant site. In the *TP53* wild-type mice, both the Lewis lung carcinoma and T241 tumour cells implanted into the midline dorsum grew at a significantly slower rate when the leg of the animal was exposed to five 10 Gy fractions of radiation compared with sham-irradiated animals. This suggests that the abscopal effect is not tumour-specific. When the radiation dose to the leg was reduced (12 × 2 Gy), the inhibition of Lewis lung carcinoma tumour growth was decreased, indicating a radiation dose dependency for the abscopal effect. In contrast, when the legs of *TP53* null animals or wild-type *TP53* mice treated with pifithrin-alpha (a *TP53* blocker) were irradiated (5 ×10 Gy), tumour growth was not delayed. These data implicate *TP53* as a key mediator of the radiation-induced abscopal effect and suggest that pathways downstream of *TP53* are important in eliciting this response.

92. Ionizing radiation can reduce tumour growth outside the field of radiation [A15, E16, K27, R9, R10], but this abscopal effect remains a rare and poorly understood event. Ionizing radiation generates inflammatory signals and in principle could provide both tumour-specific antigens from dying cells and maturation stimuli that are necessary for dendritic cells to activate tumour-specific T-cells. Demaria et al. [D23] tested the hypothesis that the abscopal effect elicited by radiation is immune-mediated. Mice bearing a syngeneic mammary carcinoma, 67NR, in both flanks were treated with growth factor Flt3-Ligand daily for 10 days after local radiation therapy to only one of the two tumours at a single dose of 2 or 6 Gy. The second, non-irradiated tumour was used as indicator of the abscopal effect. Radiation alone led to growth delay exclusively of the irradiated 67NR tumour. However, growth of the non-irradiated tumour was also impaired by the combination of radiation and ligand. Importantly, the abscopal effect was shown to be tumour-specific, because growth of a non-irradiated A20 lymphoma in the same mice containing a treated 67NR tumour was not affected. Moreover, no growth delay of non-irradiated 67NR tumours was observed when T-cell-deficient (nude) mice were treated with the combination of radiation and ligand. These results demonstrate that in this cell system the abscopal effect is in part immune-mediated and that T-cells are required to mediate distant tumour inhibition induced by radiation.

93. Abscopal effects after partial-body irradiation have also been described in earthworms [M8], White Leghorn cockerels [M9] and rats [C8, H1]. Abscopal reactions have also been described in patients with chronic leukaemias, wherein irradiation of an enlarged spleen or liver will induce a generalized remission with return of the bone marrow, white blood cell count and peripheral blood cells to normal ranges [N15]. In fact, there are a number of well-recognized effects

described by radiation therapists that occur beyond the radiation field. Goldberg and Lehnert [G7] have recently reviewed these bystander-like effects but concluded that the clinical literature does not provide strong evidence for or against the existence of radiation bystander effects, and by extension abscopal effects, in vivo. They argue that many studies can be interpreted as suggesting non-targeted effects in vivo, and recommend that prospective clinical trials be carried out that involve detailed field and dose information combined with well-documented patient risk factors in order to investigate potential bystander effects after radiation therapy [G7].

B. Conclusions

94. The few studies discussed in this section that describe the potential abscopal effects of ionizing radiation are generally descriptive in nature and provide little or no interpretation in terms of the mechanism underlying the response. This together with the lack of confirmatory studies means that definitive conclusions on the impact of any potential abscopal effects are not possible. Additional focused research involving well-designed prospective clinical trials could clarify this issue.

V. CLASTOGENIC FACTORS INDUCED BY IONIZING RADIATION

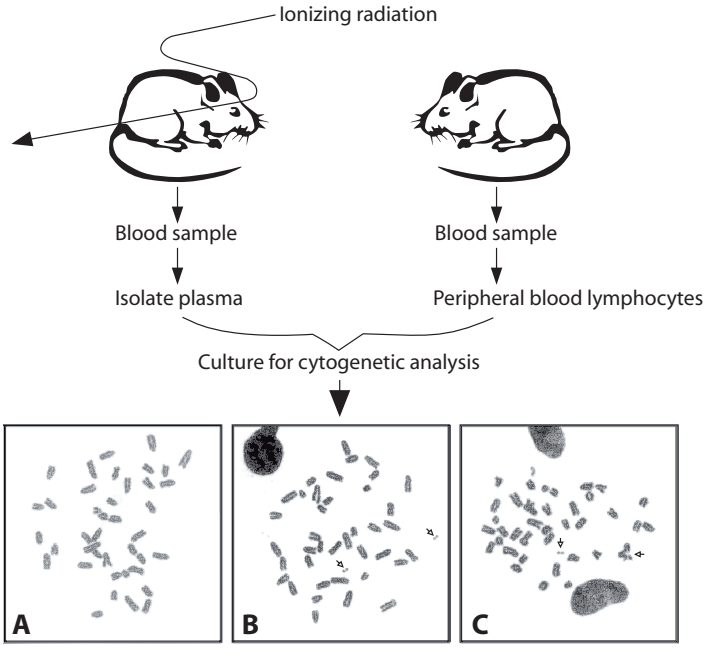
A. Review

95. Following whole-body irradiation, blood plasma from some animals and humans can contain factors that can cause chromosome damage, hence the name clastogenic factors. This indicates the persistence of soluble factors induced by radiation that are capable of causing effects in non-irradiated cells. As such, clastogenic factors are not necessarily classical bystander effect factors but are included here to support the concept of a role for secreted and released soluble factors in delayed effects associated with radiation exposure. Strictly, under the definitions presented in this review, clastogenic factors, like abscopal effects, could well be considered bystander effects. However, for historical reasons, both abscopal effects and clastogenic factors are considered separately. In reality, however, no mechanistic distinctions are implied between these non-targeted effects of exposure to ionizing radiation.

96. There is a large body of evidence demonstrating that plasma from irradiated animals and humans can contain factors capable of inducing detrimental effects in unexposed cells. These "clastogenic factors" (or clastogenic plasma factors) were first described by Parsons et al. [P5], who observed bone marrow damage in the sternum of children with chronic granulocytic leukaemia whose spleens had been irradiated. This report was corroborated by Souto [S19], who showed that rats injected with plasma from irradiated animals developed mammary tumours at a significantly higher rate than rats exposed to plasma from non-irradiated animals. A number of reports eventually followed that demonstrated that culturing normal human peripheral blood lymphocytes in medium containing plasma obtained from accidentally [G5] or therapeutically [H6, L15] irradiated individuals resulted in significantly more chromosomal aberrations than culturing lymphocytes in medium with plasma from non-irradiated individuals. These observations led to the suggestion that, after in vivo irradiation, exposed individuals can possess clastogenic factors in their blood plasma that, when transferred to cell cultures from unexposed individuals, can induce chromosome damage (figure XI).

Figure XI. Theoretical schematic for identifying clastogenic factors.

Plasma isolated from blood from an irradiated mouse is mixed with blood from a non-irradiated mouse and cultured for cytogenetic analysis. Clastogenic factors in the plasma from the irradiated mouse can cause chromosomal aberrations in the peripheral blood lymphocytes of the non-irradiated mouse. A: metaphase chromosomes from a peripheral blood sample from a non-irradiated mouse cultured in media containing isolated plasma from a non-irradiated mouse. No chromosomal aberrations are observed. B and C: metaphase chromosomes from a peripheral blood sample from a non-irradiated mouse cultured in isolated plasma from an irradiated mouse (B: chromatid deletions (arrows); C: chromatid exchange and deletion (arrows)).



97. Clastogenic factors have been described in plasma from survivors of the atomic bombings in Japan [P2], personnel involved in salvage operations after the Chernobyl accident [E8, E10] and children exposed as a consequence of the Chernobyl accident [E11, G3]. In addition, clastogenic factors have been reported in human [G1, L19, S10] and rat blood plasma [F1] after in vitro irradiation. Clastogenic factors can be induced within 15 minutes of irradiation [F1] and appear to be very persistent or continuously regenerated: times of 10 weeks post-irradiation have been reported for rats [F1], 7–10½ years for irradiated humans [E8, G5, G6] and >30 years for the atomic bombing survivors [P2]. Clastogenic factors reflect neither radiation-induced depletion of protective factors nor radiation-induced changes in normal plasma components, but rather represent products secreted or excreted by cellular elements as a result of irradiation [F1]. In vitro induction of clastogenic factors does not appear to be related to the dose [S10] or quality of radiation [G1]; however, this might not be the case in vivo [E8].

98. Emerit et al. [E8] investigated clastogenic factors in the plasma of 32 civil workers from Armenia who had been engaged as emergency workers around the Chernobyl atomic power station in 1986. They also included 15 emergency workers who had emigrated from the former Soviet Union to Israel. Reference plasma samples were obtained from 41 blood donors from the Armenian Blood Center in Yerevan. The samples were tested for their clastogenic activity in blood cultures from healthy donors. The samples from the first Armenian group, with the higher average radiation dose (0.6 ± 0.6 Gy), were more clastogenic than those from the second group, which had been exposed to 0.2 ± 0.2 Gy. The samples from the Israeli emergency workers also induced significantly increased aberration rates (14.0 ± 3.9% aberrant cells). The clastogenic activity described above could be inhibited by superoxide dismutase [E8], indicating that the chromosome-damaging effects of radiation-induced clastogenic factors are exerted via the intermediation of superoxide radicals, as is known for clastogenic factors of different origin [E18, F12].

99. It should be stressed that there is variability between individuals in their ability to produce clastogenic factors [E11, G3], and not all irradiated individuals exhibit this effect [L4]. Indeed it is difficult to evaluate how common is the induction of clastogenic factors in the human population. On the one hand, blood samples from irradiated individuals are a valuable commodity, usually studied for more conventional biomarkers of radiation exposure. On the other hand, it is perhaps not surprising that there are very few reports failing to detect clastogenic factors, as such negative results are generally less likely to be published.

100. The precise nature of clastogenic factors is unknown, but endogenous viruses and compounds that interfere with DNA repair and/or increase the production of free radicals have all been implicated [E1, E2, E3]. On the basis of a number of inhibitor studies, the bulk of evidence suggests that the mechanism of action of clastogenic factors is probably mediated by free radicals. Free radical scavengers such as superoxide dismutase, penicillamine, cysteine and various antioxidant plant extracts all reduce or eliminate clastogenic factor activity [E1, E2, E3]. The molecular mechanisms for this effect and the specific nature of the factors capable of persisting, or of being regenerated over protracted time intervals (>30 years in the case of some of the atomic bombing survivors [P2]), remain unknown. Nevertheless, it is tempting to speculate on the potential relationship between clastogenic factors and factors involved in the bystander effect. Both can be induced by ionizing radiation and the factors produced can cause genetic damage in non-irradiated cells. Given the current interest in bystander effects resulting from secreted factors produced after cellular irradiation [S39], it may be an appropriate time to revisit clastogenic factors and evaluate the biological significance and nature of these radiation-induced secreted factors.

101. At present, the biological significance of clastogenic factors remains unclear [H11]. Furthermore, it would be misleading to imply that clastogenic factors are unique to radiation exposure. Transferable clastogenic effects have been described in blood plasma after whole-body stresses as diverse as asbestos exposure [E7] and ischaemia reperfusion injury [E6], and occur spontaneously in patients with HIV-1 [E1], hepatitis C [E12], Crohn's disease [E5] and scleroderma [A2]. The reports of diffusible clastogenic factors induced by irradiation also resemble the reports of clastogenic activity in the plasma of patients with certain inherited disorders, including Bloom's syndrome [E4], ataxia-telangiectasia [S14] and Fanconi's anaemia [E9]. Individuals with these chromosome breakage syndromes show an increased incidence of cancer, which begs the question as to the role of clastogenic factors in creating a cellular environment predisposed to increased genomic instability and ultimately neoplastic transformation [H11, W11].

102. Emerit and colleagues have carried out many of the studies investigating clastogenic factors. These investigators have described these factors in plasma from individuals exposed to different types of radiation under a variety of exposure conditions. In addition, Emerit's group has described clastogenic factors after exposure to other DNA-damaging agents, as well as in individuals with a number of medical conditions and various genetic diseases. It would be misleading to imply that Emerit and colleagues are the only ones to describe clastogenic factors in various disease states. Reports from other laboratories lend support to these observations (e.g. [B10, G1, S14]), but the biological significance and potential health hazards associated with clastogenic factors remain to be determined.

103. Nevertheless, the presence of clastogenic factors in peripheral blood samples from some irradiated individuals raises intriguing questions concerning the role of chromosomal rearrangements as dosimeters of radiation exposure [S10]. Cytogenetic analysis of first division metaphase

cells from irradiated individuals reveals both asymmetrical (dicentric and polycentric chromosomes as well as ring chromosomes) and symmetrical (reciprocal translocations) exchange-type aberrations, insertions, inversions and chromosomal breaks (reviewed in reference [C9]). The asymmetrical exchange-type aberrations generally lead to proliferative cell death in subsequent mitoses and decline over time. Symmetrical translocations, on the other hand, are generally stable over time and can persist, although they also appear to decline, albeit at a much slower rate than asymmetrical aberrations [S20]. Since clastogenic factors from some irradiated individuals can induce chromosome damage, a role for these factors in the well-described persistence of chromosomal rearrangements in blood samples from irradiated individuals is possible but unlikely.

B. Conclusions

104. It remains difficult to establish a clear description of the relevance of clastogenic factors to overall cellular responses to ionizing radiation, particularly at low doses. In part, this is due to the paucity of data on the nature of the factors and on their mechanism of action. Furthermore, how such clastogenic factors might influence the dose–response curve at low doses is not possible to discern at this time.

VI. IMPACT OF NON-TARGETED AND DELAYED EFFECTS OF RADIATION ON FUTURE GENERATIONS

105. It is important to consider whether non-targeted effects of radiation influence our consideration of the consequences of irradiation of a parent on end points in offspring. The following sections summarize the data from non-human and human studies and consider them in the context of the Committee's current position on heritable effects of irradiation. Several diverse studies have provided a somewhat confusing picture of the potential non-targeted or delayed effects of ionizing radiation in humans, mice and other organisms. Many of the studies present technical difficulties and ambiguities in interpretation, for example, with respect to uncertain radiation doses in human studies, potential strain dependency of responses in mouse studies, and poorly defined criteria used to define effects in some molecular studies. These complicating factors will be highlighted throughout this section.

106. Many of the studies examine effects in the F_1 offspring of irradiated parents, while other studies consider F_2 and later generations. It is only these studies of F_2 and later generations that can be unambiguously considered transgenerational. This is because the F_1 studies may be revealing mutations that occur during parental germ cell development. Thus F_1 studies address "heritable effects", while F_2 and later generation studies address "transgenerational effects".

A. Studies in non-mammalian species

107. The first demonstrated transmission of genomic instability to subsequent generations was in *Drosophila* treated with ionizing radiation and mustard gas. However, the end point used was lethality, which revealed little about the nature or the mechanisms of the processes involved [A12].

108. Shima and colleagues have developed a "specific locus" test system using the Japanese medaka fish, *Oryzias latipes* [S40]. The genetic end points available are dominant lethal mutations, total "specific locus" mutations and viable "specific locus" mutations. The medaka has a transparent egg membrane and embryo body, and both visible mosaics and whole-body mutations can be detected during development at an early-expressed pigmentation locus [S40]. When wild-type +/+ males were gamma-irradiated and then mated with wl/wl females, the frequency of F_1 embryos with both wild-type orange leucophores (wl/+) and mutant-type white leucophores (wl/wl*) (mosaic mutants) was ~5.7×10^{-3} Gy^{-1}. The frequency of embryos with only white leucophores (whole-body mutants) was ~1.3×10^{-3} Gy^{-1}. These results suggest that delayed mutations arise frequently in medaka fish embryos that have been fertilized with irradiated sperm [S41].

109. There was also a significant dose-rate effect for this type of mutation. Shimada et al. [S43] determined the frequency of "specific locus" mutations at five pigmentation loci in medaka spermatogonial stem cells after gamma irradiation at 0.03 cGy/min and 95 cGy/min. At each total dose, the mutation frequency was significantly lower in the 0.03 cGy/min group than in the 95 cGy/min group. The ratio of the induced mutation frequency at 0.03 cGy/min to that at 95 cGy/min was approximately 0.42 for doses of less than 1.9 cGy and approximately 0.33 for doses of 1.9–4.75 Gy [S43]. There was some specificity as to when such mutation events can be induced during spermatogenesis. When sperm and late spermatids were irradiated, the mutation frequency within non-irradiated maternally derived alleles was approximately three times higher than in the control group. In the F_2 generation, however, no increase in mutation frequency was observed. Similarly, there was no significant increase in the F_1 mutation frequency when stem cell spermatogonia were irradiated. These data suggest that irradiation of sperm and late spermatids can induce indirect mutations in F_1 somatic cells, supporting the idea that genomic instability arises during F_1 embryonic development. Moreover, such instability apparently arises most frequently when eggs are fertilized just after the sperm are irradiated [S42]. It should be noted that dose-rate effects and germ-cell-stage specificities for mutational response were previously demonstrated for "specific locus" mutations in mice, and such findings have been factored into past decisions of the Committee regarding the estimation of the risk of hereditary effects. Although interesting, these new findings in fish provide no reason to modify those estimates.

110. Microsatellite mutations have also been studied in plants grown in heavily contaminated areas near Chernobyl [K15, K16]. Kovalchuk et al. [K15] investigated the mutation rates of 13 microsatellite loci in wheat plants grown in a contaminated (900 Ci/km^2) versus a control (<1 Ci/km^2) area, and found a 3.6-fold increase in germinal mutation rate in the contaminated versus the control plot. Ellegren et al. [E17] reported an increased frequency of partial albinism, a morphological aberration associated with loss of fitness, among barn swallows, *Hirundo rustica*, breeding close to Chernobyl. Heritability estimates indicate that mutations causing albinism were at least partly of germ line origin. Furthermore, evidence for an increased germ line mutation rate was obtained from segregation analysis at two hypervariable microsatellite loci, indicating that mutation events in barn swallows from Chernobyl were two- to tenfold higher than in birds from control areas in Ukraine and Italy.

B. Mouse studies

1. Irradiation of the mouse zygote

111. A long-held belief regarding radiation teratogenesis was that developmental defects are only inducible when the conceptus is irradiated during organogenesis. In contradiction to this, Streffer et al. [M26, P1, P7] demonstrated that irradiation of a single cell, the zygote, can induce developmental abnormalities, particularly gastroschisis, in the resulting animal. Indeed, when irradiated during early embryogenesis, induced teratogenesis is suppressed in a *TP53*-dependent manner, where apoptotic cell death plays a critical role [K23, N23, N24].

112. Streffer's studies used the Heiligenberger (now the HLG/Zte) mouse, which has a spontaneous frequency of gastroschisis of ~3%. One gray of X-rays to the zygote increased this to ~11%, leading the investigators to conclude that ionizing radiation enhances latent damage already present in this predisposed mouse strain [H27, S34]. Similar findings have been made by other investigators [G4, G20, J7] after exposure to chemical mutagens [G15]. Streffer and colleagues extended these studies to demonstrate congenital malformations in the 19-day-old foetuses after paternal irradiation (2.8 Gy of ^{137}Cs gamma rays) [M64]. This increased lethality occurred after exposure of all stages of spermatogenesis with the exception of early spermatogonia.

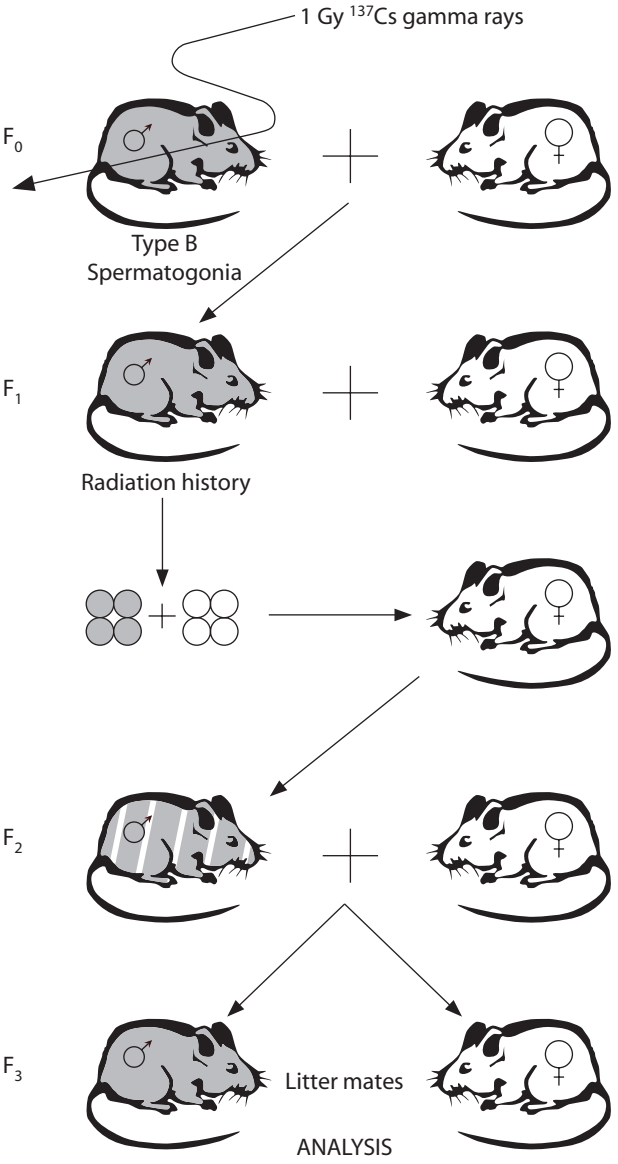
113. One possible interpretation of the gastroschisis results is that this mouse strain has a peculiarly high susceptibility to this type of gross abnormality and that the radiation treatments induced many dominant lethal mutations in germ cells or in the pronucleus that greatly exacerbated this extreme inherent susceptibility. While it is important to be aware that such situations exist, there does not seem to be any practical way to apply data on a strain of mice with a strong predisposition to a serious anomaly to the estimation of hereditary risk in human populations.

114. The effect of in utero exposure to ionizing radiation during the early phases of pregnancy has previously been reviewed [J7, P1, S34]. The risk of congenital malformations, the role of genomic instability after irradiation during the pre-implantation phase [S63], genetic susceptibility [J6] and health consequences [H17] have been reviewed in detail and will not be further considered here.

2. Pre-implantation embryo chimera assay

115. Wiley et al. have used a pre-implantation embryo chimera assay to demonstrate adverse effects in embryos after acute whole-body paternal irradiation (figure XII). They measured the competitive cell proliferation disadvantage of an embryo with a radiation history after challenge by direct cell-to-cell contact with a normal embryo in an aggregation chimera [O1, O2, W10]. The F_1 embryos conceived 6–7 weeks after paternal F_0 irradiation were most likely to display the phenotype, indicating that the type B spermatogonia were the most sensitive [W10]. Recently, Baulch et al. [B4, B5] have evaluated F_3 mouse offspring from F_0 paternal mice exposed to 1 Gy of ^{137}Cs gamma rays for gene products that can modulate cell proliferation rate, including receptor tyrosine kinase, protein kinase C and MAP kinases, and

Figure XII. Pre-implantation embryo chimera assay.
F_0 CD1 male mice were exposed to 1 Gy of ^{137}Cs gamma rays and mated to non-irradiated CD1 females six weeks after F_0 paternal irradiation. F_1 animals with a radiation history were mated with non-irradiated females to obtain F_2 embryos with a paternal F_0 radiation history. These four-cell embryos were paired with non-irradiated CD1 control four-cell embryos, and the resulting chimeras were cultured to the blastocyst stage and transferred to a foster mother. The pups resulting from the transfer were screened, and those with and without a paternal F_0 radiation history were identified and bred with normal females. Sex-matched pairs could then be evaluated for competitive cell proliferation disadvantage, protein kinase C, MAP kinase and GST activities, as well as p21^{waf1} and TP53 protein levels.



protein levels of nuclear TP53 and $p21^{waf1}$. All three-protein kinase activities were altered, and nuclear levels of TP53 and $p21^{waf1}$ protein were higher in F_3 offspring with a paternal F_0 radiation history than in non-irradiated litter-mates. While there is clear evidence of effects, it is unclear how this rather novel and artificial phenotype can be related to clinically important hereditary effects, and thus no attempt will be made to apply these results to hereditary risk estimation in humans.

3. Mouse mutation assays

116. To investigate whether preconception paternal irradiation can lead to the heritable transmission of genomic instability in mice, Luke et al. [L25] measured mutation frequency in a transgenic mouse model tagged with a lambda shuttle vector. This assay system allowed mutations in the *lacI* gene from the shuttle vector to be analysed in vitro after the animal had been irradiated in vivo. The results indicated that, as parental dose increased, there was a trend towards higher mutation frequency in vectors recovered from DNA from the bone marrow of F_1 progeny. These data demonstrate heritable transmission of factors leading to genomic instability in F_1 progeny following paternal pre-conception irradiation, although the results with a lambda shuttle vector would be complicated to apply in quantitative risk estimation for humans because they do not involve mammalian genes.

117. An increase in micronucleus frequency in bone marrow erythrocytes from the F_1 progeny of male BALB/c mice exposed to chronic low-dose gamma irradiation was observed by Fomenko et al. [F11]. Mice were irradiated with 10, 25 or 50 cGy at dose rates of 1, 5 and 15 cGy/day, and were mated with non-irradiated females on day 15 after irradiation. The obtained offspring had an elevated micronucleus frequency in bone marrow erythrocytes at the age of two months. This suggests the transmission of genomic instability from damaged germ line cells of irradiated male parents to somatic cells of the progeny. It is unclear, however, if the effects on micronuclei represent chromosome damage of consequence to clinical diseases beyond those already covered by the Committee's current methods to estimate hereditary risk.

118. The p^{un} mouse background allows visual detection of ~70 kb DNA deletions in the pink-eyed unstable (p^{un}) locus in developing mouse embryos. These are scored as black spots on the light gray fur or black cells on the transparent retinal epithelium of the offspring [R2]. In the fur spot assay, 10-day-old pups are observed for black spots on the light gray fur, and the number of animals with fur spots is counted. In the eye-spot assay, mice are sacrificed at ~20 days, the eyes are removed and the number of black cells in whole mounts of the unpigmented retinal pigment epithelium are determined [R2]. The C57BL/6Jp^{un}/p^{un} mouse strain contains a 70 kb tandem duplication of the pink-eyed dilution (p) gene [B29], the pun mutation. The p^{un} mutation is autosomal recessive and results in a dilute, light grey coat colour and pink eyes. Intrachromosomal homologous recombination between the 70 kb repeats that delete one copy of a duplicated 70 kb DNA fragment at the p^{un} locus restores the p gene and produces black pigment in the hair and retinal epithelium in wild-type mice. On the C57BL/6Jp^{un}/p^{un} inbred background, 5–10% of the mice spontaneously display fur spots and from four to six eye-spots per unpigmented retinal pigment epithelium.

119. The p^{un} fur spot assay was used to demonstrate that exposure of the parental germ line to ionizing radiation results in induction of delayed DNA deletions in mouse offspring [C16, S17]. Male p^{un} assay/p^{un} assay mice were irradiated with 1 Gy of X-rays and mated 28 days later with non-irradiated p^{un} assay/p^{un} assay females. The offspring showed a higher frequency of large fur spots. Since deletions occurring early in embryogenesis should yield larger spots than events occurring later, the large spots indicated deletion events occurring early in embryo development and many cell divisions after irradiation [C16]. Shiraishi et al. [S17] irradiated male mice with 6 Gy and observed an increase in p^{un} assay reversions resulting in eye-spots, in the irradiated paternal p^{un} assay allele as well as in the non-irradiated maternal p^{un} assay allele, indicating untargeted recombination in the offspring. The number of spots per retinal epithelium increased twofold when the male was irradiated at the spermatozoa stage [S17], but p^{un} assay instability was not observed when radiation was delivered either to spermatogonial stem cells or to late spermatids. It is noteworthy that radiation-induced instability of the p^{un} assay allele has been observed in F_1 mice but not in the F_2 generation (reported in reference [N22]).

120. Estimates by the Committee of hereditary risk for exposures of males have been based on damage to spermatogonial stem cells because they are the only germ cells in males that can accumulate appreciable doses under low-dose-rate exposure conditions. The finding that there is no induced p^{un} instability in spermatogonial stem cells suggests that there is no need to revise current risk estimates upward.

4. Alterations in tandem repeat DNA sequences

121. Alterations in tandem repeat DNA sequences, such as minisatellite DNA and expanded simple tandem repeats (ESTRs), in the genome have been used as markers of genetic change. Such loci have high spontaneous rates of mutation, which facilitates the measurement of induced mutation in relatively small numbers of samples. Alterations (mutations) are manifested as gains or losses in repeat units and are detected either by pedigree screening or by amplifications [N22, Y4]. Mutations in both minisatellite sequences and ESTRs appear to arise via indirect mechanisms rather than by direct damage to the repeat locus itself [Y4]. The significance of these DNA sequence changes is unknown. If they are genetically neutral they will not affect risk; nevertheless, the fact that they happen indicates that exposure to ionizing radiation can lead to genomic destabilization that may occur by a non-targeted mechanism.

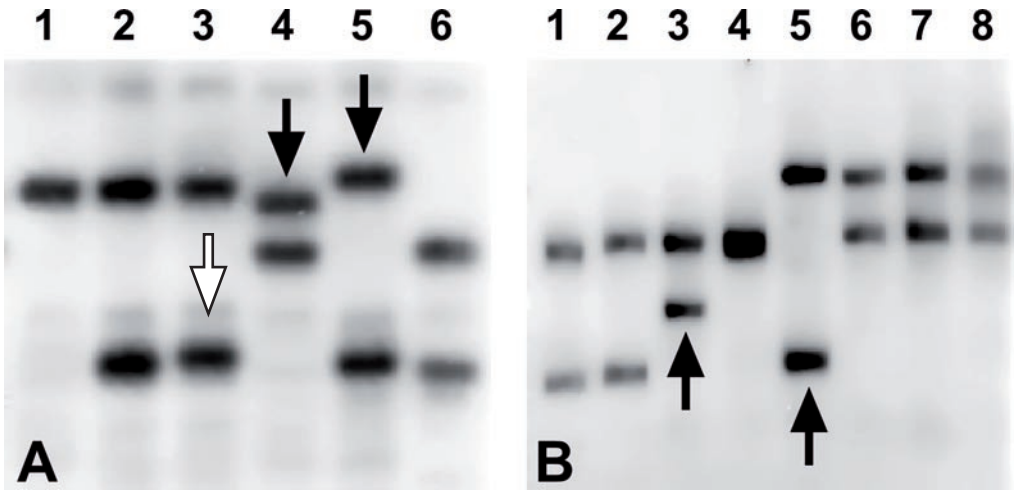
122. Minisatellites are tandem repeat loci, typically 0.5–30 kb long, with repeat units in the range 6–100 base pairs. Thousands of minisatellites exist in the genome and frequently they show variability in repeat copy number and therefore allele length [J1]. Some minisatellites are highly unstable and undergo a frequent length change mutation spontaneously in germ cells, sometimes as high as 10^{-1} per gamete [J2]. ESTRs were formerly classified as minisatellites but are now recognized as a fundamentally different entity. In contrast to minisatellites, ESTRs are composed of long arrays, up to 16 kb in length, of short (<10 bp) repeat units. Unstable ESTRs appear to be comprised almost exclusively of homogeneous arrays, with longer arrays exhibiting the highest rates of mutation [B30]. This is in contrast to the complex variant repeat distribution that makes up common minisatellite alleles. ESTRs exhibit high levels of somatic instability and also show different mechanisms of mutation from that observed in the highly mutable GC-rich minisatellites [Y4]. Examples of alterations (mutations) in an ESTR in mice are presented in figure XIII.

Figure XIII. Mutation analysis at two mouse ESTR loci.

A: Ms6-hm loci. Lanes: 1: father; 2: normal offspring; 3: offspring with a maternal mutation (open arrow); 4 and 5: offspring with two different paternal mutations (dark arrows); 6: mother.

B: Hm-2 loci. Lanes: 1: father; 2, 4, 6 and 7: normal offspring; 3 and 5: offspring with different paternal mutations (dark arrows); 8: mother.

[Figure kindly provided by Y. Dubrova, University of Leicester, United Kingdom.]



123. By analysing DNA fingerprints of the offspring of ^{60}Co gamma-irradiated mice, Dubrova et al. [D10] have shown that tandem repeat loci mutations appear to be induced in spermatogonia by low doses of ionizing radiation, with an estimated doubling dose of 0.5 Gy. This estimated doubling dose was subsequently revised downwards to 0.33 Gy [D14], which is similar to that reported earlier in the "specific locus" test in mice [R4]. This is an assay that has been relied upon by the Committee to a large extent in estimating hereditary risk.

124. Dubrova [D21] has since compared the spectra and dose response for mutations at ESTR loci in the germ line of male mice acutely exposed to low-LET X- or gamma rays at pre-meiotic stages of spermatogenesis in five strains of laboratory mice. He found that most mutation events involved the gain or loss of a relatively small number of repeat units, and the distributions of the observed length changes were indistinguishable between the exposed and the control males. Overall a significant bias toward gains of DNA repeats was detected, with approximately 60% of mutants showing gains. The values for ESTR mutation induction produced doubling doses of 0.44–0.98 Gy. Doubling dose estimations were also made by Niwa and his group, and values of 3.4, 0.893 and 4.0 Gy were reported for stem cells, spermatids and spermatozoa, respectively [F2, N14]. Inherent imprecision in the methodology used is likely to be the cause of these discrepancies [N22].

125. To date, most laboratory studies demonstrating heritable effects of radiation exposure have involved paternal irradiation [B4, B5, D14, D15, N14, S1, W10], and an approximately linear dose-response curve for paternal mutation induced at pre-meiotic stages was found [D14]. Germ line mutation in mouse tandem repeat loci appears to be a sensitive indicator of irradiation of pre-meiotic stage germ cells [D10, F2, S1], and an elevated paternal mutation rate was found after irradiation of mouse pre-meiotic spermatogonia. In contrast, post-meiotic irradiation of spermatids gave a result similar to that in control litters [D14]. However, both pre- and post-meiotic exposures were reported to increase mutation yield, suggesting that strains of mice may differ in stage susceptibility [F2, N14, S1].

126. Dubrova et al. [D14] also analysed the maternal mutation rate in mice after paternal irradiation at different stages of spermatogenesis, and found no difference in the frequency of maternal mutation. In contrast, Niwa et al. have described

a small but statistically significant increase in the maternally derived Ms6hm allele, in addition to the paternally derived allele, when only male parents were irradiated at the spermatozoa stage [F2, N14, S1]. Niwa and Kominami [N14] demonstrated a statistically significant increase in maternal allelic mutation rate in F_1 mice born to irradiated male parents. The authors concluded that, as a consequence of male (sperm) irradiation, genomic instability is triggered in the zygote, which then mutates the paternally derived allele in *cis* and the maternally derived allele in *trans*.

127. Mutation rates at two ESTR loci have been studied in the germ line of first- and second-generation offspring of inbred male CBA/H, C57BL/6 and BALB/c mice exposed to either neutrons or X-rays. Paternal CBA/H exposure resulted in increased mutation rates in the germ line of two subsequent generations. Comparable transgenerational effects were observed also in neutron-irradiated C57BL/6 and X-irradiated BALB/c mice. The spontaneous mutation rates and radiation-induced transgenerational instability varied between strains (BALB/c>CBA/H>C57BL/6). Pre- and post-meiotic paternal exposure resulted in similar increases in mutation rate in the germ line of both generations of CBA/H mice, which suggests that radiation-induced expanded simple tandem repeat instability is manifested in diploid cells after fertilization [B2]. Although there are some difficulties in interpreting these data [B41], they do suggest that excess transgenerational mutation at the unstable loci may be detected after irradiation of developing male germ cells.

128. Analysis of ESTR mutation induction in the mouse germ line suggests that mutations are likely to be due to pausing of the replication fork and subsequent polymerase slippage events [B31, D21]. The frequency of induced mutation appears to be significantly greater than the predicted frequency of radiation-induced DNA damage at a given tandem repeat locus. This has led many investigators to conclude that the increased mutation rates observed at these repeats are not necessarily the result of directly induced DNA damage at the "specific locus", but result from radiation-induced damage elsewhere in the genome or cell [D13, D14, D15, F2]. While alterations in tandem repeat DNA sequences may provide useful biomarkers of induced germ line effects, the biological significance of such mutations remains unknown. The similarity of the calculated doubling doses to those published for "specific-locus" mutations in mice supports the validity of the tandem repeat DNA sequence data, although the uncertainty in doubling doses in "specific-locus" experiments needs to be borne in mind [U1]. The comparative data between germ cell stages for mutations that affect tandem repeat DNA sequences remain of interest and potential importance.

5. Tumour induction in the offspring of irradiated parents

129. Nomura first reported a significant increase in lung tumours, mostly benign adenomas, in the F_1 offspring of X-irradiated ICR parental mice [N17, N18, N28]. This has been confirmed in some studies [H10, L20, L21, V3] but not all. For example, Cattanach et al. [C4], using the optimal experimental conditions defined by Nomura [N17], found that tumour incidence was no higher in the offspring of irradiated BALB/cJ mice than in the non-irradiated controls. They did find that the proportion of fertile females and mean litter size were affected by the radiation, showing a dose-dependent, dominant lethal response. In attempting to reconcile these differences, Cattanach et al. [C4] proposed that inconsistencies in the animal experiments may in part be due to lack of an appropriate concurrent control, whose periodic or cyclic variation in tumour incidence may have been out of phase with that in the treated animals. Alternatively, the reported differences could reflect strain differences in the mice used.

130. Selby and Priest [S52] reported no induced leukaemias when male CBA/Ca mice were injected with ^{239}Pu citrate solutions at nominal activities of 6 and 60 Bq/g, to give absorbed doses of approximately 0.3 and 4.0 cGy, and were mated to females of the same strain 54–68 days later. Nomura [N16] found no increase in leukaemia in the offspring of ICR mice derived from spermatogonia after acute irradiation. In contrast, when spermatogonia from the N5 mouse strain were irradiated, Nomura found a 10-fold-greater incidence of acute lymphocytic leukaemia in the offspring than in the non-irradiated controls. Once again, this may be explained by differences in genetic predisposition to leukaemia induction by radiation in these mouse strains.

131. Extending his original findings, Nomura hypothesized that, if radiation-induced mutations in the germ line led to heritable lung tumours in offspring, then all the cells in the lung should be at increased tumorigenic risk. Following a subsequent challenge with the carcinogen urethane, Nomura described significantly increased clusters of tumour nodules in the lung [N17, N18]. Vorobtsova and Kitaev [V3] reported similar findings, but Cattanach et al. [C21] were unable to replicate these results in C3H/HeH mice. Selby et al. [S53] suggested that the explanation for the surprisingly high rates of induction of dominant mutations that cause tumours suggested by the experiments of Nomura and others might result from the confounding effect of the radiation-induced dominant lethality that often occurs in such experiments. Other possible non-mutational explanations for the high mutation rates reported for dominant tumour mutations by Nomura have been suggested by Selby [S51] and Cattanach et al. [C4, C21].

132. Lord et al. have also investigated the heritable effects of pre-conception paternal irradiation from injected plutonium (^{239}Pu alpha particle irradiation), ^{137}Cs gamma rays or the Auger-electron-emitting radionuclide ^{55}Fe. They demonstrated perturbed haemopoiesis in offspring, as well as enhanced sensitivity to methylnitrosourea (MNU, 50 mg/kg) as a secondary carcinogenic insult [H10, L20, L21]. A major difference from the tumour experiments discussed above was that Lord et al. used much lower doses of radiation. As a result, there would have been little or no induced dominant

lethality, and the possibility that this was a confounding effect is therefore removed. The mutation rates calculated from these results suggest that Nomura's results actually underestimated the extent of this phenomenon. Since these studies rely on a secondary treatment with a carcinogen, there seems to be no way to apply their results to revise the Committee's estimates of hereditary risk, which are made for radiation alone.

C. Malformation induction in the offspring of irradiated parents

133. There are several studies addressing the question of malformation induction after irradiation of either female [K13, L50, M65, N17, N31, N32, N34, W22, W23] or male [K12, K36, M64, N17, N31, N32, N34, R15] mice and looking for malformations in the next or subsequent [L50, L51, N33, P7] generations. In all of these studies, conditions were found that resulted in transmission of radiation-induced germ cell effects in the form of malformations to the F_1 or subsequent generations. The mechanism for the development of these malformations is apparently different under these conditions from that which is responsible for the development of malformations induced by radiation exposures during major organogenesis [S34]. The following conclusions can be drawn from the experiments: comparatively high doses (>1 Gy) are required for transgenerational malformation effects to be detected. The effects seen after female exposure are not due to indirect effects because of radiation sickness of the mother, but must have, at least partly, a genetic background [L50, W22, W23]. This genetic background is quite obvious after radiation exposure of male mice. These transgenerational effects are not restricted to low-LET radiation [K36]. It seems unlikely that there is a direct relationship between chromosomal translocations and congenital anomalies [L50, N34], although some suspicion in that direction has been expressed [R15]. The basis of the malformations observed seems to be heterogeneous: some are due to genetic changes of high penetrance that are rapidly eliminated; some are due to modification of genes of low penetrance; and some are probably of non-genetic origin [L50]. There are strain-specific differences as to the extent of the transgenerational effect [R15]. There are indications that a major proportion of mutations are eliminated in the first generation and that only a minor proportion are transmitted to later generations [L50].

134. At present there are too many uncertainties about those data suggesting that dominant mutations and/or genomic instability cause tumours in progeny of irradiated mice to be able to apply such data in hereditary risk estimation.

D. Human studies

135. To date, no radiation-induced genetic, i.e. hereditary, diseases have been demonstrated in human populations exposed to ionizing radiation. Neither the offspring of individuals treated with chemotherapy and/or radiotherapy for cancer [B18] nor the offspring of women treated with radiation during infancy for haemangiomas [K6] demonstrated any significant effects attributable to parental exposure to chemicals or radiation [U1]. Furthermore, a number of studies involving the children of survivors of the Hiroshima and Nagasaki atomic bombings have failed to detect any transmitted genetic effects of radiation exposure [K14, N12, N13, S4, S5]. A cohort of 31,150 children born to parents who were within 2 kilometres of the hypocentre at the time of the bombing was compared with a control cohort of 41,066 children. During the children's early years, congenital defects, sexual development, physical development and survival were all investigated. Later, cytogenetic studies and the electrophoretic properties of a series of serum proteins or erythrocytic enzymes were analysed, in addition to a complete medical evaluation. None of these indicators was significantly modified by parental radiation exposure (reviewed in reference [N10]).

136. In addition, in a study that examined 50 families exposed after the Hiroshima and Nagasaki atomic bombings with 64 children, and 50 control families with 60 children [K14], minisatellite analyses revealed no genetic effects at six human tandem repeat. This study was later expanded to include analysis of mutations at eight hypervariable minisatellite loci in the offspring (61 from exposed families, in 60 of which only one parent was exposed, and 58 from unexposed parents) of atomic bombing survivors with mean doses of >1 Sv. They found 44 mutations in paternal alleles and 8 mutations in maternal alleles, with no indication that the high doses of acutely applied radiation had caused significant genetic effects [K28].

137. In contrast to these observations, Dubrova et al. [D11] described elevated mutation rates in DNA tandem repeat sequences in humans living in rural areas of the Mogilev district of Belarus, which was heavily contaminated with radionuclides from the Chernobyl reactor accident. The frequency of mutation was assayed both by DNA fingerprinting using one multilocus probe and by single-locus analysis using four probes, and this revealed mutation rates approximately twofold higher in the offspring of exposed parents when compared with an unexposed population from the United Kingdom. These initial observations were expanded to include analysis of families from rural areas in the Kiev and Zhytomir regions of Ukraine, which were heavily contaminated by radionuclides after the Chernobyl accident. A statistically significant 1.6-fold increase in mutation rate was found in the germ line of exposed fathers, whereas the maternal germ line mutation rate in the exposed families was not elevated [D9].

138. The initial report by Dubrova et al. [D11] generated commentary centred largely on the selection of a non-exposed population from the United Kingdom for comparison with exposed parents from Belarus [S4], the failure to exclude other contaminants such as pollutants and viral infections [N11], and questions regarding the biological significance of

increased mutations in hypervariable tandem repeat alleles [N10]. These criticisms initially appeared to detract from the significance of the findings of Dubrova et al. However, in a subsequent report, Dubrova et al. [D12] recruited more families from the affected region and used five additional minisatellite probes, and once again their data indicated a twofold higher mutation rate in exposed families than in non-irradiated families from the United Kingdom. They also used individual radiation doses for external and internal chronic exposure to ^{137}Cs as an indicator of long-term population exposure and found a significant positive correlation between radiation dose and mutation rate over multiple loci with the exposed families. Significantly, there were no obvious differences in the mutation spectrum observed between the exposed and the control families. In subsequent studies, children born to parents who participated in recovery operations after the Chernobyl accident also showed an elevated mutation rate at some loci [L18, W5], as did children born to parents living around the Semipalatinsk nuclear test site in Kazakhstan [D8].

139. It should be stressed that the analysis of tandem repeat loci mutation rate demands sophisticated molecular biology, and it is important that observed mutants be validated. Jeffreys and Dubrova [J3] suggested that technical artefacts might explain the sevenfold increase in mutation rate in children of Chernobyl recovery operations workers described by Weinberg et al. [W5]. Questions of valid paternity, sample mix-up, variation between DNA samples and the demands of the required technology are all potential sources of variability and could explain differences in results between different investigators.

140. Nevertheless, other studies have failed to reproduce these positive findings [F15, L17, S55]. A way of reconciling these apparently contradictory results was offered by Livshits et al. [L17]. They measured the frequency of inherited mutant alleles at seven hypermutable minisatellite loci in 183 children born to Chernobyl recovery operations workers and in 163 children born to control families living in non-irradiated areas of Ukraine. No significant difference in the frequency of inherited mutant alleles was found between the exposed and the control groups. The exposed group was then divided into two subgroups according to the time at which the children were conceived in relation to the fathers' work at the power plant. Eighty-eight children were conceived either while their fathers were employed at the facility or up to 2 months later (subgroup 1). The other 95 children were conceived at least 4 months after their fathers had stopped working at the Chernobyl site (subgroup 2). The frequencies of mutant alleles were higher for the majority of loci in subgroup 1 than in subgroup 2, suggesting that the timing of irradiation during spermatogenesis had affected its mutagenic potential.

141. Given the frequency of mutations in these hypervariable alleles and the lack of evidence for significant differences in the mutation spectrum between control and exposed families [D12], it is unlikely that the minisatellite loci themselves are the direct targets of the radiation. If the increased mutation rate is not caused by DNA damage directly, it might well result from non-targeted events associated with radiation-induced genomic instability [D13, D16, F2, N14, S1].

142. The reasons for the discrepancy between the data collected from the children of survivors of the Hiroshima and Nagasaki bombings and the induction of human germ line mutation in the majority of studies involving the offspring of parents living in radiation-contaminated environments are not easily reconciled. The types of radiation to which individuals were exposed differed between the two populations, and there are uncertainties associated with the doses. The bombings resulted in a single acute exposure to predominantly gamma radiation and a small amount of neutron radiation, whereas contamination from Chernobyl resulted in chronic exposures to internalized ^{131}I. However, this is unlikely to be the only reason for the discrepancy, because there are also negative results for the children of recovery operations workers exposed to low-dose-rate external/internal exposures [F15, S55].

143. The controversy surrounding the induction of mutations in tandem repeat sequences is far from resolved. May et al. [M5] examined the mutation frequency at hypervariable tandem repeats in sperm from three seminoma patients following hemipelvic radiotherapy. Scattered radiation doses to the testicles were monitored, and the mutation rates in pre-treatment sperm DNA were compared with sperm derived from irradiated meiotic and post-meiotic cells. No evidence for radiation-induced germ line mutation at these hypervariable loci was observed even though the patients were monitored for a period of 1 to 11 months.

144. Of all the reported studies, it is only those of the populations living in the Semipalatinsk region of Kazakhstan that permit comment on the potential transgenerational effects (i.e. F_2+ generations). In one such study, the F_2 offspring of those parents that received the highest external and internal doses did not show any elevation of minisatellite mutation frequency [D8].

145. These and related studies have been summarized in table 2 and have recently been subject to critical review [B41]. To summarize the conclusions of Bouffler and the expert review panel [B41]: only limited data are available indicating that germ line mutation of minisatellites can be detected in irradiated human populations. The data are inconsistent and show only limited evidence of dose dependence, and the panel found that additional work would be necessary to establish the radiation responsiveness of these loci. Furthermore, the data on mutation of human tandemly repeated DNA loci do not warrant a dramatic revision of germ line or cancer risk estimates for radiation at present [B41].

E. Cancer incidence in the offspring of irradiated humans

146. The risk of cancer in the offspring of humans irradiated prior to conception is also controversial. No excess

cancer incidence has been reported in children born to parents exposed to ionizing radiation by the atomic bombings in Japan [Y1, Y2] or in the offspring of cancer patients treated with radiotherapy [H4].

147. A major event early in this controversy was the conclusion by Gardner et al. [G2] from a case-control study that the increased incidence of leukaemia and non-Hodgkin's lymphoma among children living near the nuclear reprocessing plant in Sellafield in the United Kingdom was associated with paternal employment and the recorded external dose of whole-body radiation during work at the plant before conception. This conclusion was controversial [D6] and was not supported by the excess of childhood leukaemia observed at nearby Seascale [P4], or by an extensive study of radiation workers and childhood cancers [D7]. Nevertheless, Dickinson and Parker [D4] published results of a cohort study that supports the initial association with paternal radiation dose, which suggests that it still remains a possible explanation [B15], although population mixing [G17] is regarded as a potentially important factor in this particular cluster.

148. The United Kingdom Committee on Medical Aspects of Radiation in the Environment (COMARE), in their seventh report, reviewed the evidence concerning the incidence of childhood leukaemia and other cancers in the offspring of parents occupationally exposed to radiation prior to conception [C17]. The COMARE agreed that, while a link between parental exposure and such effects in the offspring was possible in principle, the epidemiological evidence from the offspring of radiation workers in the United Kingdom, the United States and Germany failed to support an increased rate of solid tumours in children. Furthermore, COMARE concluded that the balance of evidence indicated that the likelihood of developing childhood leukaemia or non-Hodgkin's lymphoma was not related to radiation dose.

F. Pregnancy outcomes in the offspring of irradiated humans

149. In their eighth report, COMARE reviewed pregnancy outcomes following pre-conception exposure to ionizing radiation in humans [C18]. They found that the available epidemiological data were inadequate to allow definitive statements about the effect of pre-conception radiation exposure on pregnancy outcomes. This was due to difficulties in obtaining reliable figures for the end points of concern and to the possibility that the radiation exposures in most studies may have been too low to produce a detectable effect. The conclusion reached by COMARE was that, from all the epidemiological data examined, there was little evidence that adverse reproductive outcomes in general are related to parental radiation exposure. Similarly, the limited data available did not link miscarriage or neonatal death with parental irradiation. COMARE did point out, however, that almost all of the published studies on pregnancy outcome following parental exposure to radiation in human populations lack statistical power; this is probably due to the low doses to which the populations were exposed and to the small population sample size.

150. The Scientific Committee takes note of these conclusions by COMARE, which support its own view that the types of genetic data discussed in this annex imply no modification of its own estimates of hereditary risk. It has been the Scientific Committee's position that the mutation rates in experimental organisms upon which it has based its estimates of hereditary risk are sufficiently low as to make it unlikely that analyses of the radiation-exposed human populations available for study would show statistically significant increases in hereditary diseases.

G. Genetic damage and malformation induction in the offspring of irradiated parents

151. The densely populated costal regions of Kerala state in southwest India have deposits of radioactive monazite-bearing sand and provide a unique opportunity to investigate the effects of high levels of natural radiation on human populations [N35]. The background radiation levels range from ≤1.0 mGy to >35.0 mGy per year owing to naturally occurring thorium and its decay products. There is a comprehensive programme to assess the biological and health effects of this radiation exposure in humans, focusing mainly on constitutional chromosome abnormalities and the incidence of congenital malformations in newborns. To date, the data do not reveal any effect on cytogenetic aberrations in lymphocytes [C24] or the incidence of congenital malformations in newborns [J11] that can be associated with exposure to ionizing radiation.

H. Impact of non-targeted and delayed effects of radiation on future generations

152. Heritable effects are observed in first-generation offspring and/or in later generations after one or both parents have been irradiated prior to conception. Since it was established, the Committee has made estimates of the genetic effects of radiation in humans in offspring of irradiated parents based upon clear demonstrations that mutations can be induced in experimental organisms, including experimental mammals. The UNSCEAR 2001 Report on the hereditary effects of radiation emphasized that no radiation-induced genetic (i.e. hereditary) diseases have so far been demonstrated in human populations exposed to ionizing radiation [U1]. No demonstrable adverse reproductive outcomes were described for the survivors of the atomic bombings in Japan, or for women irradiated during infancy for skin haemangiomas. No demonstrable hereditary effects of radiation exposure resulting from the Chernobyl accident have been described [U2]. Likewise, no increase in cytogenetic abnormalities [W19] or genetic effects [B39] has been reported in survivors of childhood cancer exposed to ionizing radiation before reproduction.

153. Ionizing radiation is considered a universal mutagen. Experimental studies in plants and animals have demonstrated that radiation can induce hereditary effects, and humans are unlikely to be an exception in this regard. It was for this reason that the Committee estimated hereditary risk in humans in the absence of direct evidence in humans. This annex presents a re-evaluation of some of the controversial data, in view of newer findings, and also a review of some types of damage not considered in earlier UNSCEAR reports. Two assumptions are commonly made in the estimation of genetic risk: (1) that the seven "specific loci" in the mouse constitute a suitable basis for extrapolation to genetic disease in humans; and (2) that heritable mutations are induced by radiation damage (energy-loss events leading to double-strand damage) occurring within the genome and are induced linearly with dose, at least at low doses. The issues of main importance in this section are whether the information on the types of mutation considered below might (1) be used to improve the Committee's estimates of hereditary risk, and (2) indicate some type of genetic instability that could lead to modification of risk through subsequent generations.

154. Overall it is clear that irradiation of the parent can lead to some changes in the offspring, but it is likely that most of these are due to the manifestation of direct damage caused by radiation in the original germ cell. The high incidence of offspring with "mutations" in ESTRs and the detection of mutations in maternal alleles after paternal irradiation suggest that non-targeted instability may be induced in specific circumstances. There is only very limited evidence that instability is transmitted across into the F_2 generation, and human data are negative. Therefore the Committee considers that these data are insufficient to justify modification of current risk estimates for hereditary effects or cancer in humans.

VII. IMPLICATIONS OF NON-TARGETED AND DELAYED EFFECTS

155. A wealth of information has been reviewed that deals with possible radiation-induced non-targeted and delayed genetic effects, as well as genetic end points that can occur spontaneously. Most of the estimates of hereditary risk made by the Committee in the past have been based on classical mutation experiments. Such studies often require exceedingly large samples of offspring. For the reasons described above, it does not appear that the new findings necessitate changes in the Committee's estimates of hereditary risk. It is possible that certain types of genetic damage detected by some of the assays have no relationship to clinically important phenotypes. It is also possible that the methods of hereditary risk estimation used by the Committee in the past adequately incorporate any genetic risks of clinical relevance that might be associated with the damage detected by the various assays considered. While it is clear that some of the assays permit demonstration of effects of radiation using much smaller sample sizes than more classical methods, uncertainty remains as to whether these effects correlate with the rare types of mutation that cause clinically serious conditions. There is no convincing evidence of transgenerational instability in humans caused by radiation that would lead to the propagation of clinically important effects over succeeding generations. Because the Committee's current risk estimates already assume transmission of many of the effects found in the first generation to later generations, rare instances of such propagation would have little impact on total risk estimates.

156. The relevance of non-targeted and delayed effects to the development of cancer and hereditary effects is not yet clear. Carcinogenesis involves a progression of genetic events that are associated with specific stages of the malignant process. It is tempting to speculate that induced genomic instability can drive the progression of genetic changes and thus provide the impetus for acquiring those genomic alterations associated with carcinogenesis. Yet this must be tempered by the high frequency with which instability is observed both in vitro and in vivo, and the observation that instability generally tends to saturate at low doses of radiation. Nevertheless, if radiation exposure induces a transgenerational instability that could be passed through the germ line and increase a child's susceptibility to cancer or genetic effects, this would have health ramifications. Bystander effects could also have significant implications for human exposures, particularly to very low fluences of high-LET radiation, e.g. to radon, where only a small fraction of the cell population would be hit, i.e. subject to energy deposition events. However, bystander effects appear to be limited to the irradiated organ, and since risk estimates are to an organ and not a cell, bystander effects are essentially encompassed in current radiation risk estimates for carcinogenesis.

157. Radiation-induced instability and the existence of bystander effects are well established and incontrovertible. A common observation of these responses is that they dominate at low doses and saturate with increasing dose (reviewed in references [P13, S18]). In addition to damage directly induced by the deposition of energy in the nucleus of the irradiated cell, consideration must now be given to these indirect effects of radiation. An irradiated cell can send out a signal and induce a response in a cell whose nucleus was not hit by radiation. This might result in genetic damage, genomic instability or lethality in non-irradiated cells. These non-targeted effects in essence "amplify" the biological effectiveness of a given radiation dose by increasing the number of cells that experience effects over those directly exposed to the radiation.

158. Understanding of these non-targeted effects is still in its infancy, and much of the data to date have been obtained from in vitro studies. While the significance of these indirect effects for human health remains to be elucidated, it would seem prudent to consider the implications of non-targeted delayed effects of radiation exposure when considering models of radiation carcinogenesis, particularly at low doses.

159. In vivo non-targeted effects are not new and have previously been implicated in radiation-induced carcinogenesis [S22]. The recent revival of interest in these non-targeted effects and the subsequent influx of new data suggest that it is time to re-examine the concepts of radiation dose and target size. Many of the indirect effects described indicate that the tissue volume in which detrimental effects of radiation may be observed is larger than the precise volume irradiated. This issue may have important implications for human health. Life exists in a radiation environment, the use of radiation has become an integral part of modern life, and the applications of radiation in medicine and industry bring tremendous benefits to society. Over time, biological systems have demonstrated a remarkable ability to adapt to environments to which they are gradually exposed, and low doses of radiation are no exception. Higher doses of radiation can cause neoplasia. This presumably occurs through a combination of direct damage and non-targeted effects, and models of radiation-induced carcinogenesis should incorporate both direct and indirect effects when evaluating radiation risks. Ultimately, understanding the multitude of multicellular responses to radiation may provide a framework for evaluating health risks associated with radiation exposure and a logical means of intervening in the development of suspected radiation-induced cancers.

CONCLUDING REMARKS

160. This annex has reviewed a multitude of studies on non-targeted and delayed effects of exposure to ionizing radiation. In addition to damage directly induced by the deposition of energy in the nucleus of an irradiated cell, consideration should now be given to these indirect effects of radiation. An irradiated cell can send out signals and induce a response in a cell whose nucleus was not subject to energy deposition events following irradiation. These non-targeted effects in essence "amplify" the biological effectiveness of a given radiation dose by increasing the number of cells that experience effects over those directly exposed to the radiation.

161. In spite of the large body of new information available, considerable disagreement remains concerning any definitive relationship between these non-targeted effects and the observed health effects attributable to radiation.

162. The Committee stresses that direct epidemiological observations and associated quantification of the health effects of radiation incorporate all mechanistic elements, including the targeted (direct) effects of irradiation as well as the non-targeted and delayed effects described in this report.

163. A specific role for non-targeted effects in the observed health effects associated with radiation exposure cannot be determined directly. Such effects can provide mechanistic information at doses of below ~200 mGy that could be pertinent to evaluating health effects at these low doses. However, in ascribing a mechanism to a particular biological effect, the data in question should be independently replicated and show a strong coherence with the particular end point considered. The UNSCEAR 2000 Report considered the conventional view that the deposition of energy in the nucleus and the subsequent cellular processing of induced DNA damage were consistent with the observed cancer/heritable effects induced by ionizing radiation.

164. In light of these considerations, the overall view of the Committee is that the data currently available do not require changes in radiation risk coefficients for cancer and hereditary effects of radiation in humans. The Committee will maintain surveillance of developments in the area of non-targeted and delayed effects, and recommends that future research pay particular attention to study design emphasizing replication, low-dose responses and associations with health effects particularly in the human population. Ultimately, understanding the range and multitude of multicellular responses to radiation will provide mechanistic insights into how radiation induces its observed health effects.

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ANNEX D

Effects of ionizing radiation on the immune system

Contents

INTRODUCTION

1. DNA lesions and defective DNA repair are usually considered to be the key mechanisms that explain the biological effects of ionizing radiation. Recently, radiation-induced non-targeted effects, e.g. genomic instability or the bystander effect, have been described; they may represent other critical mechanisms in the initiation and development of late radiation-induced effects at the cellular or tissue level. Acute effects of whole-body irradiation may manifest as multiple organ failures (involving essentially the haematopoietic, digestive and central nervous systems as well as the skin). In this context, alterations of the immune system by ionizing radiation represent a field that has not recently been thoroughly assessed.

2. The effects of ionizing radiation upon immune system function were first reviewed in detail by the Committee in 1972 [U10]. Aspects of the subject were discussed in further UNSCEAR reports, as summarized in table 1 [U2, U4, U6, U7, U8, U9]. A large number of new papers and technological developments since then have fostered progress in understanding the basic concepts concerning mechanisms underlying the effects of ionizing radiation on the immune system. In addition, there exists today a considerable amount of new data related to impaired immunological functions and the risk of diseases or mortality from causes other than cancer.

3. The scope of this annex includes reviews of:

- Radiation-induced alterations of the immune response, including immunosuppression (depression) or immunostimulation (activation);
- Possible mechanisms by which the immune system is altered following exposure to ionizing radiation;
- Epidemiological assessments of immune system alterations in various diseases, with emphasis on the effects of ionizing radiation.

Table 1 Aspects covered by UNSCEAR documents concerning the effects of ionizing radiation upon immune system function

Content	*Reference*
Volume II, annex F ("Effects of radiation on the immune system"): the general components of the immune response, effects of radiation on susceptibility to infection, effects of radiation on antibody formation, effects of radiation on cellular immune reactions, radiation and immunological tolerance, immunological aspects of radiation-induced carcinogenesis, effects of variation of condition of irradiation on immunological responses	[U10]
Radiocarcinogenesis and the immune system. Effects of prenatal irradiation on the haematopoietic and immune systems	[U9]
Effects of ionizing radiation on the haematopoietic and immune systems	[U8]
Radiation-induced cancer: impairment of immunological surveillance	[U7]
Haematological and immunological effects of ionizing radiation	[U6]
Annex B, part III: Effects of ionizing radiation on the immune system in the context of adaptive response	[U4]
Radiosensitivity and defective recombination in the immune system; immunological effects of exposure to radiation from the Chernobyl accident	[U2]

I. GENERAL FEATURES OF THE IMMUNE SYSTEM

A. Introduction

4. The immune system consists of cells and tissues spread widely throughout the body that protect against infections and cancer. Following the recognition of foreign or novel antigens, which are present in an immense variety of organisms or neoplastic cells, this system executes a complex response in order to give protection. Organs or tissues transplanted from other individuals are also recognized by the immune system as foreign agents. Immune recognition of these antigens generally leads to their elimination through the destruction and removal of the foreign cells.

5. The immune response is mediated by a number of different cells and molecules. The majority of these cells are white blood cells (leucocytes) produced in the bone marrow as precursors. Some develop into mature cells within the bone marrow, and others are transported by the blood to other tissues where they develop and mature further. Many molecules of the immune system are soluble or cell surface proteins. The immunocompetent cells are strategically located in areas that come into close contact with foreign substances. In these locations, they are perfectly positioned to recognize those substances as "non-self" or foreign. Upon such recognition, immune cells are activated and function to neutralize or destroy the invading foreign substance.

6. Immunophenotyping with combinations of antibodies to various cell surface and cytoplasmic proteins allows the identification of specific cell types, determination of the degree of cell differentiation and recognition of abnormal cells. Most of these antibodies are against surface glycoproteins, which are often not only associated with particular cell lineages but also vary in expression with maturation, and are thus referred to as differentiation antigens. These antigens have been grouped together in clusters of differentiation (CD), numbered in the order in which they were identified. Therefore, by incubating cell suspensions with monoclonal antibodies that bind selectively to these cell membrane components, it is possible to identify the phenotype of different subsets of immune cells and determine their relative and absolute abundance (see table 2).

Table 2 List of CD antigens cited in the text
Adapted from reference [J1]

CD	*Cell population*[a]	*Function*[b]	*Molecular family*
CD1 a, b, c, d, e	Dendritic cells, Langerhans cells, B-cells, thymocytes	Antigen presentation (glycoplipids)	Immunoglobulin
CD2	Thymocytes, NK cells, T-cells	Adhesion molecules; binding to CD58 and Lck activating T-cells	Immunoglobulin
CD3	T-cells, thymocytes	TCR-associated	Immunoglobulin
CD4	Subpopulation of thymocytes, Th1 cells, Th2 cells, monocytes, macrophages	Co-receptor of TCR for class II MHC; receptor of HIV-1/2 gp120; fixation of Lck (src-family tyrosine kinase) to the inner surface of the cell membrane	Immunoglobulin
CD5	Thymocytes, mature T-cells and B-cells	Positive or negative modulation of TCR signalling	Receptor
CD8	Subpopulation of thymocytes, cytotoxic cells	Co-receptor of TCR for class I MHC; binding to Lck on the inner surface of the cell membrane	Immunoglobulin
CD11b	NK cells and myeloid cells	Binding with CD54, iC3b components and αM subunit of integrins	α-integrins
CD14	Monocytes, macrophages, weakly expressed in neutrophils and myeloid dendritic cells	Receptor for the complex of LPS and LPB	Receptor
CD16	Neutrophils, NK cells, macrophages	Low-affinity FcR component (FcRγIII); mediator of phagocytosis and cytotoxicity	α-integrins
CD19	B-cells	Formation of complexes with CD21 and CD81; B-cell co-receptor	α-integrins

CD	*Cell population*[a]	*Function*[b]	*Molecular family*
CD20	B-cells	Ca^{2+} channel formation; possible role in B-cell activation	Possesses four transmembrane segments
CD23	Mature B-cells, activated macrophages, eosinophils, megakaryocytes, dendritic cells	Receptor with low affinity for IgE	Lectin-C-like
CD25	Activated T-cells, B-cells, monocytes	Subunit α of the human interleukin-2 receptor	α-chain type I glycoprotein containing two complement control protein domains
CD27	Medullar thymocytes, T-cells, NK cells and certain B-cells	Co-stimulator of T- and B-cells	TNF receptor
CD28	T-cell subpopulations, activated B-cells	Naive T-cell activation; co-stimulatory pathway involving CD80 and CD86	Immunoglobulin and CD86
CD38	Activated T- and B-cells	Multifunctional enzyme (NAD glycohydrolase, ADP-ribosyl cyclase, cyclic ADP ribose hydrolase); regulation of T- and B-cell activation; also functions in cell adhesion, signal transduction and calcium signalling	Type II glycoprotein
CD40	B-cells, macrophages, dendritic cells, basal cells, epithelial cells	Co-stimulator of B-cells; cytokine production by macrophages and dendritic cells	TNF receptor
CD43	Leucocytes (except resting B-cells)	Anti-adhesive	Mucine
CD44	Leucocytes, erythrocytes	Link with hyaluronic acid; adhesion receptor	Adhesion molecule
CD45	All haematopoietic cells	Tyrosine phosphatase	Fibronectin type III
CD45RO	T- and B-cell subpopulations, monocytes, macrophages	Isoforms of CD45 without A, B and C exons	Fibronectin type II
CD45RA	Naive T-cells, monocytes	Isoforms of CD45 with the A exon	Fibronectin type II
CD48	All leucocytes except neutrophils	Involved in lymphocyte activation through the interaction with its receptor (CD2)	Adhesion molecule
CD56	NK cells	Isoform of NCAM molecules	Immunoglobulin
CD69	Activated B- and T-cells, activated macrophages and NK cells	Antigen of early activation	Lectin C
CD80 (B7.1)	B-cell subpopulation	Co-stimulator; ligand for CD28 and CTLA-4	Immunoglobulin
CD86 (B7.2)	Monocytes, activated B-cells, dendritic cells	Ligand for CD28 and CTLA-4	Immunoglobulin
CD95	Broadly expressed in different cell lines	Binding with FasL (TNF-like), apoptosis induction	TNF receptor
CD117 (c-Kit)	Haematopoietic progenitor cells	Stem cell factor receptor	Immunoglobulin, tyrosine kinase
CD119	B-cells, monocytes, macrophages, endothelium	Interferon-γ receptor	Fibronectin type III
CD120	Broadly expressed	TNF receptor	TNF receptor
CD121	T-cells, thymocytes, B-cells, macrophages, monocytes	IL-1α and IL-1β receptor	Immunoglobulin
CD124	T-cells, B-cells, haematopoietic precursors	IL-4 receptor	Cytokine receptor, fibronectin type III
CD125	Activated B-cells, eosinophils, basophils	IL-5 receptor	Cytokine receptor, fibronectin type III
CD127	Monocytes, pro-B-cells, T-cells, lymphoid precursors	IL-7 receptor	Fibronectin type III
CD130	Broadly expressed	Common subunit shared with IL-6, IL-11 and leukaemia inhibitor factor	Immunoglobulin, cytokine receptor, fibronectin type III
CD132	B-cells, T-cells, NK cells, mastocytes, neutrophils	γ-chain of the IL-2 receptor; common subunit shared with IL-4, IL-7, IL-9 and IL-15	Cytokine receptor

a NK = natural killer.

b TCR = T-cell receptor; MHC = major histocompatibility complex; HIV = human immunodeficiency virus; LPS = lipopolysaccharide; LPB = LPS-binding protein; TNF = tumour necrosis factor.

B. Organs and tissues of the immune system

7. Like red blood cells (erythrocytes) and platelets (thrombocytes), the cells of the immune system arise in the bone marrow from pluripotent stem cells by a process called haematopoiesis. Myeloid precursors develop into a group of white blood cells known as phagocytes. Phagocytes include monocytes, macrophages and neutrophils. Other myeloid descendants become granule-containing inflammatory cells such as eosinophils, basophils and mast cells. Lymphoid precursors develop into the small white blood cells called lymphocytes. The two major classes of lymphocytes are B-cells and T-cells. Cells and molecules of the immune system are discussed in detail in section I.C.

8. The bone marrow and thymus are termed the primary (or central) lymphoid tissues, because mature lymphocytes are produced within these organs. Mature lymphocytes then enter the blood and travel to the secondary (or peripheral) lymphoid tissues such as lymph nodes, spleen and mucosa-associated lymphoid tissue. A notable feature of lymphocytes is that they can cross from the blood into the lymphatic circulation and then return, a phenomenon referred to as lymphocyte traffic or recirculation. The direct migration of lymphocytes to specific tissues is called homing.

1. Central lymphoid organs

9. All cells of the immune system originate from just one cell type, called a haematopoietic stem cell, in the bone marrow of adults (for prenatal life, see section II.F below). Pluripotent stem cells multiply to produce more pluripotent stem cells (self-renewal), thus ensuring the steady and lasting supply of stem cells. Homeostatic regulation stabilizes the number of pluripotent stem cells. Some of the pluripotent stem cells differentiate to become committed to one of two blood cell lineages: lymphoid or myeloid. Then lymphoid and myeloid stem cells become progenitor cells for each type of mature blood cell. These cells have lost the capacity for self-renewal and are committed to a given cell lineage: T- and B-cell progenitors, and progenitor cells for granulocytes, monocytes, eosinophils, basophils, mast cells, platelets and erythrocytes. Dendritic cells belong to the myeloid cell lineage, and it is likely that monocytes and dendritic cells arose from a common myeloid precursor. Progenitor commitment depends upon the acquisition of responsiveness to different growth factors such as colony-stimulating factors (CSFs), erythropoietin (EPO) and interleukins (ILs). The particular microenvironment within which the progenitor cell resides controls differentiation. The haematopoietic cells grow and mature on a meshwork of stromal cells, which are non-haematopoietic cells that support the growth and differentiation of the haematopoietic cells (figure I). In the absence of infection, bone marrow stromal cells are the major source of haematopoietic cytokines. In the presence of infection, cytokines produced by certain activated immunocompetent cells induce additional haematopoietic activity, resulting in the rapid expansion of the white blood cells that participate in fighting infection. After a period of maturation and expansion within the bone marrow, more differentiated cells enter the bloodstream and are distributed throughout the body in different tissues. B-lymphocytes develop and mature in the bone marrow before entering the bloodstream, in contrast to T-lymphocytes, which leave the bone marrow as T-cell precursors and complete further maturation in the thymus [A25].

10. The thymus is an organ located anterior in the upper mediastinum. Immature T-lymphocytes migrate from the bone marrow into the thymus, where they become mature immunocompetent T-cells. A minority of lymphocytes become mature T-lymphocytes by extrathymic maturation processes. After thymic involution (see below), this pathway of extrathymic lymphocyte maturation becomes more relevant [B32]. Thymus cells are called thymocytes and are predominantly immature T-cells at various stages of development. There are also scattered epithelial cells, macrophages and dendritic cells [A25]. The thymus is very large in the first years of life, reaches maximum size at puberty and then becomes smaller in a process called involution. During this degenerative process, connective tissue, fibres and fat cells replace the previously functional tissue. Although only a few pieces of functional tissue remain, they suffice to continue to supply the organism with enough mature lymphocytes. Cervical lymphoid organs with thymic structure and function have recently been found in BALB/c and C57BL/6 mouse strains. Although cervical thymus tissue has been observed in other species, the presence of cervical thymus tissue in humans is considered rare after birth [T18].

11. The main functions of the thymus include:

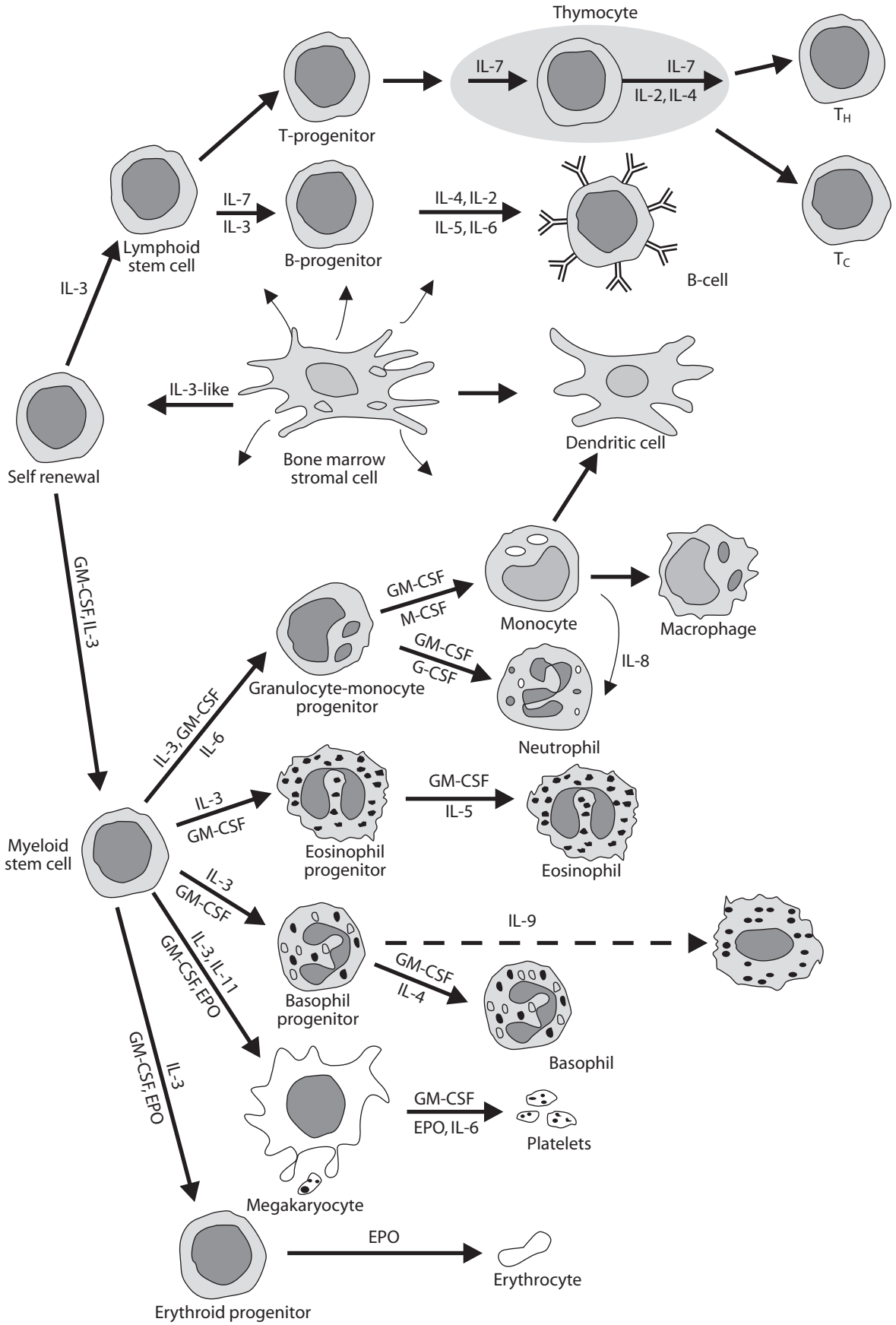
- Production of immunocompetent T-lymphocytes;
- Production of mature T-cells for peripheral tissues and circulation;
- Regulation of T-cell maturation, proliferation and function;
- Immunological self tolerance via positive and negative selection.

2. Peripheral lymphoid tissues

12. After maturing in the thymus, T-cells move through the circulation to other organs. Lymph nodes are small, bean-shaped structures that are spread throughout the body along the lymphatic routes. They contain specialized compartments where immune cells congregate and where they can encounter antigens. Lymph nodes constitute groups in areas where lymphatic vessels come together to form larger vessels, such as in the groin, neck and axilla. All lymphatic vessels draining back to the venous circulation from the tissues pass through lymph nodes, which filter and purify the drain fluid of the lymphatic vessels (lymph) before it flows

Figure I. Regulation of haematopoiesis in the bone marrow by cytokines that stimulate the proliferation and/or differentiation of various haematopoietic cells (adapted from reference [G14]).

GM-CSF: Granulocyte–macrophage colony-stimulating factor.



into the venous system [J1]. Several immune functions take place in the lymph nodes:

- Phagocytosis of microorganisms by fixed macrophages;
- T-lymphocyte activation;
- B-lymphocyte activation and proliferation (plasma cell formation and antibody production);
- Interaction between antigen-presenting cells and circulating lymphocytes.

13. The spleen is a soft organ located in the left hypochondrium that removes abnormal blood cells through phagocytosis, collects and disposes of senescent red blood cells, provides storage of iron from recycled red blood cells and plays a role in foetal and sometimes adult haematopoiesis. Spleen cells are called splenocytes. It is in the spleen that the initiation of immune responses by B-cells and T-cells takes place in response to antigens circulating in the blood. The spleen has a "red pulp" (its colour due to the presence of large numbers of erythrocytes in the blood vessels) that is characterized by a parenchyma that consists of macrophages and blood cells surrounded by numerous venous sinuses. The "white pulp" of the spleen is characterized by the lack of these sinuses and consequently the presence of fewer erythrocytes, and consists of B- and T-lymphocytes located in two different areas of the spleen. B-cells are located in the lymphoid follicles scattered throughout the organ. T-cells are located around the central arteries and form a kind of sheath (periarteriolar lymphoid sheath) [G27].

14. Mucosa-associated lymphoid tissue (MALT) consists of connective tissue containing lymphocytes and is located beneath mucous membranes of the respiratory, gastrointestinal, urinary and reproductive tracts. MALT has no distinct capsule like that of the lymph nodes but does often have a germinal centre containing actively dividing lymphocytes. Waldeyer's throat ring (comprising the adenoids and the palatine, lingual and pharyngeal tonsils), Peyer's patches of the small intestine, and the appendix are all examples of mucosa-associated lymphoid tissue [J1].

15. The skin is the largest organ in the human body. It protects against injury, infection, heat and cold, and it stores water, fat and vitamins [C30]. Although classically described as an anatomic barrier of the innate immune system (see section I.C.1 below), the skin may also be considered as an organ of the immune system. The ability of the cutaneous barrier to help defend the body against pathogens depends on both acquired and innate immune responses [B17, B27].

16. The "skin immune system" (SIS) has been proposed as the term for the complex of immune-response-associated cells present in normal human skin. Approximately half of human skin cells are directly or indirectly involved in the immune response (e.g. keratinocytes, endothelial cells, dendritic cells, T-lymphocytes, monocytes/macrophages, granulocytes and mast cells). Some of these cells are skin residents, others can be recruited or are recirculating cells. This concept of the SIS also includes human skin humoral constituents such as immunoglobulins, cytokines, chemokines, complement, cathelicidins, defensins, dermcidins, prostaglandins and free radicals [B28, R13].

3. Remarks concerning organs and tissues of the immune system

17. On the basis of the preceding discussion, some concepts may be highlighted. The bone marrow and the thymus are the central lymphoid tissues. All cells of the immune system originate in the bone marrow from haematopoietic stem cells. After maturation and expansion, more differentiated immune cells enter the bloodstream to be distributed in the different tissues. Some immature lymphocytes move from the bone marrow to the thymus, where they become immunocompetent T-cells. Spread throughout the body, lymph nodes are peripheral lymphoid tissues where immune cells congregate and where these cells can encounter antigens. It is in the spleen, another peripheral lymphoid organ, that the initiation of the B- and T-cell immune responses to circulating antigens takes place. MALT consists of connective tissue containing lymphocytes. The skin and mucous membranes are the first line of defence against pathogens. Classically they have been described as anatomic barriers of the innate immune system alone; however, around half of human skin cells are involved in both innate and acquired immunity.

C. Cells and molecules of the immune system

18. The cells and molecules of the immune system execute two different but related forms of immunity: innate and acquired. While innate immunity is fully functional before any foreign agent enters the body, acquired immunity is activated only after a pathogen has entered the organism. Acquired immunity mediated by B- and T-lymphocytes recognizes pathogens by rearranged high-affinity receptors. However, as acquired immunity involves activation and gene expression as well as cell proliferation, it is often not rapid enough to eradicate microorganisms. Innate immunity provides more rapid defence mechanisms.

1. Innate immunity

19. The innate immune system comprises several defensive barriers:

- Anatomic barrier: skin and mucous membranes;
- Physiological barrier: temperature, pH, lysozymes and circulating factors such as interferon and complement;
- Inflammatory barrier: cellular and chemical mediators of the inflammatory response (histamine, acute phase proteins, fibrin, kinin);
- Phagocytic barrier (see below).

20. Phagocytes include granulocytes, peripheral monocytes, tissue macrophages and dendritic cells. Phagocytes are cells capable of surrounding, engulfing and digesting complete microorganisms by phagocytosis. Some of them play an important role in producing molecules involved in the inflammatory response associated with infections. They migrate towards the site of infection by margination, diapedesis and chemotaxis.

21. Granulocytes include three types of cell: neutrophils, eosinophils and basophils. Neutrophils play an essential role in the body's innate immune defence and are one of the primary mediators of the inflammatory response. They are highly specialized for their primary function, which is the phagocytosis and destruction of microorganisms. To defend the host, neutrophils employ a wide range of microbicidal products, such as oxidants, microbicidal peptides and lytic enzymes. The generation of microbicidal oxidants by neutrophils results in a respiratory burst with generation of highly reactive oxygen and nitrogen species (ROS/RNS) [G13, Q1]. Eosinophils attack parasites and phagocytose antigen–antibody complexes. Basophils secrete anticoagulant and vasodilatory substances, such as histamines and serotonin. Even though they possess phagocytic capability, their main function is the secretion of substances that mediate hypersensitivity reactions.

22. Monocytes in peripheral blood are young cells that already possess phagocytic capabilities. After migration into tissues, monocytes undergo further differentiation to become multifunctional tissue macrophages. Macrophages and dendritic cells present antigens to be recognized by lymphocytes, and are thus called antigen-presenting cells (APCs). (Although less efficient, B-lymphocytes are also APCs, as will be discussed later.) Macrophages play a central role in the immune response. Among other functions, they seek out, ingest and destroy bacteria, viruses, tumour cells and other foreign material. They present foreign material to the cells of the immune system and in this way regulate the immune response. The understanding of the role of macrophages has changed since the identification of a particular family of receptors called pattern recognition receptors (PRRs). These PRRs recognize highly conserved antigenic structures, termed pathogen-associated molecular patterns (PAMPs), shared by large groups of pathogens. PRRs are secreted (complement, lectins) or expressed at the surface of cells (Toll-like receptors (TLRs)) [B23].

23. The TLR family constitutes an important component of the innate immune system. Although most commonly considered to be expressed on immune cells, e.g. phagocytes and T-regulatory cells, TLRs are also known to be functionally expressed on a variety of other cell types, such as airway and gut epithelial cells [A3, A16, C3, G31, J2, K40]. Ten members of the TLR family have been identified in humans; each recognizes a small range of conserved pathogen molecules (table 3). The binding of PAMPs to TLRs induces the production of pro-inflammatory cytokines and the up-regulation of surface co-stimulatory molecules. Recent studies have identified intracellular signalling pathways specific for individual TLRs that lead to the release of cytokine profiles specific for particular PAMPs. The ability of individual TLRs to discriminate among different PAMPs is an important determinant of the unique gene expression profiles activated in the host by different invading pathogens or environmental "danger signals" [V6]. Thus TLRs confer a certain degree of specificity to the innate immune response. Moreover, TRL-mediated recognition represents a cross-talk between the innate and the acquired immune system [A2, N8, R4].

Table 3 Ligands recognized by Toll-like receptors

TLR	*PAMP*	*Ligand*
TLR2	Gram (+) bacteria Gram (+) bacteria Bacteria Spirochaetes Leptospiras and porphyromonas Mycobacteria Yeast Trypanosoma Schistosoma Neisseria Klebsiella Host	Peptidoglycan (PGN) Lipoteichoic acid (LTA) Lipoproteins Glycolipids Lipopolysaccharide (LPS) Lipoarabinomannan Zymosan Glycosylphosphatidylinositol (GPI) Phosphatidylserine (PS) Porin Membrane protein A Heat shock proteins (HSPs)
TLR2/TLR1	Chemicals Bacteria Neisseria meningitides	JBT-3002 Lipoproteins Soluble factor
TLR2/TLR6	Staphylococcus Streptococcus (Group B) Mycoplasma	Modulin Soluble factor Lipoprotein
TLR3	Chemicals Virus	Poly (I:C) Double-stranded RNA

TLR	*PAMP*	*Ligand*
TLR4	Gram (–) bacteria Chlamydia Murine retroviruses RS virus Plant Host	LPS, LTA HSP60 Envelope proteins F protein Taxol HSPs
TLR5	Bacterial flagellum	Flagellin
TLR7	Chemicals	Imidazoquinolines
TLR9	Bacteria, virus, insects Host	Unmethylated DNA rich in CpG Chromatin-IgG complexes
TLR10	Unknown	Specific ligands and functions are currently unknown

24. Natural killer (NK) cells are large granular lymphocytes able to kill a number of different target cells and that, in contrast to T- or B-lymphocytes, do not express clonally distributed receptors for antigens [M10]. They belong to the innate arm of the immune response because their cytotoxic activity is spontaneous, although activation may be mediated by cytokines. NK cells do not show secondary or memory responses. NK cells may recognize, bind and kill virus-infected host cells and tumour cells [H18, T12].

25. The heterogeneity of NK cells presents a major problem in their identification. They do not express CD3, but exhibit subsets expressing CD16 and CD56. Most human NK cells are mainly involved in cytotoxicity, have low-density expression of CD56 and have high levels of CD16 ($CD3^{-}$ $CD56^{low}$ $CD16^{high}$), but around 10% of NK cells play a role in cytokine-mediated immunoregulation and are $CD3^{-}$ $CD56^{high}$ $CD16^{-/low}$ [C14, S19]. It was recently reported that peripheral $CD56^{high}$ NK cells are terminally differentiated cells indistinguishable from mature $CD56^{low}$ NK cells activated by IL-12, and do not constitute a functionally distinct NK cell subset [L23].

26. The different NK cell subsets have receptors on their surface that are not antigen specific. Depending on their function, NK cell receptors can be divided into activation and inhibition receptors; NK cells are regulated by the integration of these opposing signals [L3, M10]. Normally NK cells are prevented from lysing "self" cells by the interactions of inhibitory receptors on the NK cell surface with the major histocompatibility complex (MHC). The MHC in humans is called the human leucocyte antigen (HLA), which will be discussed in more detail in section I.C.3 below. Upon self class I HLA binding, inhibitor NK receptors trigger a signalling pathway resulting in inhibition of cytotoxicity, thus providing protection for normal cells. Cells that have lost class I HLA molecules, or express insufficient amounts of them, are frequently found in viral infections or tumour transformation, are not able to trigger these inhibitory signals and thus are lysed by NK cells. Although a combination of several activating receptors may also boost lysis by NK cells, the higher affinity of the inhibitory receptors by self class I HLA prevents autoimmunity [B7, C5, L2, M11]. NK receptors may be classed within several families: killer cell immunoglobulin-like receptors (KIRs), lectin-like receptors (NKG2 and CD95), leucocyte Ig-like transcripts (ILTs), natural cytotoxicity receptors (NCRs) and Fcγ receptor III (CD16). These are described in table 4.

Table 4 Natural killer cell receptors

Family[a]	*Receptor*	*Function*[b]	*Ligand*	*Comments*
KIR	KIR2DL (KIR2DL4)	(–)	HLA-C (HLA-G)	2D: two extracellular Ig-like domains; L: long cytoplasmic tail
	KIR3DL	(–)	HLA-B HLA-A	3D: three extracellular Ig-like domains; L: long cytoplasmic tail
	KIR2DS	(+)	HLA-C	2D: two extracellular Ig-like domains; S: short cytoplasmic tail
	KIR3DS	(+)	HLA-B	3D: three extracellular Ig-like domains; S: short cytoplasmic tail

Family[a]	*Receptor*	*Function*[b]	*Ligand*	*Comments*
Lectin C	CD94/NKG2A (heterodimer)	(–)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	CD94 functions as a chaperone (transports NKG2 to cell surface)
	CD94/NKG2C (heterodimer)	(+)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	
	CD94/NKG2E (heterodimer)	(+)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	
	NKG2D/NKG2D (homodimer)	(+)	MICA MICB ULBP	MICA/MICB: up-regulated on stressed cells and overexpressed in many tumours; ULBP: UL16-binding proteins from human cytomegalovirus
ILT	ILT2	(–)	HLA-A HLA-B HLA-F HLA-G	Also termed leucocyte Ig-like receptors or CD85j
NCR	NKp46	(+)	Viral and tumour proteins	Expressed in resting and activated NK cells
	NKp30	(+)	Viral and tumour proteins	
	NKp44	(+)	Viral and tumour proteins	Expressed only in activated NK cells
Fcγ receptor III	CD16	(+)	Low-affinity receptor for IgG	Facilitates antibody-dependent cellular cytotoxicity

[a] KIR = killer cell Ig-like receptor (Ig superfamily); ILT = Ig-like transcript; NCR = natural cytotoxicity receptor.

[b] Functions: (–) = inhibitory; (+) = activating.

27. A particular type of lymphocyte called an NKT cell exhibits certain characteristics of both T-cells and NK cells. NKT cells, by definition, are T-lymphocytes, as they express a T-cell receptor (TCR) on the surface of their membranes. This distinguishes them from NK cells, although NKT cells do share some markers characteristic of NK cells. In contrast to conventional T-lymphocytes, the NKT cell TCR does not interact with peptide antigen presented by classical class I or II HLA molecules but instead recognizes glycolipids presented by CD1d, a non-classical antigen-presenting molecule. NKT cells also express a far more limited range of TCR variable (V) region genes. Unlike NK cells, NKT cells develop in the thymus and are either CD4+ or CD4–. When activated, NKT cells secrete large amounts of cytokines, such as interferon-γ (IFN-γ) and interleukin-4 (IL-4). The activation of NKT cells can lead, paradoxically, either to suppression or to stimulation of immune responses, depending on the type of signal received. The cells play a critical and probably unique role in the immune system by linking innate and acquired immunity. NKT cells have been implicated in the regulation of immune responses associated with a broad range of diseases, including autoimmunity, infection and cancer [S9].

28. Mast cells (or mastocytes) are considered part of the immune system, where they play a role in innate immunity. Although best known for their role in allergy and anaphylaxis, mast cells are also involved in wound healing and in defence against pathogens. Mast cells circulate in an immature form, only maturing once in a tissue site. They are present in most tissues in the vicinity of blood vessels, and are especially prominent near the boundaries between the outside world and the internal milieu (skin and mucosa). When activated, a mast cell rapidly releases its characteristic granules and various molecules into the intercellular environment: histamine, heparin, serine proteases, prostaglandin, leukotriene and cytokines.

29. The complement system refers to a series of serum proteins produced by different tissues and cells, including hepatocytes, macrophages and gut epithelial cells. These proteins circulate in an inactive form, but in response to the recognition of molecular components of a microorganism, they become sequentially activated in a cascade where the binding of one protein promotes the binding of the next one. Three pathways may activate the complement system: the classical complement pathway, the lectin pathway and the alternative complement pathway. These pathways differ in the manner in which they are activated. Antibody–antigen complexes activate the classical pathway. The lectin pathway is mediated by the mannan-binding protein (MBP), a protein that binds to the mannose groups found in many microbial carbohydrates but not usually found in human carbohydrates. The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defence against a number of infectious agents. Activation of the complement system results in the production of several

biologically active molecules (e.g. kinins, chemotactic factors and opsonins), which may lead to lysis, opsonization, inflammation or clearance of immune complexes.

2. Acquired immunity

30. The main features of acquired immunity are the following:

- Memory: recovery from one infection frequently protects against subsequent infection by the same organism (the individual is said to have become "immune");
- Specificity: recovery from infection by one pathogen does not usually give protection against another;
- Diversity: the immune system can respond to an immense variety of foreign antigens;
- Self and non-self discrimination: an individual does not normally make an immune response against the antigens usually present in the body and distinguishes such antigens from those that do not belong to that individual (immune tolerance).

31. Lymphocytes are the predominant cells involved in acquired immunity, which includes humoral and cell-mediated responses. Soluble antibodies present in the serum mediate humoral responses, while cell-mediated responses result from the interaction between different types of cell. This distinction correlates, respectively, with the existence of two types of lymphocyte: B-cells and T-cells.

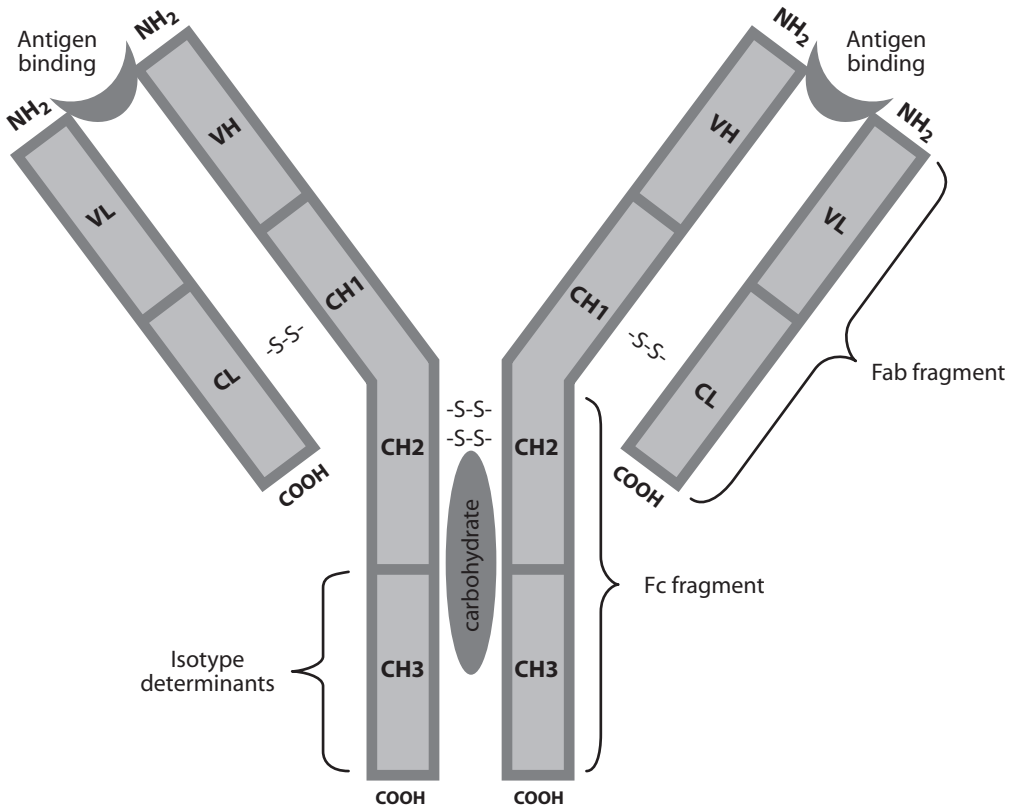
32. Cells involved in immune recognition have surface molecules called receptors that allow them to interact with other cell-attached or soluble molecules in their environment. Each receptor has a conformation complementary to the specific molecule (ligand) to be recognized. The ligand becomes bound to the receptor when recognition occurs. The term "antigen" includes any molecule that can be recognized by an antibody or by a lymphocyte receptor. However, lymphocyte receptors recognize only a small part of a whole molecule or ligand. The different parts of an antigen that can be recognized by lymphocyte receptors are called antigenic determinants or epitopes. The diversity and specificity of immune response imply that an enormous number of different specific receptors should exist. The diversity of the receptor repertoire is acquired during the maturation process of lymphocytes, which involves a large number of gene rearrangements and results, among the clones existing in the final mature population, in more than 10^8 types of receptor for different specific epitopes [J1].

33. B-lymphocytes are the effector cells of the humoral response. They originate and mature in the bone marrow, then they move through the circulation to various sites throughout the body. Upon interaction with a foreign antigen, B-lymphocytes become mature antibody-secreting cells called plasma cells. Plasma cells are rarely found in the circulation but reside mostly in connective tissue, beneath epithelia, in the medullary cords of lymph nodes and in the white pulp of the spleen. Most plasma cells in the spleen and lymph nodes migrate into bone marrow.

34. B-cell receptors (BCRs) are able to recognize "free" soluble antigens in unmodified form, referred to as native antigens. B-cells can secrete antibodies in a soluble form when they are activated and develop into plasma cells. The BCRs found on mature B-cells consist of a membrane immunoglobulin (Ig) acting as an antigen-binding subunit, associated with a signalling subunit, which is a disulphide-linked Ig-α/Ig-β heterodimer [M31]. Immunoglobulins are usually Y-shaped and consist of two light chains (λ or κ) and two heavy chains (α, δ, γ, ε or μ). The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE and IgM, respectively). Each light chain has a molecular weight of ~25 000 daltons and is composed of two domains, one variable domain (VL) and one constant domain (CL). The heavy chain has a molecular weight of ~50 000 daltons and contains one variable domain (VH) and either three or four constant domains (CH1, CH2, CH3 and CH4), depending on the antibody class or isotype. Variable regions are contained within the amino (NH_2) terminal end of the polypeptide chain and serve as the antigen-binding sites. Constant regions are, in comparison, rather uniform from one antibody to another within the same isotype. The four polypeptide chains are held together by covalent disulphide (-S-S-) bonds (figure II). While IgG is the predominant serum form, IgA is the main class of immunoglobulin found in secretions such as saliva, breast milk and tears, and in the digestive tract [G27].

35. Although in certain conditions B-cells may be susceptible to T-cell-independent activation [F15], the majority of B-cells require interaction with helper CD4+ T-cells to become activated and proliferate. The B-cell first expresses Ig on the cell surface as the B-cell receptor. If the B-cell receptor binds specific antigen, then the cell internalizes the antigen and presents it to T-cells, where it is recognized by the T-cell receptor (TCR). T-cell interaction with the B-cell involves additional regulatory signals involving stimulation, e.g. interactions CD40/CD40L and CD28/CD80. Fas ligand binding to Fas between B- and T-cells may negatively modulate B-cell activation, inducing apoptosis that limits B-cell proliferation and activation. Cytokines such as IL-2, IL-4 and IL-10 also play an important role in B-cell activation. B-cells express class II HLA molecules. They can find T-cells in secondary lymphoid organs shortly after antigen entrance, BCR-mediated endocytosis allows them to concentrate small amounts of specific antigen, and BCR signalling and class II HLA expression direct their antigen-processing machinery to favour presentation of antigens internalized through the BCR. These characteristics allow B-cells to be considered as antigen-presenting cells (APCs).

Figure II. Molecular structure of immunoglobulins (adapted from reference [J1]).



36. T-lymphocytes move from the bone marrow into the thymus as immature cells, take up residence and become thymus-dependent or mature T-lymphocytes. Prothymocytes in the superficial cortex of the thymus (CD2+) give rise to cortical thymocytes (CD1a+, CD2+, CD3+) and mature T-cells (CD4+ or CD8+). Medullary thymocytes are fewer and larger, and express CD4 or CD8 [A25]. Mature T-cells pass through the circulation to find homes in lymph nodes, mucosa-associated lymphoid tissue or the spleen. Most of the T-cells belong to one of two subpopulations, distinguished by the presence on their surface of one of two glycoproteins, designated CD8 and CD4. The type of these glycoproteins present determines the cell type to which T-lymphocytes can bind (see section I.C.3 below). The majority of CD8+ T-lymphocytes are cytotoxic T-cells, and the majority of CD4+ T-lymphocytes are helper T-cells. Cytotoxic and helper T-lymphocytes perform very different functions in the immune system. Cytotoxic T-lymphocytes are effector cells that, once activated, can remove foreign organisms. Helper T-lymphocytes induce other immune cells to become better effectors. There are at least two subsets of helper T-cells (Th1 and Th2); they secrete very different cytokines upon activation [S36]. While the Th1 subset produces large amounts of cytokines that promote cell-mediated immune response, the Th2 subset produces an environment favouring humoral immunity by providing B-cell help for antibody production. The Th1 response (now sometimes called "type 1 immunity") is characterized by production of IFN-γ, IL-2, TNF-β and TNF-α. Characteristics of the Th2 response ("type 2 immunity") include production of IL-4, IL-10, IL-13 and IL-5, and stimulation of the production of IgE and IgG1 antibodies [K11]. Immune regulation involves homeostasis between Th1 and Th2 activity directing different immune response pathways (table 5).

Table 5 Characteristic molecules produced by CD8+ and CD4+ T-cells
Adapted from reference [J1]

T-cell type	*Molecule*	*Action*
CD8+ Production of molecules with cytotoxic activity	Perforins Granzymes Fas ligand IFN-γ TNF-β TNF-α	Pore-forming proteins Proapoptotic intracellular proteases Transmembrane death activator Inhibition of viral replication Cell death Cell death

T-cell type	*Molecule*	*Action*
CD4+/Th1 Production of molecules capable of activating macrophages	IFN-γ TNF-α Fas ligand GM-CSF CD-40-L IL-3 TNF-β IL-2	Control of viral replication, Th2 inhibition Cell death Transmembrane death activator Granulocyte–macrophage colony stimulation Activation of target cells (via CD40 receptor) Growth and differentiation of target cells Cell death Lymphocyte activation and proliferation
CD4+/Th2 Production of molecules capable of activating B-cells	IL-4 IL-5 IL-6 CD-40-L IL-3 GM-CSF IL-10 TGF-β Eotaxin	B-cell activation Eosinophil growth and differentiation Plasma cell and stem cell differentiation, antibody secretion, acute phase response Activation of target cells (via CD40 receptor) Growth and differentiation of target cells Granulocyte–macrophage colony stimulation Macrophage inhibition Chemotaxis and IL-1 synthesis Attracting eosinophils

37. Although most CD4+ T-cells belong to either the Th1 or Th2 subsets, 5–10% of CD4+ T-cells do not belong to these two subsets; these cells express CD25, the receptor for IL-2, and the αβ TCR. Their repertoire of antigen specificities is as broad as that of naive T-cells. These "T-regulatory" (Treg) cells are activated by binding to the class II HLA molecule, and they also receive co-stimulatory signals that serve to initiate, maintain and regulate the activation cascade. Most CD25+ CD4+ Treg cells are produced by the normal thymus as a functionally distinct and mature subpopulation of T-cells [S5]. The major function of Treg cells is to inhibit other T-cells from mounting an immune response against self components, thus protecting against autoimmunity. Upon TCR stimulation, Treg cells secrete large amounts of powerful immunosuppressor cytokines, which inhibit both Th1 help for cell-mediated immunity and Th2 help for antibody production.

38. Treg cells also express several members of the TLR family. Stimulation of Treg cells through TLRs can expand their numbers and strengthen their suppressive activity [S4]. This effect is apparently in opposition to the TLR-dependent stimulation of APCs, which enhances production of pro-inflammatory cytokines, thereby augmenting T-cell-mediated acquired immunity. However, the PAMP concentrations required for stimulation of Treg cells through TLRs are several orders of magnitude higher than those required for activation of APCs. If a large amount of PAMP, e.g. bacterial lipopolysaccharide (LPS), is produced, Treg cell activation may prevent severe systemic reactions, such as septic shock due to the production of large amounts of pro-inflammatory cytokines [S4]. Therefore CD4+ CD25+ Treg cells are an evolutionarily unique T-cell subpopulation bearing two kinds of receptor: TCRs and TLRs. This dual signalling source, together with other signals, may enable CD4+ CD25+ Treg cells to finely tune their activity to modulate acquired response against self and non-self antigens.

39. Normally lymphocytes are in a resting state, and before any contact with an antigen they are referred to as "naive". They become activated to carry out their specific functions when an immune response is triggered. Activated lymphocytes begin to proliferate in a process termed "clonal expansion", giving rise to a clone of descendant cells. This activation also induces the cells to differentiate into primary effector T-cells, which can secrete cytokines and kill infected cells, and leads to rapid clearance of the pathogen. Once the response has ceased, lymphocytes revert to their previous inactive state, the primary effector cell population begins to contract and the majority of the cells die by apoptosis over the following weeks. A minority of cells, however, escape this period of cell death; the cells that survive constitute an expanded population of "memory" cells able to trigger a vigorous and effective secondary response should the same antigen be encountered in the future. The maturation status of CD4+ and CD8+ lymphocytes (naive/memory) can be determined on the basis of expression of CD45RA molecules. The phenotypes are CD45RA+ for naive T-cells and CD45RA– for memory T-cells. Although T-cells express CD45RO, the expression of CD45RO by T-cells is not a marker per se for memory cells, because other types of immune cell also express it. Phenotypically naive T-cells also express high levels of CD27 and CD28 molecules (CD45RA+ CD27+ CD28+). T-cells belonging to the memory pool have lost CD45RA expression (CD45RA– CD27+ CD28+). After repeated stimulation by antigen, memory T-cells down-regulate CD28 and subsequently CD27, and give rise to memory/effector cells (CD45RA– CD27– CD28–) and terminally differentiated effector T-cells (CD45RA+ CD27– CD28–) [V2]. The number of memory cells remains remarkably constant over time; this is due to their ability for self-renewal by undergoing slow, periodic turnover, referred to as "homeostatic turnover", and these cells can protect against secondary infections [A10, C1, J3].

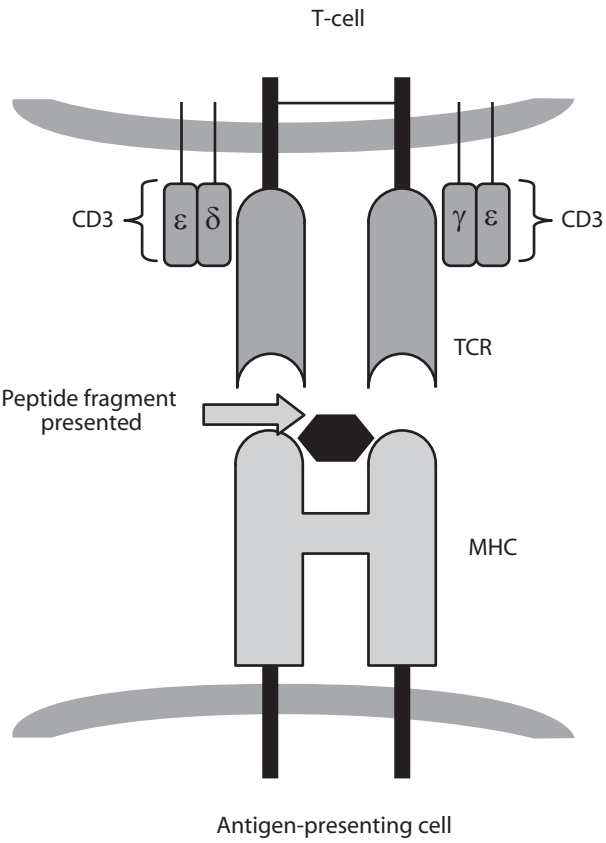
40. T-cells can be divided into two subsets on the basis of the structure of their TCRs. Most T-cells express a TCR heterodimer consisting of α- and β-chains (αβ T-cells), whereas a minor subpopulation expresses an alternative TCR isoform made of γ- and δ-chains (γδ T-cells). Each chain consists

of a variable domain, a constant domain, a transmembrane domain and a cytoplasmic domain. The TCR is encoded by multiple constant (*C*), joining (*J*), diversity (*D*) and variable (*V*) gene segments that are selectively recombined to generate TCR diversity. This diversity is necessary for the recognition of the many antigenic peptides possible. First, *V(D)J* recombination assembles unique *BCR* and *TCR* genes from three separate gene segments—the *V*, *D* and *J* genes—during lymphocyte differentiation. In addition to this recombination, which occurs in the central lymphoid tissues, somatic hypermutation may take place during a late phase of the immune response in peripheral lymphoid tissues [G37]. As immature T-cells undergo maturation in the thymus, the TCR and other molecules are responsible for the selective processes involved in creating immunocompetent T-cells [J1]. While αβ T-cells only recognize ligands presented within class I or class II MHC (see section I.C.3), most γδ T-cells recognize intact proteins, as well as a variety of other types of organic molecule that are fundamentally different from the short peptides seen by αβ T-cells in the context of MHC. Most of these γδ T-cells have neither CD8 nor CD4 on their surface (they do not recognize class I and class II HLA) [H28].

41. The multichain TCR/CD3 complex is one of the most elaborate cell surface signalling receptors and plays a key role in antigen recognition, T-cell activation and triggering antigen-specific immune response. This process is induced by direct interaction of the TCR with an antigen presented by the MHC on APCs. Upon the structural and functional cooperation of the TCR with the CD3 complex, the activating signal is transmitted through the cell membrane to the nucleus [F10, S2]. The structure of the TCR/CD3 complex is represented schematically in figure III.

42. Engagement of the TCR by the antigen leads to a series of intracellular biochemical events culminating in the transcription of new genes and T-cell activation. One or more tyrosine kinases phosphorylate first the CD3 chains themselves and subsequently other substrates. Subsequent to tyrosine kinase activation, a series of secondary events follow TCR engagement, including activation of serine/threonine kinases, activation of the guanosine triphosphate (GTP)-binding protein p21ras and activation of transcription factors for receptors and growth factors such as the major T-cell growth factor IL-2. The CD4 and CD8 co-receptors bind a tyrosine kinase (p56Lck) by their intracytoplasmic tail, which plays a critical role in T-cell signalling. However, TCR binding is not sufficient to activate T-cells, and a second (co-stimulatory) signal is required. Indeed, as originally envisaged in the "two-signal hypothesis", T-cell activation required stimulation both by the TCR (signal 1) and through additional co-stimulatory molecules (signal 2). The principal co-stimulatory molecules expressed on APCs belong to the B7 family: B7-1 (CD80) and B7-2 (CD86). T-cells display receptors for these B7 molecules—CD28 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)—with similar structures but opposite functions. While CD28 helps to initiate T-cell activation, CTLA-4 down-regulates the response [I14, L30]. Activated T-lymphocytes may be identified by the expression of certain surface markers, e.g. CD25, CD38 and HLA-DR. Only a low percentage of circulating lymphocytes are in an activated state in healthy individuals; these activated lymphocytes can be rapidly mobilized against aggressors and they help in the further recruitment of defending cells [J1].

Figure III. Structure of the TCR/CD3 complex (adapted from reference [J1]).



43. The stages of T-cell development within the thymus are identified by the expression of specific cell surface markers, such as TCR, CD3 (which serves as the signal transduction component of TCR) and CD4/CD8. Direct cell-to-cell interaction between these cells and thymic cells induces their proliferation and also differentiation. Pre-T-cells do not express any of the above-mentioned T-cell markers; at this stage they are referred to as "double-negative" cells (CD4– CD8–). At this point the β-chain of TCRs undergoes rearrangement. The successful rearrangement of this chain serves as a signal for these cells to undergo further proliferation. During this time, both CD4 and CD8 start to be expressed; thus these cells are referred to as "double-positive" cells (CD4+ CD8+). It is only at this point that the α-chain of the TCR undergoes rearrangement. At the double-positive stage, a second molecular sensor assembles and controls the transition to the single-positive CD4+ CD8– or CD4– CD8+ stage on the basis of the specificity of the TCR αβ heterodimers (table 6). Likewise, thymocytes committed to the γδ lineage also find a checkpoint at the penultimate double-negative stage (CD44– CD25+), which counterbalances the stochastic nature of the concurrent TCR γ and δ rearrangements and allows only cells expressing a γδ TCR to mature rapidly into CD25+ CD4– CD8– γδ cells and leave the thymus [N15].

Table 6 T-cell αβ+ development and its correlation with CD expression on the cell surface
Adapted from reference [J1]

Double-negative (CD4- and CD8-negative)				*Double-positive (CD4- and CD8-positive)*			
Surface molecule	*CD44+ CD25–*	*CD44+ CD25+*	*Pre-TCR*	*Proliferation*	*CD4/CD8*	*TCR*	*Single-positive*[a]
CD2				→	→	→	→
C-Kit	→	→	→				
CD44	→	→	→				
CD25		→	→				
CD3			→	→	→	→	→
CD4					→	→	→ *a*
CD8					→	→	→ *a*
CD24						→	

[a] Single-positive: CD4+ or CD8+.

44. The expression of CD4, CD8, CD3 and TCR chains changes during the different stages of thymocyte development, and this is shown in figure IV. First, immature thymocytes do not express the above-mentioned markers. These double-negative CD4– CD8– cells are precursors of two populations of cells: a minor proportion are CD3+ γδ+ thymocytes and a larger proportion are CD3+ αβ+ thymocytes. CD3+ γδ+ thymocytes express neither CD4 nor CD8, even when they reach maturation and are exported to the periphery. In contrast, CD3+ αβ+ thymocytes go through further stages of development, including changes in CD4 and CD8 expression. Large double-positive thymocytes (CD3+ αβ+ CD4+ CD8+) differentiate into small double-positive cells, and the majority (97%) die by apoptosis within the thymus. The remaining cells (3%) lose the expression of either CD4 or CD8 and become single-positive thymocytes (CD3+ CD4+ CD8– or CD3+ CD4– CD8+), which are exported to the periphery after maturation.

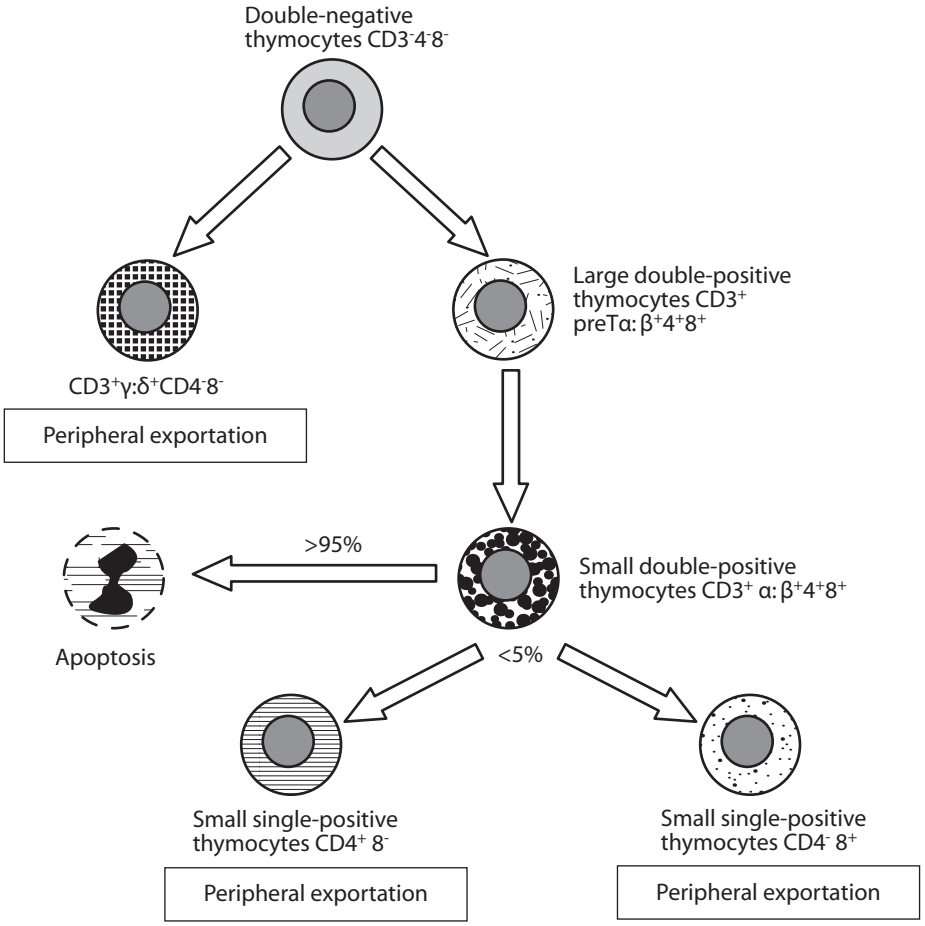
45. The immune system is a site of intense DNA modifications, which result from programmed and specific mechanisms during its maturation, or as a consequence of non-specific injuries inflicted during cellular proliferation and/or cellular activation. The V(D)J recombination is initiated by lymphoid-specific proteins through the introduction of a DNA double-strand break. The terminal maturation of B-lymphocytes, which occurs during an immune response in the germinal centre of secondary lymphoid organs such as the spleen, is characterized by two important modifications of the rearranged immunoglobulin genes. The isotype class switch recombination and the generation of somatic hypermutations ensure the production of efficient antibodies of various isotypes. These two B-cell-specific processes are triggered by the activation-induced cytidine deaminase protein through DNA modification within immunoglobulin genes. Beside these three DNA-altering mechanisms, B- and T-lymphocytes are also exposed to general DNA injuries known to occur, for example, during DNA replication, as several waves of intense cellular proliferation accompany not only their maturation but also their expansion during immune responses. Lastly, one important aspect of an immune response relies on the inflammatory reaction, during which several soluble factors and/or natural reactive metabolites are produced that can be considered as possible causes of DNA damage. Altogether this demonstrates that the lymphoid tissue is at particular risk for mutagenic events inflicted through defective DNA repair machineries [R20].

3. Human major histocompatibility complex

46. TCRs recognize specific epitopes on the surface of APCs, once these are degraded into small peptides, a form referred to as “processed antigen”. The major histocompatibility complex (MHC), which in humans is called the human leucocyte antigen (HLA), plays a fundamental role in enabling T-cells to recognize antigens by forming complexes with the peptides. Antigen processing and presentation are intracellular processes that result in fragmentation (proteolysis) of proteins, association of the fragments with MHC molecules and expression of the peptide–MHC complexes at the cell surface, where they can be recognized by the TCR on a T-cell (figure V). The peptide-binding cleft of MHC molecules is the location where an antigen is attached for display to a T-cell. However, not the entire antigen is bound to the cleft. Only a few “anchoring” residues of the antigen need actually to attach to the MHC molecule for the antigen to be displayed. This makes it possible for only a few common

residues on antigens to allow binding to one form of MHC. This is important because it allows many different antigens with only a few residues in common to be displayed by one form of an MHC molecule, giving these molecules broad specificity. Furthermore, the size of the peptide-binding cleft (larger in class II MHC) is proportional to the size of the peptide that it can accommodate (class II MHC molecules bind larger residues).

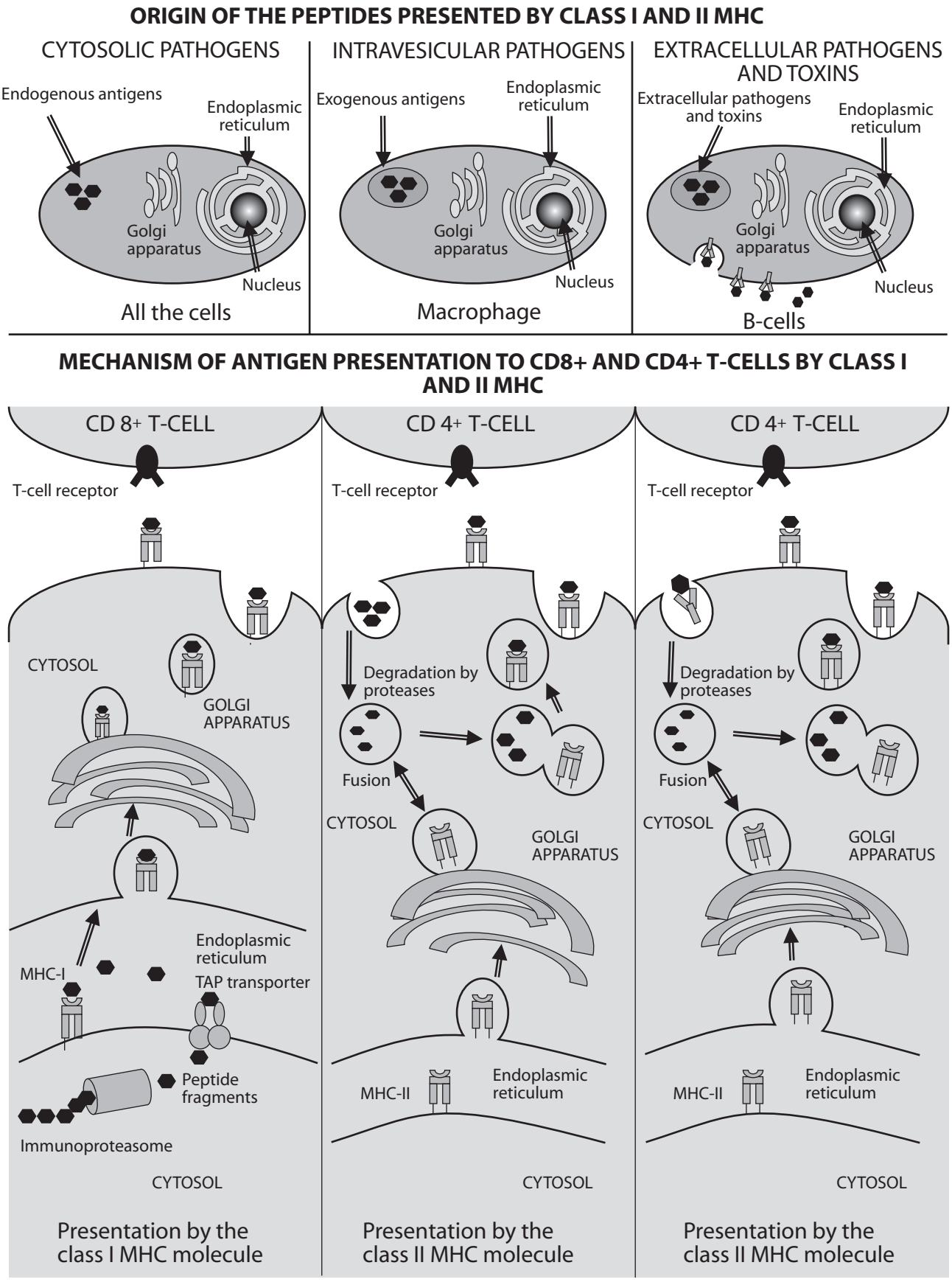
Figure IV. Stages of thymocyte development. The expression of CD4, CD8, CD3 and TCR chains changes during the different stages of thymocyte development (adapted from reference [J1]).



47. The MHC is a complex of closely linked genes that code for two families of glycoproteins (class I and class II MHC). While class I molecules are constitutively expressed by virtually all somatic nucleated cells, class II molecules are expressed only by APCs. The class I MHC family in humans is divided into classical and non-classical subfamilies composed of HLA-A, -B and -C, and HLA-E, -F and -G, respectively. The Class II MHC family in humans includes HLA-DR, -DP and -DQ. Classic class I and class II HLA molecules are characterized by polymorphisms; within each class, and even at a single locus, an enormous number of variants (alleles) are found among different individuals in a population. A haplotype is a group of alleles that are inherited together on a single chromosome. Even though the human population is outbred, because the HLA genes are tightly linked, they are frequently inherited together, and patterns of inheritance have been identified among certain groups of HLA antigens. Each individual has only a very small set of different HLA genes and expresses one (homozygous) or a maximum of two (heterozygous) alleles for each locus.

48. HLA-G is a non-classical class I MHC molecule involved in immunotolerance, with restricted tissue distribution. It was first found in extravillous cytotrophoblast cells [C4] and was subsequently demonstrated in a variety of normal tissues, including T-cells. The class I MHC chain-related molecule MICA belongs to a novel family of heat-shock-induced proteins, by which the immune system can recognize and destroy stress-induced cells. This represents an immune surveillance mechanism for the detection of damaged, infected or transformed cells [D4, S12]. MICA molecules are recognized by TCR γδ. It has been demonstrated that HLA-G mediates a dominant negative signal leading to tumour escape from immune surveillance by counteracting the MICA-activating signal [R9].

Figure V. Upper panel: origin of the peptides presented by MHC; lower panel: mechanism of antigen presentation to CD8+ and CD4+ T-cells by class I and class II MHC (adapted from reference [J1]).



49. The central MHC region or human class III encodes, within a ~700 kb sequence located between the centromeric class II MHC and the telomeric class I MHC regions, a heterogeneous collection of more than 60 genes where a few families do emerge. These genes include those involved in the activation cascades of the complement system, steroid hormonal synthesis, inflammation and cell stress (tumour necrosis factor, lymphotoxins, heat shock proteins) and extracellular matrix organization (tenascin), as well as immunoglobulin superfamily (Ig-SF) members. The remainder (the majority) of the loci are involved mainly in more "core" biological functions with no immediate implication for the immune system [H21].

50. T-cells are grouped functionally according to the class of HLA molecules that associate with the peptides they recognize. CD8+ cytotoxic and CD4+ helper T-cells tend to recognize peptides bound to class I and class II HLA molecules, respectively. Endogenous antigens may be viral proteins synthesized within the infected cell or the specific proteins synthesized by tumour cells. These cytosolic pathogens are degraded into peptide fragments that form complexes with class I HLA and are then transported through the endoplasmic reticulum towards the cell surface. Proteasomes are the major non-lysosomal protein degradation machinery in eukaryotic cells. They deal primarily with endogenous proteins. Specialized proteasomes called immunoproteasomes are responsible for the processing of antigens for presentation by the class I HLA pathway. In mammals, activation of the immune system leads to the release of cytokines, causing the activation of immunoproteasomes and degradation of the antigenic protein into peptides about 10 amino acids in length. These peptides are then transported from the cytosol into the endoplasmic reticulum, where each enters the groove at the surface of a class I HLA molecule. This peptide–HLA complex then moves through the Golgi apparatus and is inserted into the plasma membrane, where it can be recognized by CD8+ T-cells to induce cell death (cytotoxicity) [R6, T3] (figure V). Exogenous antigens are degraded by APCs by endocytosis. The pH of the endosomes containing the engulfed pathogens progressively decreases, activating proteases that reside within these acidified endocytic vesicles to degrade the engulfed material. The resulting peptides are located within class II HLA molecules, which are then exported towards the cell surface. Toxins are extracellular pathogens, for example the majority of bacteria, which mainly reside and replicate extracellularly. They are degraded inside intracellular acidified vesicles and associate with class II HLA molecules to be presented to CD4+ T-cells that can help B-cells to secrete Ig against these bacteria. Other exogenous antigens, for example some bacteria and parasites, grow intracellularly (intravesicular pathogens). Once degraded in acidified vesicles, their peptides are also bound to class II HLA and presented to CD4+ T-cells. Upon recognition of these peptides by the TCR, the presenting cell is activated to kill the pathogens (table 7).

Table 7 Origin of the peptides presented by class I and II HLA

Property	*Cytosolic pathogen*	*Intravesicular pathogen*	*Extracellular pathogen or toxin*
Degraded in	Cytoplasm	Acidified vesicles	Acidified vesicles
Peptides bind to	Class I HLA	Class II HLA	Class II HLA
Presented to	CD8+ T-cells	CD4+ T-cells	CD4+ T-cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B-cells to secrete Ig to eliminate extracellular bacteria or toxin

4. Antigen-presenting cells

51. The immune system contains three types of antigen-presenting cell (APC): macrophages, dendritic cells and B-lymphocytes. These three types of APC present different sets of antigens and may also serve to activate helper T-cells at different points during the immune response. As previously discussed, T-cell activation requires stimulation both by the TCR and through additional co-stimulatory molecules. The most relevant property of APCs is that, in addition to antigen presentation, they provide co-stimulatory signals. APCs (except dendritic cells) do not constitutively express these co-stimulatory molecules. Since these cells potentially phagocytose both self and infectious materials, there has to be some mechanism for the recognition of self and non-self. Upon this recognition, the APCs will up-regulate their co-stimulatory molecules (namely B7 molecules), and only then activate T-cells, by interacting with B7 receptors.

52. Macrophages are part of the innate response. Unlike T- and B-cells, they do not contain specific receptors but do express TLRs that allow them to recognize differential PAMPs on foreign cells. Stimulated macrophages up-regulate class II HLA and express co-stimulatory molecules (B7 molecules). It is at this point that antigen presentation by class II HLA will activate CD4+ helper T-lymphocytes.

53. Dendritic cells are mostly found in the skin and mucosa epithelium (Langerhans cells). Dendritic cells also possess TLRs that can recognize PAMPs, and they continuously express high levels of co-stimulatory molecules (B7 molecules). Upon recognition of infectious particles, these

cells migrate through the lymphatic system to the nearest lymph node, where they come into close contact with naive T-cells. Unlike macrophages, however, dendritic cells can also recognize viral particles as non-self. In addition, they can present antigen via both class I and class II HLA. Thus they can directly activate both CD8 and CD4 T-cells. Once the T-cells are activated, they leave the lymph nodes and travel to the sites of inflammation. Since dendritic cells present viral particles, these should also activate CD8 cells, the main effector cells for fighting viral infections. Dendritic cells are also very numerous in the thymus, where they act in T-cell selection during development. While a role of dendritic cells in the negative selection in the thymus has been well established [W13], their role in the positive selection is still questionable [W11].

54. The skin is equipped with specialized cells called Langerhans cells (LCs) which play a central role in the skin's immune system as an integral part of the body's total defence system. LCs are epidermal antigen-presenting dendritic cells originating in the bone marrow. They migrate to the epidermis, where they form a regularly ordered network representing 1.86% of all epidermal cells. A constant numerical ratio (1:53) exists between LCs and the other epidermal cells. The surprisingly constant relationship of LCs to other epidermal cells supports the hypothesis of an epidermal LC unit where one LC seems to be responsible for the immune surveillance of 53 epidermal cells [B21].

55. After contact with the corresponding antigens (viruses, contact allergens, skin transplants), LCs migrate from the epidermis to the regional lymph nodes for presentation of antigenic peptides to T-cells. On their journey, LCs undergo a maturation process leading to the presentation of the antigen on the cell surface. The migrating LCs are replaced by a corresponding number of new LCs from the bone marrow. In the lymph nodes the mature LCs activate the helper T-cells that have the matching antigen-specific receptors on their surfaces. In this way they direct the reaction of the immune system [K42].

56. Epidermal LCs have a spectrum of different functions with implications that extend far beyond the skin. They have the potential to internalize particulate agents and macromolecules, and display migratory properties that endow them with the unique capacity to journey between the skin and draining lymph nodes where they encounter antigen-specific T-lymphocytes. In addition, LCs are considered to play a pivotal role in infectious disease, allergy, chronic inflammatory reactions, tumour rejection or transplantation [V10]. Factors influencing the activity of the LCs in the epidermis include cytokines such as IL-10, immunosuppressive drugs such as corticoids, and ultraviolet and ionizing radiation [K39].

57. B-cells are the least efficient APCs. Unlike the other two APCs, they possess specific antigen receptors (surface immunoglobulins). B-cells effectively ingest soluble antigens that bind to cell surface immunoglobulin receptors by receptor-mediated endocytosis. Thus B-cells can present specific antigens to activated T-cells. However, resting B-cells do not express co-stimulatory molecules; in order to do so, most of the B-cells need to be activated by helper T-lymphocytes. The role of B-cells as APCs in vivo is not very well understood [R21].

5. Self tolerance and self-HLA-associated recognition

58. As seen, a diverse and polymorphic T-cell repertoire is generated in the thymus by random recombination of discrete *TCR* gene segments. This repertoire is then shaped by intrathymic selection events to generate a peripheral T-cell pool of self-HLA-restricted, non-autoaggressive T-cells. To ensure that self tolerance is achieved, self antigens are presented to the matured T-cells by APCs in the thymus. At this stage, these cells undergo the processes of positive and negative selection. During positive selection, double-positive T-cells that can recognize self HLAs are selected for proliferation, and those T-cells that do not recognize self HLAs die by apoptosis. Positive selection has been associated mainly with cortical thymic epithelial cells and their associated HLA molecules, both of which are necessary and sufficient for positive selection of double-positive thymocytes and the development of single-positive cells [G15]. By negative selection, those T-cells that are strongly activated by self HLA and self peptides are eliminated in the thymus. This process of clonal deletion prevents lymphocytes from subsequently reacting against self antigens and causing autoimmune diseases. Mature T-cells leave the thymus, go into the circulation and eventually find their way to lymph nodes, mucosa-associated lymphoid tissue or the spleen [J1] Although the majority of self-reactive T-cells are clonally deleted in the thymus, some mature lymphocytes may remain capable of responding to self antigens. Intrinsic biochemical and gene expression changes, as well as a lack of co-stimulation, can reduce this ability by triggering a process generally termed clonal anergy. Finally, even if the lymphocytes have evaded these controls, mechanisms of extrinsic control, such as active suppression by Treg cells, can prevent the danger of self-reactive receptors [G37].

59. The achievement of immunological self tolerance raises the question of how T-lymphocytes that are reactive to proteins expressed only by non-thymic tissues can be identified and addressed. The clinical relevance of this question is related to the fact that many of these tissue-restricted proteins (e.g. insulin, thyroglobulin, myelin, retinal S-antigen) are associated with organ-specific autoimmune diseases (e.g. type 1 diabetes, thyroiditis, multiple sclerosis, uveitis). A classical explanation for this phenomenon was that, while tolerance to ubiquitously expressed or blood-borne antigens is centrally achieved in the thymus, tolerance to tissue-restricted antigens is secured by peripheral extrathymic mechanisms. However, ectopic synthesis of these peripheral tissue-restricted proteins has recently been demonstrated in thymic medullar epithelia

cells [A22]. A startling discovery for the understanding of this ectopic synthesis is the autoimmune regulator *AIRE* gene that enables a battery of tissue-restricted antigens to be expressed in thymic medullar epithelia cells, thus playing an important role in controlling tolerance induction. In the absence of *AIRE*, autoimmunity, and ultimately overt autoimmune disease, develop [A23, M16]. Although *AIRE* transcripts are by far most abundant in the thymus, they were also detected in peripheral lymphoid organs at significantly lower levels [A22].

60. Although 1–24% of T-cells are alloreactive, i.e. they respond to MHC molecules encoded by a foreign haplotype, it is generally believed that T-cells cannot recognize foreign peptides binding foreign HLA molecules [D21]. The term "MHC restriction" refers to the phenomenon whereby T-cells from one individual recognizing an antigen fail to recognize cells presenting the same antigen unless the presenting cells express one or more HLA alleles identical to those on this individual's cortical thymic epithelial cells, in which those T-cells matured. This phenomenon derives in part from the requirement to recognize self HLA (self-HLA-associated recognition) and in part from the different peptide-binding specificity of different HLA alleles. Through positive and negative selection in the thymus, self peptides bound to autologous HLA molecules determine the repertoire of peripheral T-cells, which then respond to infection by recognizing foreign peptides bound to those same HLA molecules [P13].

6. Cytokines

61. As described above, the immune system has many different types of cell acting together to deal with unwanted infections and altered cells. Cytokines are small proteins produced by the immune cells for signalling and for orchestrating the attack. They generally act over short distances and short time spans and at very low concentrations by binding to specific membrane receptors, which then signal via second messengers, often tyrosine kinases. Responses to cytokines may include increased or decreased expression of membrane proteins, secretion of effector molecules and cell proliferation. By these actions cytokines mediate and regulate immunity, inflammation and haematopoiesis.

62. Many cell populations make cytokines, but the predominant producers are CD4+ helper T-cells and monocytes/macrophages. Cytokines made by lymphocytes are called lymphokines, and cytokines made by monocytes are called monokines. Other groups of cytokines include interferons (IFNs) and chemokines. While IFN-α and IFN-β inhibit virus replication in infected cells, IFN-γ has the additional property of stimulating HLA expression. Chemokines are cytokines with chemotactic properties that attract leucocytes to infection sites. The general term interleukins (ILs) is used to define cytokines made by one leucocyte and acting on other leucocytes. Interleukins have been numbered in the order in which they were identified. Thus the first IL identified was named IL-1; about 30 different ILs have been identified so far (table 8).

Table 8 Main cytokines and their functions
Adapted from reference [J1]

Cytokine[a]	*Main source*[b]	*Target cells*	*Function*
IL-1 α and β	APCs	Th cells	Co-stimulation
		B-cells	Maturation and proliferation
		NK cells	Activation
		Various	Haematopoiesis, inflammation, fever, acute phase response
IL-2	Th1 cells	T-, B- and NK cells	Activation, growth, proliferation
IL-3	Th1 cells, NK cells	Stem cells	Growth and differentiation
		Mast cells	Growth and histamine release
IL-4	Th2 cells, mast cells	B-cells	Proliferation, differentiation, IgG and IgE synthesis
		T-cells	Proliferation
		Macrophages	Expression of class II HLA
IL-5	Th2 cells	Eosinophils	Proliferation
		B-cells	Proliferation, differentiation, IgA synthesis
IL-6	APCs, Th2 cells, stromal cells	B-cells	Differentiation (into plasma cells)
		Plasma cells	Antibody secretion
		Stem cells	Differentiation
		Various	Acute phase response

Cytokine[a]	*Main source*[b]	*Target cells*	*Function*
IL-7	Marrow stroma, thymus stroma	Stem cells	T- and B-lymphopoiesis
IL-8	Macrophages, endothelial cells	Neutrophils T-cells	Chemoattractant
IL-9	T-cells	Stem cells, thymus cells	Haematopoiesis and thymopoiesis
IL-10	Th2 cells, macrophages, Tc cells, B-cells	Macrophages, B-cells, mast cells	Inhibition of cytokine production Proliferation, differentiation, Ig synthesis Inhibition of growth Suppression of cell-mediated immunity
IL-11	Stromal cells	Marrow cells	Haematopoiesis and thrombopoiesis
IL-12	Macrophages, B-cells	Tc cells NK cells	Differentiation, promotion of cell-mediated immunity Activation, proliferation, IFN-γ production
IFN-α and -β	Macrophages, neutrophils, fibroblasts	Various	Inhibition of viral replication Induction of class I HLA expression
IFN-γ	Th1 cells, NK cells	Various Various B-cells Th2 cells	Inhibition of viral replication Induction of HLA expression Ig switch to IgG2a Inhibition of proliferation
TGF-β	T-cells, monocytes	Macrophages B-cells Various	Chemotaxis and IL-1 synthesis IgA synthesis Inhibition of proliferation
TNF-α	Monocytes, NK cells, mast cells	Macrophages Tumour cells	Cytokine expression Cell death
TNF-β	Th1 cells, Tc cells	APCs Tumour cells	Phagocytosis, NO production Cell death

[a] IL = interleukin; IFN = interferon; TGF = transforming growth factor; TNF = tumour necrosis factor.

[b] Th = CD4+ helper T-cells (Th1- or Th2-type); Tc = CD8+ cytotoxic T-cells.

63. Cytokines have several important characteristics:

- The same cytokine may be made by a number of different cells;
- Pleiotropy: the same cytokine may have different effects and/or may act on several different cell types;
- Redundancy: similar functions can be induced by different cytokines;
- Synergy: cytokines often act together and increase one another's effects;
- Antagonism: some cytokines may cause opposing effects;
- Cascade effect: cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines;
- Paracrine actions: cytokines act on cells near to them or that they actually touch;
- Autocrine functions: cytokines may act on the same cells that secrete them;
- Endocrine functions: some cytokines may act in some instances on distant cells.

7. Remarks concerning cells and molecules of the immune system

64. The preceding paragraphs concerned cells and molecules of the immune system. Anatomic, physiological, inflammatory and phagocytic barriers constitute the innate immune system. Phagocytes (granulocytes, monocytes/macrophages and dendritic cells) engulf and digest pathogens. They express a special family of receptors (TLRs) capable of recognizing PAMPs. NK cells also belong to the innate arm of the immune response. NK cells can recognize, bind and kill virus-infected and tumour cells. Spontaneous NK cytotoxic activity is regulated through activating and inhibiting membrane receptors. NKT cells are a particular type

of lymphocyte expressing some NK surface markers and TCRs, and that bridge innate and acquired immunity. As part of the innate immunity, mast cells are involved in allergy, anaphylaxis, wound healing and defence against pathogens. The complement system circulates in an inactive form. In response to the recognition of certain molecular patterns of pathogens, complement activation results in the production of biologically active molecules, which may lead to lysis, opsonization and inflammation.

65. Lymphocytes are the predominant cells involved in acquired immunity. B-lymphocytes and T-lymphocytes are the effector cells of the humoral and the cellular response, respectively. B-cells recognize native antigens through the immunoglobulins expressed on their surface as BCRs. T-cells recognize antigens previously processed into small peptides through the TCR/CD3 complex expressed on their surface. B-cell/T-cell interactions involve additional co-stimulatory signalization. Most of T-cells belong to the CD8+ or CD4+ subpopulations. CD8+ cytotoxic T-cells are effectors that, once activated, can remove foreign organisms. CD4+ helper T-cells induce other immune cells to become better effectors.

66. By secreting different cytokines, Th1 and Th2 CD4+ helper T-cells promote cellular or humoral immunity, respectively. A minority of CD4+ T-cells belong to neither Th1 nor Th2 subsets and are called regulatory T-cells that express CD25, the receptor for IL-2. These CD4+ CD25+ Treg cells protect against autoimmunity and prevent severe systemic reactions such as septic shock. Naive (CD45RA+) lymphocytes have never encountered an antigen. When an immune response is triggered, they become activated and proliferate (clonal expansion). Once the response ceases, most of the activated lymphocytes die by apoptosis, but a minority of them remain as memory (CD45RA–) T-cells.

67. TCRs recognize antigenic peptides bound to the MHC (in humans called HLA) on the surface of APCs (dendritic cells, macrophages and B-cells). While CD8+ T-cells recognize endogenous antigens bound to class I HLA, CD4+ T-cells recognize exogenous antigens bound to class II HLA. Through positive and negative selection in the thymus, self antigens bound to autologous HLA molecules determine the repertoire of T-cells to ensure immunological self tolerance. Cytokines are proteins, mainly produced by CD4+ T-cells and monocytes/macrophages, that bind to specific membrane receptors that act as second messengers.

D. Physiological immunosenescence

1. Concept of immunosenescence

68. Immunosenescence can be defined as the progressive decline in immune function observed in the elderly; it results in a higher susceptibility to infections and increased morbidity and mortality [B6, G2, H16]. These age-related changes in immune function have been well documented [H13, R7]. A reduction with age has been reported in the overall capacity for renewal of haematopoietic stem cells, indicating that some of the deficits of immunosenescence may be initiated at the stem cell level [H1, L1].

2. Main features of immunosenescence

69. The immune property most sensitive to ageing is the production and export of T-cells from the thymus, which is manifested by a decrease of naive cells with age [H16]. Naive cells have the greatest diversity of TCR repertoire. With ageing, the thymus involutes, the supply of naive T-cells falls and there is a gradual accumulation of memory T-cells. Thus in elderly persons the T-cell population shifts to a lower naive/memory T-cell ratio, and the TCR repertoire available to respond to new antigens is reduced [H1, V2, V4].

70. There is a progressive increase with age in the proportion of CD8+ T-cells that lack expression of CD28, a critical co-stimulatory molecule [E2, H16]. This results in lower proliferative capacity, decreased IL-2 production, telomere erosion and less response to TCR stimulation. Taking into account the fact that specific effector cells should be able to proliferate sufficiently to fight an infection, the age-related limitation of cell division could have devastating consequences for immune function [E1]. Immunosenescence also involves hyporesponsiveness to mitogens, lowered lytic capacity, decline in transmembrane signalling and higher oxidative stress [B6, B15, V2].

71. As a result of thymic involution, the kinetics of the transition from CD4– CD8– (double-negative) to CD4+ CD8+ (double-positive) thymocytes is altered in old age. It has been demonstrated that thymic levels of p56Lck, a factor involved in the maturation of T-cells from CD4– CD8– double-negative into CD4+ CD8+ double-positive T-cells, are negatively correlated with age, which could lead to the accumulation of CD4– CD8– double-negative T-cells in the elderly [H15].

72. There exists a chronic inflammatory state in the elderly due to an increased release of pro-inflammatory cytokines such as TNF-α and IL-6 [B6, E1]. Dysregulation of TNF-α and IL-6 may be involved in age-related diseases such as osteoporosis, atherosclerosis, Alzheimer's disease, diabetes, cardiovascular disease and cancer [H16]. The balance between Type 1 and Type 2 cytokines, important for the outcome of several infectious diseases, also changes with age. Evidence suggests that, while a Th1-type response predominates in adults, a Th2 response predominates in the elderly. This shift from a Th1 to a Th2 cytokine profile is a possible mechanism for age-associated immune dysfunction [R1, S7].

73. As a result of ageing, the immune system may lose the ability to distinguish self versus non-self antigens.

Age-related changes in humoral immunity involve reduced vaccine responses and increased production of autoantibodies. Although the ability of B-cells to generate antibody responses declines with age, many of the humoral changes observed in the elderly are related to declining T-cell function with dysregulation of T-cell/B-cell interactions [B15, F3, H1, L5]. The B-cell repertoire changes with age, and the altered spectrum of expressed immunoglobulins may affect the quality of the antibody response in the elderly and be highly relevant for health [V4].

74. The innate response is not free from the effects of ageing. The production of reactive oxygen and nitrogen species (ROS/RNS) by neutrophils and macrophages in the elderly is significantly impaired, which diminishes the capacity to destroy bacteria. In contrast to the case with T- and B-cells, the absolute number of NK cells is increased in elderly persons, but their cytotoxic capacity on a per-cell basis is impaired [B15, H1]. NK cells from elderly people show a decreased proliferative response to IL-2 [H16]; NK cells are thus less able to destroy virus-infected and tumour cells in the elderly [P8]. The immune system in older people is also characterized by an increased proportion and number of NKT cells in the peripheral blood [M9].

75. Several aspects of immune response exhibit diurnal and seasonal circadian rhythmicity related to the level of melatonin, which exerts immunoenhancing and antioxidant actions. Such rhythms play an important role in immune homeostasis [S22]. The gradual decline of pineal melatonin synthesis and secretion over the lifespan could cause immunocompetence to deteriorate in the elderly [K6].

3. Remarks concerning immunosenescence

76. The main features of immunosenescence are:

- Thymic involution;
- Progressive decrease of the naive cell pool with age (shift to a lower naive/memory ratio);
- Reduced TCR repertoire;
- Increase in the proportion of CD8+ CD28– T-cells;
- Accumulation of CD4– CD8– T-cells;
- Chronic inflammatory status;
- Progressive loss of the ability to distinguish self versus non-self antigens;
- Dysregulation of T-cell/B-cell interaction, resulting in humoral changes;
- Impaired phagocytic activity;
- Increase in the absolute number of NK cells, with a decrease in their cytotoxic capacity;
- Gradual decline in melatonin synthesis.

E. Summary

77. The general features of the immune system can be summarized as follows:

- The main function of the immune system is to protect against infections and cancer;
- The bone marrow and the thymus are the primary lymphoid tissues where maturation of lymphocytes takes place;
- Mature lymphocytes travel by the bloodstream towards the lymph nodes, spleen and mucosa-associated lymphoid tissue, which are considered the secondary lymphoid tissues;
- The ability of the cutaneous barrier to help defend the body against pathogens relies on both acquired and innate immune responses;
- Monocytes, macrophages, polymorphonuclear leucocytes, dendritic cells, natural killer cells and mast cells are the immune cells involved in the innate immune response;
- Lymphocytes are the predominant cells involved in the acquired immune response. While B-lymphocytes are the effector cells of the humoral response, T-lymphocytes (with their two subsets CD4+ helper T-cells and CD8+ cytotoxic T-cells) are responsible for cell-mediated responses;
- The main features of the acquired immune response are memory, specificity, diversity and self/non-self discrimination;
- There is a crosstalk between the innate and the acquired immune response;
- Cells involved in immune recognition have surface receptors that bind to specific antigens;
- The lymphoid tissue is naturally prone to DNA modifications that occur during the maturation of B- and T-lymphocytes and during immune responses;
- The MHC complex (HLA in humans) plays a fundamental role in enabling T-cells to recognize antigens;
- APCs present antigens and activate helper T-cells by co-stimulatory signals;
- Cytokines are small proteins that mediate and regulate immunity, inflammation and haematopoiesis;
- Immunosenescence is a complex process involving dysregulation, rather than a simple unidirectional decline of the whole immune system function.

II. RADIATION-INDUCED ALTERATIONS OF THE IMMUNE SYSTEM

A. Introduction

78. Radiation-induced effects on the immune system have attracted interest from the research community for several decades, and lymphocyte radiosensitivity was one of the earliest subjects of experimental radiobiology [A13, A27, A28, A29, D11, D29, H24, K54, M30, P12, P15, T19]. Immunosuppression is a consequence of whole-body irradiation (WBI) at medium to high doses. Localized radiotherapy can also result in immunosuppression. In contrast, it has been reported that very low doses of ionizing radiation may give rise to immunostimulatory effects, particularly at short times after irradiation. Because of these divergent effects, ionizing radiation is probably better considered as an immunomodulatory rather than as an immunosuppressive agent [M2, U4]. This section summarizes the main alterations induced by ionizing radiation in the immune system and considers the influence of dose, dose rate and radiation quality. Data concerning the radiosensitivity of the different lymphocyte subpopulations are analysed. Particular attention is given to alterations of the developing immune system following prenatal irradiation. Several human immune pathologies associated with hypersensitivity to ionizing radiation are also reviewed.

79. For the purposes of this annex, low doses are defined as <0.2 Gy to the whole body. Low-dose-rate exposures are considered to be those delivered at <0.1 Gy/h. These are the levels below which the International Commission on Radiological Protection [I2] deems that the dose and dose-rate effectiveness factor (DDREF) should be applied.

B. Data concerning low-dose irradiation

80. The effects of low doses of ionizing radiation on the immune system were reviewed by the Committee in 1994, in the context of adaptive response [U4]. At that time, some evidence in animals seemed to indicate that low doses of ionizing radiation may enhance immune response, but the evidence for a similar effect on the human immune system was sparse. It was concluded that further investigations were needed on these effects and their clinical significance.

1. Animal data

81. The effects of external low-dose irradiation upon the blood and the immune system of experimental animals have been studied at several centres. The dose–response relationship of immunological parameters following exposure to ionizing radiation is affected by a number of factors, the most important of which are the target cells under observation, dose range, dose rate and dose spacing, as well as the temporal relationship of the changes and the strain of animal used [L20]. Table 9 summarizes the most commonly observed alterations of immunological parameters in low-dose/low-dose-rate (LD/LDR) irradiated animals.

Table 9 Effects of ionizing radiation on the immune system of animals irradiated at low doses and low dose rates

Parameter	*Animal species*	*Dose rate (mGy/d)*	*Time of detection of effect, or total dose*	*Trend of change*	*Reference*
Leucocyte score (blood)	Dogs	3–128	0–200 d	Decrease	[S40, N23]
			200–1700 d	Accommodative phase	
Lymphocytes (spleen, thymus)	CBA mice	10–60	30 d	Decrease	[Y1]
	C57BL mice	50	100–200–800 mGy	No change	[J6]
	C57BL mice	100	2 a	No change	[C16]
Haematopoietic progenitors					
CFU-GM	Dogs	75	0–200 d	Decrease	[S39]
			200–800 d	Recovery phase	
CFU-GM	Dogs	18.8	150 d	Decrease	[N23]
			700–1200 d	Partial recovery	

Parameter	*Animal species*	*Dose rate (mGy/d)*	*Time of detection of effect, or total dose*	*Trend of change*	*Reference*
Lymphocyte mitogenic response	Dogs	>20	>300 d	Increase (T-cells)	[Y1]
	C57BL mice	50	100–200–800 mGy	Increase (T-cells)	[J6]
	C57BL mice	40	20 d	Increase (T-cells) No change (B-cells)	[N20]
	C57BL mice	40	200 mGy	Increase (T-cells)	[P11, S16, S17]
	BALB/c mice		75 mGy	Increase (T-cells)	[L18]
Cytokines	Mice		75 mGy	IL-10 decrease	[L19]
				IL-12 increase	
	Mice		75 mGy	IL-1, IL-2 increase	[J8]
	C57BL mice		40 mGy	IL-1β, IFN-γ increase	[I6]
	Rats		200 mGy	IFN-γ, TNF-α increase	[H20]
				TGF-β decrease	
	C57BL mice	40	200 mGy	IFN-γ decrease	[P11]
Macrophage phagocytosis and NK activity	CBA mice	0.02 + nuclides	180 d	Decrease	[G18]
	BALB/c mice		100–200 mGy; 48 h	Increase	[C21]
	BALB/c mice		200 mGy; 24–72 h	Increase	[C21, N24]
	C57BL mice	40	200 mGy	Increase	[P11]

82. Yagunov et al. [Y1] reviewed extensively the haematopoietic and immune system effects of LD/LDR irradiation in animal studies. These long-term effects of LD/LDR irradiation seem largely dependent on the capacity of radiosensitive tissues to repair DNA damage induced by a given daily dose. Post-irradiation changes in the peripheral blood counts of experimental animals returned to control levels within several months of the start of irradiation. The precise mechanisms by which these adaptive or accommodative processes occur are largely unknown, although repair, cell cycle and cell selection are considered to play a role [S40]. The effect of low-daily-dose gamma irradiation (3–128 mGy/d) on the blood-forming system of canines was studied by Seed et al. [S40]. Low but significant suppression of blood leucocytes, including granulocytes, monocytes and lymphocytes, occurred at 3 mGy/d. As the dose rate increased from 3 mGy/d to 128 mGy/d, the rate of suppression increased approximately eightfold. The time required to achieve accommodation decreased as the daily dose rate increased. Within the time required to reach a cumulative dose of 700 mGy, none of the dose rates affected blood cells sufficiently to compromise short-term immune function.

83. Clear associations were found between the tissue responses and marrow progenitor responses of chronically irradiated dogs. The granulocyte–macrophage (GM)-committed progenitor marrow numbers showed a suppressive phase and a later recovery phase preceding the changes in blood leucocyte concentration [N23, S39]. Results also showed that 75 mGy/d represented a threshold below which the haematopoietic system retained either partial or full trilineal cell-producing capacity (erythropoiesis, myelopoiesis and megakaryopoiesis) for periods of exposure longer than one year [S41]. One might interpret these observations as evidence of an adaptive effect and acquisition of resistance to radiation exposure. However, at long times after exposure, animal populations experienced a high incidence of myeloid leukaemia and related myeloproliferative disorders [S49]. These animal data also indicated that a high degree of individual variability existed.

84. Post-irradiation recovery of marrow precursors and mature cells was incomplete, as evidenced by a deficient response to challenge stimuli (acute haemorrhage or gamma irradiation). Several factors may account for deficient haematological recovery after chronic irradiation [Y1]:

- Persisting post-irradiation deficiency of haematopoietic stem cells observed after exposures of >10 mGy/d for several months;
- Accelerated cell cycling of marrow precursors in irradiated animals, leading to increased ratios of S-phase populations among stem cells (colony-forming units (CFUs)) and committed marrow precursors;

– Shortened lifespan of immature erythroid and myeloid cells due to decreased cell viability in irradiated animals.

85. Internal irradiation of animals (using tritiated water incorporation) caused much more severe and prolonged immune depression than did external irradiation at the same total doses, thus indicating the higher relative biological effectiveness (RBE) of incorporated radionuclides, even for this low-LET (linear energy transfer) beta emitter [Y1].

86. The experimental findings of studies, mainly conducted in rodents and canines, reviewed by Yagunov et al. [Y1] are summarized as follows:

– Haematopoietic stem cells and blood cell progenitors seem to be the main target of chronic LD/LDR irradiation;
– Direct radiation damage to the blood/immune precursor pool results in a decrease of stem cell fraction;
– Radiation-induced depletion of the stem cell and progenitor pools results in accelerated cycling of bone marrow precursors;
– Decreased viability of mature blood cells results from ineffective haematopoiesis, thus causing restriction of myeloid (and probably lymphoid) cell reserves;
– Disturbances of cellular and humoral immunity are likely to be caused by extreme radiosensitivity of lymphoid tissues and by a restricted progenitor cell pool;
– Post-irradiation recovery is characterized by gradual reconstitution of peripheral blood and bone marrow patterns. However, residual deficiency of haematopoietic and lymphopoietic precursors may be a limiting factor in blood/immune system recovery;
– An increased percentage of S-phase marrow cells well after prolonged radiation treatment presents a typical response of the haematopoietic system to LDR irradiation, since this is not observed after acute or subacute irradiation or following chronic exposure to certain heavy metal ions;
– DNA misrepair following chronic irradiation may result in stable chromosome aberrations, increased incidence of micronuclei, and detectable point mutations in blood/immune populations.

87. Courtade et al. [C16] irradiated C57BL/6 female mice at 100 mGy/a for two years. No changes were found in cellular immunity parameters regarding CD4+ and CD8+ cells in the thymus and the spleen. In this work, cell subsets were evaluated by flow cytometry before and after stimulation with lectins. While the number of B-cells in the spleen also remained unchanged, a significant decrease in IgG1, IgG2a and IgG2b was observed, at 12, 24 and 18 months post-irradiation, respectively. Using mice irradiated with a single LDR exposure (100 mGy, 10 mGy/min), Sharetskii et al. [S18] observed increased thymus-dependent humoral immune response and polyclonal activation of B-cells. The study of the dynamics of primary immune response showed that the period of radiation-induced elevation was followed by a phase of profound reduction of antibody formation.

88. On the other hand, a large number of studies have described stimulative effects of low-dose irradiation, including stimulation of growth rate, enhancement of survival after lethal high-dose irradiation, prolongation of lifespan, down-regulation of tumour incidence and activation of immune function.

89. Regarding the activation of the immune function, LDR WBI of mice increased the proliferative response of splenic and thymic lymphocytes to mitogens such as concanavalin A (Con A), phytohaemagglutinin (PHA) or anti-CD3, with an acute exposure of 20 or 75 mGy [I5, L16, L18] or fractionated doses of 200–800 mGy, 40 mGy/d [J6, J7, N20, S16, S17]. James and Makinodan [J6] investigated the proliferative capacity of differentiated effector cells in the spleen and its correlation with alterations in thymic precursors and peripheral T-cell subsets. In C57BL/6J mice exposed to 40 mGy/d over 20 d, the increase in spleen cell proliferative response was associated with an increase in the proportion of thymic progenitor cells (L3T4– Lyt2– equivalent to CD4– CD8–) and an increase in the proportion of mature L3T4+ (CD4+) thymocytes. In the spleen, the L3T4+ and Lyt2+ (CD8+) cell proportion was increased and the double-negative cell proportion was decreased. Interestingly, caloric restriction independently altered functional activity and T-cell subpopulations in the same direction as low dose rates. The changes observed are consistent with an increase in proliferative capacity and could reflect adaptive mechanisms operating with LDR irradiation and/or caloric restriction. Interpretation could be complicated by the metabolic status of the irradiated animals. Using the same irradiation protocol, comparable results were obtained by Nogami et al. [N20]. A finding of interest in this last study is the demonstration that LDR exposure can significantly enhance the proliferative activity of splenocytes in response to T-cell mitogens, but the response to LPS, a B-cell mitogen, was not influenced by the LDR treatment. Recently, Pandey et al. demonstrated a preferential activation of CD8+ T-cells as compared with CD4+ T-cells in mice following stimulation with Con A after fractionated 200 mGy exposure [P11].

90. Assays of mitogenic-induced proliferation were also performed using co-cultures of non-irradiated splenolymphocytes and peritoneal macrophages preirradiated with 20 and 40 mGy. The response was increased to 120% and 145% of the control, respectively, suggesting that the enhancement in Con-A-induced proliferation resulting from LDR exposure was caused not by direct activation of splenocytes but by activation of macrophages in the spleen, and that the lymphocytes were activated indirectly [I5]. LDR exposure acts on both the APCs and

the T-lymphocytes, facilitating the intercellular reactions within the immunologic synapse formed between these two categories of immune cells [L20].

91. Data also indicate that enhancement of immune response parameters takes place at a certain very narrow range of dose rate and dose. When WBI with an acute dose was changed from 20 to 200 mGy, the proliferation was inhibited [I5]. Likewise, a change in the dose rate from 40 to 100 mGy/d decreased the proliferative response to PHA or produced no effect [J7, N20].

92. The enhancement of T-cell-dependent immune response as measured by plaque-forming cell counts after immunization with sheep red blood cells (SRBCs) was reported to be stimulated by both single-dose WBI of mice (75 mGy, 12.5 mGy/min) [L15] and continuous 1.2 mGy/h irradiation for up to 140 days [I9, I13]. The in vivo T-cell response was also evaluated by delayed-type hypersensitivity (DTH) after immunization with *Myccobacterium vaccae* or dinitrofluorobenzene (DNF). While DTH to *M. vaccae* and DNF was suppressed in C57BL/6 mice, DTH to *M. vaccae* was increased in BALB/c mice, and DTH to DNF was not significantly changed [S17]. In the same study, the authors reported an inverted proliferative response to Con-A, with enhancement in C57BL/6 mice and suppression in BALB/c mice. Thus the outcome may depend on the strain of animal, the type of antigen and the type of response [S17]. Ina and Sakai [I13] examined the effects of continuous LDR irradiation of the whole body of several wild-type mouse strains and observed a significant activation of the immune system both before and after the immunization with antigens. The different strains displayed different levels of response but with the same tendency: significant increases of CD4+ T-cells, CD8 molecule expression and CD40+ B-cells, and significant enhancement in SRBC-antibody-producing cells by immunization. The age at exposure may also determine the intensity of immunoenhancement, since the presence of a non-involuted thymus contributes to this response [P11].

93. Furthermore, the efficiency of the immune response was measured by the NK activity of splenocytes [C21, N24], functional response of macrophages and cytokine secretion [G11, H20, L18, L19, P11, S33]. Enhanced cytotoxic activity of NK cells was found between 24 and 72 h post-irradiation in whole-body-irradiated BALB/c mice with single doses of 100 and 200 mGy [C21, N24]. LDR irradiation also enhanced the phagocytic activity of macrophages from C57BL/6 mice exposed to a total dose of 200 mGy (40 mGy/d) [P11].

94. Regarding the modulation of cytokine expression by LDR irradiation, increased secretion of interleukins that activate T- and NK cells, such as IL-2 by mouse splenocytes, and IL-1 and IL-12 by APCs, has been described, as well as down-regulation of IL-10 synthesis in splenocytes following WBI with 75 mGy [J8, L14, L18, L19]. There have been observations of the increased secretion of TNF-α and IL-1β by macrophages in response to WBI of mice with both low and high doses [I6, S33]. These two pro-inflammatory cytokines exert a regulatory effect on lymphocytes, promoting their activation. Hashimoto et al. [H20] showed increased expression of the genes coding for TNF-α and IFN-γ in splenocytes of tumour-bearing rats given 200 mGy of WBI, while mRNA of transforming growth factor β (TGF-β) had decreased. These findings suggest immune activation, since IFN-γ plays a key role in both innate and acquired immune defences and also has antitumour properties, while TGF-β is an immunosuppressive cytokine that allows tumour escape from immune destruction. All of these changes might contribute to a shift of the immune response in favour of Th1 differentiation.

95. In contrast to these observations, Gridley et al. found that LDR irradiation (50 mGy at 0.030 mGy/h) enhanced the intracellular expression of IFN-γ in CD3+ CD4+ T-cells from C57BL/6 mice. Surprisingly, it did not result in increased levels of secreted IFN-γ after protracted irradiation alone or when mice were exposed to a protracted low-dose followed by an acute high-dose irradiation [G11]. In the carefully conducted study of Pandey et al. on immunomodulation induced by LDR irradiation, the secretion of IFN-γ by C57BL/6 mouse spleen cells stimulated by Con A was considerably reduced in the group irradiated at 200 mGy. This may appear contradictory to the enhanced cytotoxic T-cell response observed in the same study, but IFN-γ may also have other important regulatory roles in the immune system [P11].

96. Evidence for the suppressive effect of LDR irradiation on tumour growth, metastases and carcinogenesis has been presented. Ishii and colleagues reported a decreased incidence of spontaneous thymic lymphoma in AKR mice as a result of chronic fractionated low-dose whole-body X-irradiation [I1]. In another well-recognized model of thymic lymphoma induced in C57BL mice by fractionated WBI (four acute doses of 1.8 Gy, one per week), preirradiation with 75 mGy given before each 1.8 Gy dose decreased the frequency of tumours from 90% to 63%. This level was further lowered to 43% by continuous WBI at 1.2 mGy/h for 450 days starting 35 days before the first 1.8 Gy dose. Interestingly, continuous irradiation to a total dose of 7.2 Gy over 258 days yielded no thymic lymphoma. In parallel, CD4+ T-cells, CD40+ B-cells and plaque-forming-cell counts in the spleen were significantly increased by continuous 1.2 mGy/h irradiation alone, indicating the involvement of immune activation in tumour suppression by LDR irradiation [I9]. However, other modifying factors, such as DNA repair and elimination of injured cells by apoptosis, are involved in the mechanisms for suppression of tumours. In addition, the complex nature of murine thymic lymphoma, with involvement of cell killing in the aetiology of the tumour, makes the interpretation of the data difficult [C34].

97. Significant suppression of the development of pulmonary tumour nodules was reported by Ju et al. [J8] and Cai [C33], who irradiated mice with single doses of X-rays ranging from 50 to 150 mGy, 24 h before injection of B16 melanoma or Lewis lung cancer cells. These results were corroborated later by Cheda et al. [C21], who injected syngeneic

low-immunogenic sarcoma cells into BALB/c mice 2 h after WBI with 100 or 200 mGy. This resulted in significantly reduced pulmonary tumour colonies. The authors associated the effect with stimulation of NK-cell-mediated cytotoxicity detected in splenocyte suspensions obtained from irradiated mice but not from sham exposed mice.

98. A report of the French Academy of Sciences extensively reviewed published data concerning the dose–effect relationship and carcinogenic risk of low doses of ionizing radiation [F11]. A database of cancer induction by LDR irradiation obtained from 472 different animal experiments was analysed [D30]. The meta-analysis showed that the spontaneous cancer rate fell significantly after LDR irradiation in only 40% of those experiments that could potentially have revealed the effect. It was suggested that, together with other mechanisms, the finding could partly be explained by the stimulation of immunological mechanisms. However, the author states that the statistical strength of the overall observations has not yet been determined. Although many observations show a reduction in the cancer rate and a longer life in low-dose-irradiated animals, these studies should act as a focus for further research in order to confirm or disprove the generality of the effects.

99. The adaptive response to radiation is a biological defence mechanism in which low-dose ionizing radiation (a "priming dose") elicits cellular resistance to the genotoxic effects of subsequent irradiation (the "challenge dose") [S37]. The adaptive response to radiation in animal and human populations, as well as its effects on the immune system, have been extensively considered in the UNSCEAR 1994 Report, annex B [U4].

100. The adaptive response has been observed after WBI. The experiments show that a priming exposure to chronic irradiation can induce radioresistance in mice. The manifestation of this resistance is reduced mortality (from the haematopoietic syndrome) of pre-exposed mice after a challenge acute irradiation [S43]. Yonezawa et al. have reported that pre-exposure of mice to 500 mGy two weeks before a lethal (7–8 Gy) WBI induced marked radioresistance and survival of the mice [Y7, Y8]. Later, using the same priming dose and a challenge dose of 5 Gy for haematopoietic studies, they investigated whether preirradiation favours recovery of pluripotent haematopoietic stem cells. They found that radiation-induced resistance to lethality appeared to be closely related to the recovery of endogenous colony-forming units of the spleen (CFU-S), with a maximum response when the priming dose was given 14 days before the challenge dose [Y5]. In addition, they demonstrated that the adaptive response at a challenge dose of 5 Gy seemed to be induced through a reduction of p53-dependent apoptosis in haematopoietic stem cells [H29].

101. Gong and co-workers [G28] studied thymocyte apoptosis and cell cycle progression induced by WBI in Kumming mice, using priming doses of 25–200 mGy and challenge doses of 1–2 Gy given 6 h after the priming dose. Their results indicated that the percentage of thymocyte apoptotic bodies decreased, the arrest of G1 and G2/M phases diminished and the frequency of cells in S-phase increased. However, when the priming dose was 200 mGy, the adaptive response was no longer induced. Furthermore, a dose-dependent increase in thymocyte apoptosis was found with doses of 250 mGy or higher [M27]. The question of threshold dose for the immune-enhancing effects is critical. It depends on the end point tested, but the dependence on other factors such as animal species and the radiation dose rate is still an open question [S34].

102. Selective changes in the expression of proteins are reported to accompany LDR exposure [S17]. Increased levels of stress proteins were observed in mitogen-stimulated splenocytes of mice exposed to LDR radiation. The biological relevance of this was supported by the demonstration that splenocytes that failed to elevate their constitutive levels of heat shock proteins following LDR irradiation also were unable to increase their capacity to proliferate [N20]. Later, Chen et al. isolated a 10 kD protein from thymocytes after LD WBI. This protein, named RIP10, potentiates spontaneous thymocyte and mitogen-induced splenocyte proliferation, and modulates apoptosis [C23]. In addition, changes in cell cycle and apoptosis-related intracellular and extracellular proteins accompanying increased response to Con A in mouse lymphocytes following WBI were reported [S16].

103. Although growing evidence suggests that low-dose WBI can be immunostimulatory, many of the questions about immunoenhancement remain unanswered and require further experimental studies. One important aspect is the molecular basis of the stimulatory effect of LDR irradiation. Experimental data have been accumulating in this field. It has been reported that the intracellular free Ca^{2+} concentration increases after LDR irradiation and that protein kinase C (PKC) also increases in response to different doses of radiation, leading to the activation of early genes. Another signal pathway involved is the cyclic adenosine monophosphate/cyclic guanosine monophosphate protein kinase A (cAMP/cGMP-PKA) cascade: the cAMP/cGMP ratio falls after LDR irradiation, with PKA responding in the same pattern. A third pathway is phospholipase 2-prostagalandin E2 (PLA2-PGE2), which is also down-regulated. An adaptive response mediated by a feedback signalling pathway involving p38 mitogen-activated protein (p38 MAP) kinase, phospholipase C (PLC) and PKC has been demonstrated [L20].

2. Human data

104. Although few data are available on the effects of low-dose exposures on humans, some reports suggest that chronic low-dose radiation exposure can lead to effects on the human immune system. Chang et al. analysed the immune status in residents of buildings constructed using ^{60}Co-contaminated steel rods [C7]. They evaluated CD3+, CD4+, CD8+ and HLA-DR+ markers in lymphocyte subsets in 196 exposed subjects with a mean cumulative excess

dose of 169 mSv (range 8–1,662 mSv) protracted over 2–13 years. These results were compared with those obtained in 55 close relatives considered to be the non-exposed reference population. They analysis was restricted to individuals with no apparent history of medical conditions that could compromise their immune profile. The mean percentages of CD4+ T-lymphocytes and HLA-DR+ lymphocytes and the CD4+/CD8+ ratio in the exposed individuals were significantly lower than those in the reference population, while total CD8+ cell counts in the exposed individuals were moderately increased compared with the reference population. In addition, changes in the percentages of CD4+ T-cells and HLA-DR+ activated T-cells were significantly associated with radiation dose, while CD4+/CD8+ ratios were only moderately associated with dose [C7]. Low CD4+/CD8+ ratios are observed in primary or secondary immune deficiencies, and this ratio has been proposed as a method for estimation of the cellular immune status [H25]. The results presented by Chang et al. suggest that protracted gamma radiation exposure in a residential environment may induce a dose-dependent decrease of cellular immunity. However, these findings should be interpreted cautiously, taking into account the wide range of cumulative doses and their protraction among the exposed subjects. A new analysis of these results separating subgroups of people with narrower dose ranges could allow higher-quality conclusions to be drawn.

105. Immune status was evaluated by Godekmerdan et al. in 50 radiology workers [G26]. A decrease of CD4+ helper T-cells with diminished levels of immunoglobulins (IgA, IgG and IgM) and complement (C_3 and C_4) was found, suggesting impairment of cellular and humoral immunity. The authors make reference to the fact that there was no significant difference between subjects exposed for more than or less than 5 years. This study does not provide data concerning either cumulative doses or dose rates. There is no mention of the type of medical procedure performed by the subjects involved in this study or the area of the radiology department in which they worked. Thus it is not possible to establish a relationship between these findings and radiation dose.

106. Rees et al. did not find significant changes in the immune profiles of 325 male workers occupationally exposed to external low-LET radiation at the British Nuclear Fuels facility at Sellafield, United Kingdom. The cumulative exposures were >200 mSv in a period of from 19.1 to 45.7 years in one group and <27.5 mSv in a period of from 15.1 to 32.5 years in the other. No statistically significant differences in circulating T- and B-cell total counts, CD4+ and CD8+ T-cell subsets, CD4+/CD8+ ratio or CD3+/HLA-DR+ were observed [R3]. This study took account of possible confounding factors such as age, sex and cigarette smoking, and the sample size was sufficient to substantiate the conclusion that occupational exposure to low doses does not affect the immune profile of workers.

107. Similar findings were reported by Tuschl et al. in employees working at the research reactor of the Austrian Research Centre (Seibersdorf), exposed during the preceding 3 months to very low doses of gamma radiation (from 0.2 to 4.9 mSv). The percentages of CD2+, CD4+, CD8+ and NK cells were investigated in peripheral blood lymphocytes. Data were pooled in two groups of individual doses: <0.5 mSv and >0.5 mSv. Except for a slight increase in the relative number of cells expressing CD2 (a marker of T-cell activation), radiation-associated changes were not observed [T8].

108. Previously, the same group of authors had reported that lymphocytes of radiation workers exposed to 0.14–0.98 mGy/month exhibited an enhanced capacity to repair DNA damage inflicted by an in vitro challenge dose of ultraviolet (UV) radiation [T17]. More recently, Mohankumar et al. have analysed the UV-induced DNA repair capacity of the lymphocytes of 16 healthy, non-smoking radiation workers of the Indira Gandhi Centre for Atomic Research, Kalpakkam, India, who received whole-body gamma exposures ranging between 1 and 6.3 mGy during a period of 3 months prior to the study [M25]. At very low gamma doses (1–1.9 mGy), they found higher UV-induced unscheduled DNA synthesis (UDS) levels in samples receiving gamma irradiation in vitro than in control samples, but there was no such increase in the radiation workers' samples, owing mainly to the large standard deviation values of the means. For doses of over 2 mGy, both in vitro and in vivo irradiated samples show higher UDS levels. De novo synthesis of repair enzymes induced by low-dose ionizing radiation exposure was suggested as an explanation for these results. Particularly in the dose range 3–7 mGy, the UV-induced DNA repair capacity of radiation workers' lymphocytes was higher than that of cells exposed in vitro. These authors tried to explain these findings as an adaptive response of lymphocytes to radiation, due to cell renewal mechanisms, that led to a shift in the lymphocyte population in favour of a cell type with greater DNA repair capacity. They also postulated a possible involvement of the endocrinological system. However, the sample size was too small to confirm the observations and to sustain these hypotheses.

109. Tuschl et al. [T9] investigated some immunological parameters in 10 nuclear power plant (NPP) workers exposed during a 4-week period to external radiation (1.4–9.8 mSv) and tritium inhalation (committed effective doses of 1.2–2.8 mSv). Blood samples were taken 25 days after the start of this exposure period for quantification of lymphocyte subsets and evaluation of their mitogenic response to PHA. Data were compared with reference values obtained in healthy donors. CD4+/CD8+ ratios were increased in NPP workers owing mainly to an increase in absolute numbers of CD4+ T-cells. The authors interpreted these findings as a potentiation of the immune response by low radiation doses and suggested selective cell renewal of CD4+ T-cells as a possible underlying mechanism. Although the tritium burden in these workers was very small (0.47–6.3 kBq/24 h in urine), they postulated that the RBE of beta particles from tritium may account for this effect, which was not observed in other studies of occupationally exposed workers. Nevertheless, the

sample under investigation was too small, and further studies are needed to substantiate this hypothesis.

110. Beta particles from tritium are of greater biological effectiveness than gamma rays and X-rays. At low doses or low dose rates, RBE values of 2–3 have been proposed for the oxide form and even higher values when tritium is bound to organic molecules [S47]. However, depending on the end point and the irradiation conditions, RBE values for beta particles from tritium may greatly differ [M26, T10, T14]. The total number and percentage of leucocyte subpopulations were determined in 54 workers exposed to tritium in the workplace. Tritium contamination was well below the annual limit on intake for occupationally exposed subjects (mean tritium activity in urine: 1.9 kBq/litre). The functional status of leucocytes was evaluated by alkaline phosphatase (AP) and myeloperoxidase (MP) activity staining. While total leucocyte counts did not differ from those of the control group, lymphocyte and eosinophil counts were higher in radiation workers. AP and MP activities were lower in exposed workers [M23]. The author interpreted the increase in lymphocyte counts as a stimulation of the immune system by tritium and eosinophilia as a compensatory reaction of the bone marrow, where tritium had entered and disturbed enzyme synthesis in leucocyte precursors. The author stated that the workers had no clinical manifestations of immunity disorders. The selected end points seem inappropriate for evaluation of both innate and acquired immune response. The biological significance of these data cannot be readily discerned, and these results are inconclusive.

111. Few data have been published concerning the impact on the immune system of people living in high-level natural radiation areas (HLNRAs). Comparison of cord blood samples from newborns from the Kerala coast in India (average dose rates of greater than 1.5 mSv/a) with samples from newborns from areas with lower levels of natural radiation (less than 1.5 mSv/a) did not show any significant difference in the frequency of dicentrics, translocations, inversions or other types of aberration known to be associated with radiation exposure [C26]. Ghiassi-Nejad et al. reported in 2002 that no differences were found either in laboratory tests of the immune system or in haematological parameters among people living in Ramsar, an HLNRA in the Islamic Republic of Iran, where the mean dose rate is 260 mSv/a [G1]. Similar results had previously been reported for people living in Yangjiang, an HLNRA (6.4 mSv/a) in China [Z1]. In this preliminary study, Ghiassi-Nejad et al. did not observe significant differences in the basal frequency of chromosome aberrations in peripheral blood lymphocytes between people living in Ramsar and people living in normal background areas. Following a dose of 1.5 Gy of gamma rays in vitro, lymphocytes of Ramsar residents showed a significantly reduced frequency of chromosome aberrations. The authors interpreted their findings as an adaptive response induced by chronic radiation exposure.

112. Two years later the same group published new results showing a higher frequency of chromosome aberrations in Ramsar residents [G20]. Concerning humoral immunity, IgG and IgA levels were not different, whereas a significant increase in IgE levels was observed in Ramsar residents. The authors interpreted this finding as radiation-induced immunostimulation due to a shift from a Th1 to a Th2 response. While no differences were found in the expression of CD69 (a marker of lymphocyte activation) in unstimulated samples, the expression of CD69 was higher in PHA-stimulated CD4+ helper T-cells of Ramsar residents, a finding that the authors considered as an indication of a higher risk of proto-oncogene activation. However, taking into account the lack of consistency between these two papers [G1, G20], the results are not conclusive, and these hypotheses appear rather speculative. The frequency of chromosome aberrations was also higher in residents from other HLNRAs in Brazil [B18] and China [C22]. It has been reported that smoking plays a more significant role than natural radiation in the induction rate of stable lymphocyte aberrations in those areas [Z2]. However, this statement should be interpreted with caution since a recent meta-analysis from retrospective biological dosimetry data from seven European laboratories indicated that there was a strong variation of translocation yield with age, but no variation was detectable with sex or smoking habits [W17].

3. Remarks concerning low-dose/low-dose-rate irradiation

113. On the basis of the previous paragraphs, the following remarks may be made concerning LD irradiation data. It has been reported in some animal studies that under protracted gamma ray exposure at low dose rates, the normally highly radiosensitive haematopoietic system adapts and becomes radioresistant. Depending on the dose and dose rate, induced the pattern of changes in leucocyte populations can be described as an initial suppressive phase followed by a stable accommodative phase. This pattern of changes is preceded by similar changes in the haematopoietic progenitor cell compartment. However, it was suggested that the recovery of marrow precursors might be incomplete.

114. In animal experiments there is evidence demonstrating that LDR irradiation can produce activation of the immune function. Enhancement of the proliferative response of splenic and thymic lymphocytes to mitogens, enhancement of NK activity and increased secretion of cytokines with regulatory effect on immune cells promoting their activation, inter alia, have been reported. Nevertheless, the data are not entirely consistent, and the observed effects are highly dependent on the range of dose and dose rate, and upon the animal and strain of animal studied.

115. Data demonstrating suppressive effects of LDR exposure on tumour growth, metastases and carcinogenesis have been reported. An association of these effects with enhanced NK activity of splenocytes, higher antibody-dependent cellular toxicity and increased levels of CD4+ cells, CD40+ B-cells and plaque-forming cells in the spleen has been found.

116. An adaptive response to radiation is another phenomenon observed in many systems. Interestingly, radiation-induced resistance to lethality after WBI with a high challenge dose in mice appeared to be closely related to the recovery of CFU-S after the priming dose. It was also related to the reduction of apoptosis in the haematopoietic stem cells, giving insight into the possible mechanisms by which these adaptive processes occur.

117. Regarding human data, while some authors have reported evidence for effects after chronic LDR irradiation, others have not found such evidence. A dose-dependent decrease of cellular immunity, mainly evaluated by the CD4+/CD8+ ratio and HLA-DR+ activated T-cells, was described for residents of buildings constructed with ^{60}Co-contaminated materials, although these findings should be interpreted cautiously. In contrast, no significant changes were observed in the same parameters of workers occupationally exposed to external low-LET radiation in nuclear facilities.

118. Concerning tritium incorporation, it would appear to have a higher RBE than external irradiation at the same doses. This has been observed in both experimental studies and human studies, but the results are inconclusive.

119. In the same way, when the impact on the immune system of living in areas with high levels of natural radiation was analysed, the results were controversial, and the significance of these findings remains unclear.

C. Data concerning high-dose irradiation

1. High-dose-induced immunosuppression

120. The effects of radiation on the immune system generally intensify with the dose received. Massive cell death, inflammation and infection are the acute effects of high-dose radiation exposure. Human data concerning the effects on the immune system of WBI have been widely studied in victims of radiation accidents and in patients undergoing WBI as a conditioning regime for bone marrow transplantation. The effects of high doses of ionizing radiation upon immune system function were reviewed by the Committee in 1988 [U6].

121. Acute radiation syndrome (ARS) occurs after WBI or substantial partial-body irradiation of greater than 1 Gy, delivered at a relatively high dose rate [G22]. With the exception of the haematopoietic syndrome, the other clinical components of ARS (gastrointestinal and cerebrovascular) are not the subject of this annex, and they are reviewed elsewhere [K45, W9]. Since granulocytes and lymphocytes are an essential part of the immune system, profound abnormalities of immune function are expected as a consequence of high-dose WBI.

122. A patient who receives acute external WBI in the range 0.5–1 Gy is generally asymptomatic, and blood counts may be normal or minimally depressed below baseline levels 3–5 weeks after exposure [G23]. The haematopoietic syndrome may be seen following doses of >1 Gy. Acute whole-body doses of below 2 Gy induce mild cytopenia without significant bone marrow damage. Laboratory analysis in cases with WBI of greater than 2 Gy can show an initial granulocytosis, with pancytopenia evident within the first month after exposure [G23]. Mitotically active haematopoietic progenitors are unable to divide after a whole-body dose of >2–3 Gy, resulting in haematological crisis in the following weeks [G22]. During this symptom-free period of ARS (latency phase), the blood-producing cells in the bone marrow begin to diminish and are not replaced, leading to a severe shortage of white blood cells, followed by a shortage of platelets and then red blood cells. The shortage of white blood cells can lead to severe immunodeficiency, increasing the risk of infectious complications and impairing wound healing [D1, G23].

123. The rate of decrease for different leucocytes in the blood after WBI is dependent on their particular cell cycle kinetics. Neutrophils have a relatively short lifespan, and thus they have a tendency to be depleted over a matter of days following acute WBI, owing to radiation-induced damage to their progenitor cells. They show an initial increase within the first few days after doses of >2 Gy; this increase is greater after higher doses. This first "abortive rise" may be due to a cytokine-dependent transient mobilization from bone marrow or extramedullar sites and to accelerated maturation of granulocyte precursors. A progressive neutropenia then occurs, the rate and extent of which are dose-dependent; this may be followed by a second abortive rise following doses of <5 Gy as a result of haematopoiesis recovering from precursor cells. This second abortive rise is not seen following doses of >5 Gy, indicating the failure of haematopoiesis to recover permanently after very high doses, a finding that may be clinically helpful as a prognostic indicator [G22]. The duration of neutropenia may be long, requiring prolonged administration of haematopoietic growth factors, blood product support and antibiotics.

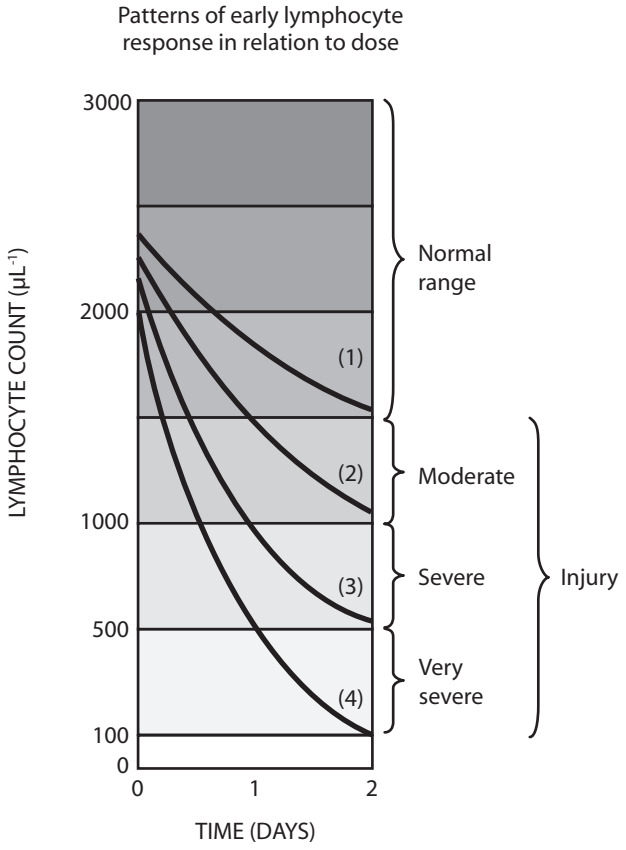
124. In non-uniform exposures, evaluation of cytopenia may be somewhat misleading because the cumulative curve of the granulocytes "averages" the actual production by different portions of the bone marrow that have received different doses. Temporal parameters in such cases are more relevant to the magnitude of the dose, and blood counts are more correlated to the volume of damaged bone marrow. Lymphopenia in non-uniform WBI is more prolonged than in uniform WBI [G34]. When the ARS is associated with cutaneous radiation syndrome, the lymphohaematopoietic suppression impairs wound healing and increases the risk of wound bleeding and infection [B16].

125. A follow-up of neutrophil values for several days post-irradiation was found to correlate well with dose. The time to reach the critical level of 500 granulocytes/mL has been proposed as a dosimetric bioindicator [B30]. However, in many cases of overexposure, an earlier approximation of

dose is required for efficient medical intervention. This can be achieved by counting the decrease of lymphocytes, as their nadir is reached much earlier than for other cell types. The predictability of lymphocyte depletion following high doses of ionizing radiation, which may be recognized within hours of exposure, has allowed the development of biodosimetric models. This approach was originally developed to give a rough categorization of the magnitude of exposure (figure VI).

Figure VI. Classical Andrews lymphocyte depletion curves and clinical severity ranges.

Whole-body doses: curve (1), 3.1 Gy; curve (2), 4.4 Gy; curve (3), 5.6 Gy; curve (4), 7.1 Gy [G3].



126. A mathematical biodosimetric method for evaluating uniform WBI by peripheral blood lymphocyte and neutrophil counts was widely used and validated following the Chernobyl accident [B31, U6]. The doses evaluated by peripheral blood cell counts, chromosome aberrations and electron spin resonance of tooth enamel were highly correlated [B30].

127. A mathematical model for lymphocyte depletion following gamma irradiation, intended only for providing a first approximation of dose, was developed on the basis of accident cases with recorded haematological data and physically reconstructed doses. During the first 8 hours after exposure, the decrease in lymphocytes followed a single-term exponential curve, and the rate constant for this decrease correlated well with dose estimates obtained from other sources of dosimetry [G3]. This technique was further extended to include analysis of various types of criticality accident. Lymphocyte depletion in high-level mixed gamma–neutron accidents was found to be approximately equal, at a given effective dose, to that for gamma ray accidents. This finding indicates that, in terms of lymphocyte depression, the RBE of neutrons could be close to unity [G24].

2. Immune reconstitution

128. Reconstitution of the immune system after radiation-induced bone marrow aplasia has been widely studied in patients undergoing bone marrow grafting. Bone marrow transplantation is characterized by a subsequent period of immunodeficiency, the duration and severity of which vary according to graft manipulation, choice of graft type (donor and source), development of graft-versus-host disease and level of residual thymic activity.

129. During the first month, T-lymphocytes reconstitute by peripheral expansion of the T-cells present in the graft. Thereafter, starting from 100 days after transplantation, the production of substantial numbers of new naive T-cells by the thymus can be detected [D9]. Rapid early expansion of transferred or residual mature T-lymphocytes of extrathymic origin results in inversion of the CD4/CD8 ratio, which persists primarily as a result of delayed CD4+ T-cell recovery. Normalization of the CD8+ subset after 60 days was reported, while persistent CD4+ reduction has been observed after 2 years, and normalization of the CD4+ subset was achieved only after 6 years in a long-term study [L4]. The different behaviour in this immune reconstitution may be explained by an extrathymic origin of CD8+ cells, while the CD4+ subset recovery, which is thymus-dependent, is impaired in the adult population. In children, who are characterized by having greater amounts of active thymic tissue and more effective thymopoiesis than adults, there is a more rapid recovery of CD4+ T-cells, which express a naive phenotype (CD45RA+). This finding may have significant implications for attempts to generate protective immunity against pathogens and clinically relevant antitumour responses by vaccination strategies in the post-transplantation period [P7].

130. Oligoclonal B-lymphocyte repopulation can be demonstrated in the early post-transplantation period, achieving normal values after 90–120 days [L4]. Serum immunoglobulin levels usually decrease post-transplantation, followed by a gradual increase and normalization in a sequential pattern similar to that identified in neonates. Recovery initially occurs in IgM levels (2–6 months), followed by IgG levels (3–18 months) and finally IgA levels (6–36 months), the time depending to some degree on conditioning and graft characteristics [P7]. Cooperation between T-cells and B-cells is necessary for antibody production. Thus, although B-cells transferred with the graft may confer temporary protection against pathogens, antigen-specific T-cells must be regenerated in order to ensure sustained B-cell competence [L4].

131. Reconstitution of innate immunity following transplantation is characteristically more rapid than for acquired immunity. Most studies have shown early normalization or even rebound increases in NK cell numbers in the early months [P7]. NK cells might provide an efficient defence against pathogens and residual tumour cells, especially immediately after transplantation, where the lack of cooperation between T-cells and B-cells results in an inability to produce antibodies.

132. The results presented all support the conclusion that normalization of immunological function is achieved within several years after transplantation. The functional capacity of the recipient thymus appears to be the dominant influence on thymus-dependent reconstitution. Thymus-independent reconstitution, which accounts for the majority of the early reconstitution, might be predicted to depend more heavily on stem cell sources.

3. Remarks concerning high-dose irradiation

133. As discussed in the preceding paragraphs, acute WBI in the range 0.5–1 Gy is generally asymptomatic, and leucocyte counts may be minimally depressed or even normal. Acute radiation syndrome (ARS) occurs after acute whole-body or substantial partial-body exposure of >1 Gy. While mild cytopenia is observed within the range 1–2 Gy, initial granulocytosis (first abortive rise) followed by pancytopenia within the first month result from whole-body doses of greater than 2 Gy. A second granulocyte abortive rise may be seen when the whole-body dose is below 5 Gy. In non-uniform exposures, temporal blood cell parameters are more relevant for dose estimation than the magnitude of cytopenia. Mathematical models have been developed for correlating the dose with the kinetics of lymphocyte depletion during the first hours after exposure.

134. Acquired immune reconstitution after radiation-induced bone marrow aplasia includes both thymus-dependent and thymus-independent pathways. T-lymphocytes reconstitute during the first month by peripheral expansion, and, during the following months, new naive T-cells begin to appear from the thymus. However, an inversion of the CD4/CD8 ratio persists for several years. While CD8+ T-cells normalize within two months, the recovery of CD4+ T-cells may be achieved within 6 years. Central T-cell recovery in adults is delayed relative to that in children, probably owing to differences in thymic function. B-lymphocyte repopulation takes place during the first 4 months. Following an initial decrease of immunoglobulin levels, recovery gradually occurs in IgM levels (2–6 months), IgG levels (3–18 months) and Ig A levels (6–36 months). Innate immune reconstitution is more rapid than reconstitution of acquired immunity. NK cells normalize or even increase in the early months.

D. Influence of dose rate and radiation quality on immune response

135. Several studies have examined the immunomodulating effects of whole-body exposure to different qualities of radiation. The driving forces for these investigations are the challenges created by exposures in the space flight environment (which are dominated by charged particles [S21, S28]), clinical use of proton radiation in the management of cancer [L9, S23] and exposures due to radon and its progeny [N1].

1. Low-LET exposures

136. The effects on the immune system of radiation, such as gamma rays or X-rays, have been extensively investigated and reviewed [A13, S1, U8, U10]. Variability in the biological response may reflect differences in the total dose, dose rate, end points measured and time of evaluation post-exposure. Analysis of the influence of each of these variables is of importance not only in radiation therapy but also for environmental or occupational radiation exposure [G8].

137. Pecaut et al. [P6] investigated early effects on mice of gamma ray doses of up to 3 Gy at low and high dose rates. They observed a significant dose-dependent loss of spleen and thymus mass, which was somewhat independent of dose rate. Decreasing lymphocyte and leucocyte numbers in the blood and the spleen with increasing dose, as well as dose-dependent decreases in lymphocyte subpopulations (CD4+ helper T-cells, CD8+ cytotoxic T-cells and CD19 B-cells) were observed at both dose rates. While the percentages of CD4+ increased with increasing dose (with some differences between blood and spleen), the percentages of the CD8+ population remained stable in both compartments, and CD19+ cells declined markedly with high or low dose rate. The number and proportion of NK cells remained stable. Because of the differences in phenotypic radiosensitivities, a decrease in lymphocyte counts sometimes results in a proportional enrichment of specific cell types. Overall, these data indicate that the changes observed were highly dependent on dose but not on dose rate, and that cells in the spleen are affected more by dose rate than those in the blood.

138. Similar data have been reported for high-dose treatment under comparable experimental conditions [H11, W5]. A more pronounced dose-rate effect was seen in spleen mass, probably owing to a change in homing receptors in circulating populations of splenic endothelial cells [P6]. It has been demonstrated that ionizing radiation can up-regulate the expression of ICAM-1 and E-selectin, both of which are important in leucocyte trafficking. CD44, a molecule involved in the migration and homing of immune cells, was altered in irradiated lymphoid cells [H3, N1].

139. Using the same animal model, the functional characteristics of splenocytes and cytokine expression was evaluated after WBI at various total doses and at low and high dose rates [G8]. An assay of spontaneous blastogenesis in

leucocytes is sometimes performed in order to determine the proliferative capacity and activation of lymphocytes. Mitogen-induced cell proliferation is employed extensively to assess the general responsiveness of lymphocytes to stimulating agents. The data showed that at 4 days post-irradiation, increasing doses resulted in a rise in spontaneous blastogenesis in blood and spleen leucocytes. This finding was correlated with a reduction in white blood cells [P6], and suggested that cell regeneration was in progress. Splenocyte response to LPS (bacterial lipopolysaccharide), a potent non-specific stimulator of B-cells, was inversely related to radiation dose. This finding is consistent with the demonstration that B-cells are the most radiosensitive lymphocyte subpopulation [C6, P6, W14]. Lymphocyte responsiveness to T-cell mitogens (PHA and Con A) was shown to depend on experimental conditions. A decreased ability of splenocyte mononuclear cells to respond to mitogens after WBI (0–7 Gy) was described by Harrington et al. [H5].

140. Acute doses of some centigrays, as well as chronic WBI, can enhance mouse splenocyte response to these mitogens [L15, N13]. Furthermore, WBI induced changes in expression of CD25 (IL-2 receptor) and CD71 (transferring receptor) activation markers [M7]. IL-2 secretion by PHA-activated splenocytes was reduced in a dose-dependent manner. Plasma levels of transforming growth factor β1 (TGF-β1) and splenocyte secretion of tumour necrosis factor α (TNF-α) were not affected by either the dose or the dose rate of radiation [G8]. The data demonstrate that the responses of the blood and the spleen for the immune parameters studied were largely dependent upon the total dose of radiation and that dose rate was not a significant factor.

141. Even though the quantity of energy deposited is minimal, a repetitive irradiation inflicts a series of small insults on the tissue. Each delivered fraction contributes to the development of inflammation. For a repetitive radiation exposure with low to medium doses, the amount of the cumulative dose is more important than the number of fractionated doses. Gremy et al. [G32] showed a modulation of expression of inflammation mediators during fractionated gamma radiation restricted to a colorectal zone in the rat (5 Gy 3 times a week, maximum dose 45 Gy). The IL-1β mRNA showed an overexpression from a cumulative dose of 20 Gy. The level of IL-10 mRNA was highly repressed at 45 Gy. The monocyte chemoattractant protein 1 (MCP-1) chemokine expression progressively increased with the cumulative dose, while IL-8, less overexpressed, went to a maximum expression at 20 Gy. Some signalling pathways were altered, exacerbated or suppressed, triggering a neutrophil recruitment to the inflammatory site at the end of the irradiation protocol.

2. High-LET exposures

(a) Neutrons

142. Neutrons have been shown to have high values of relative biological effectiveness (RBE) for some biological end points, including chromosome aberration induction in human lymphocytes. Immediate early death of lymphocytes and thymocytes after neutron irradiation seems to be comparable to that for low-LET gamma rays and X-rays when assayed by apoptosis, suggesting that the neutron RBE is close to unity for this end point. Moreover, the route to cell death is independent of dose rate. These observations are in marked contrast to the much larger RBE values and dose-rate effects typically seen in many systems of clonogenic survival, replicative death and chromosome aberration formation.

143. Warenius and Down compared apoptosis in mouse thymocytes following exposure to low doses of fast neutrons (62.5 MeV) and photons (4 MeV). Both the time course and the radiation dose–response curves were similar for high- and low-LET radiation modalities [W16]. Vral et al. examined the effectiveness of fast neutrons compared with ^{60}Co gamma rays for inducing apoptosis in human lymphocytes. Doses ranging from 0.05 to 5 Gy were applied at 0.2 Gy/min (fast neutrons) or 1.5 Gy/min (gamma rays). To investigate the role of DNA repair in apoptosis induction, they also performed irradiations at low dose rates (0.006 Gy/min). They found that apoptosis induction was independent of LET and dose rate (the calculated RBE for fast neutrons was close to unity), suggesting that the initial DNA damage, as opposed to DNA repair, dominates the induction of apoptosis in resting lymphocytes [V7]. Ryan et al. examined apoptosis in human lymphocytes following doses of between 0.25 Gy and 5 Gy of low-energy fast neutrons (280 keV mean energy) and of ^{137}Cs gamma radiation. They found that neutrons are equally as effective as gamma radiation at inducing apoptosis in lymphocytes [R23]. Goans et al. analysed lymphocyte depletion kinetics in various types of criticality accident (high-level mixed gamma/neutron fields). The results were approximately equal, at a given effective dose, to those for gamma accidents, suggesting that the RBE of neutrons could be close to unity for this end point, regardless of the structure of their energy spectra [G24].

144. Huiskamp et al. compared the effect of WBI with fission neutrons (1 MeV mean energy) and 300 kVp X-rays on the supportive role in T-cell differentiation of the thymic stroma of neonatal CBA-H mice, after transplantation to athymic nude mice. Doses varied from 2.75 Gy to 6.88 Gy (0.1 Gy/min) for neutrons and from 6 to 15 Gy (0.3 Gy min) for X-rays. Although irradiation had no effect on the stromal and T-cell composition of grafts, the graft size decreased in a dose-dependent manner with an RBE of 2.1 for fission neutrons [H32]. Holl et al. evaluated the effect of WBI with 65 MeV neutrons or 15 MeV X-rays on spleen cells of BALB/c mice following doses ranging from 0.2 to 3 Gy. Their results indicated that the RBE of neutrons differs depending on the end point selected, since they found that the level of apoptosis was equal for high- and low-LET radiation (RBE = 1), whereas spleen weight and cellularity were reduced to a greater extent by fast neutrons (RBE = 2) [H31].

(b) Protons

145. Since cells of the immune system are among the most radiosensitive in the body, an investigation of the effect of proton radiation on these cells would support more realistic estimates of the risks associated with space flight. Furthermore, a better understanding is needed of the biological interactions following exposure to protons, in view of the increasing number of patients undergoing proton radiotherapy.

146. Both early and long-term effects on the immune system of mice undergoing whole-body proton irradiation have been reported [G9, K2, P4]. Gridley et al. drew attention to lymphoid organs and specific leucocyte populations of mice receiving WBI with 0.5–3 Gy of 250 MeV protons at two dose rates: 1 cGy/min and 800 mGy/min [G9]. Four days post-irradiation, highly significant dose-dependent reductions were observed in the mass of the thymus and the spleen, and in the numbers of leucocytes and CD3+ T-cells, CD4+ helper T-cells, CD8+ cytotoxic T-cells and CD19+ B-cells in both blood and spleen. A less pronounced dose effect was noted for NK cells in the spleen. Monocyte (but not granulocyte) counts in blood were highly dose-dependent; the numbers for each population tended to be lower with high-dose-rate than with low-dose-rate irradiation. Increases in the percentage of CD3+, CD4+ and NK cells, and in the CD4+/CD8+ ratio, were noted with increasing doses. A significant dose-rate effect was found in the percentages of T- and B-cell monocytes and granulocytes, and in the CD4+/CD8+ ratio. The majority of the dose-related decreases in the spleen were highly linear. Results from gamma- and proton-irradiated groups were similar, although proton irradiation gave consistently lower values in some measurements. Very rough calculations of RBE for protons ranged from 0.82 (based on percentage of B-cells in the spleen) to 1.55 (based on percentage of NK cells in the spleen) [G9]. RBE values for CD4+ T-cells ranging from 0.9 to 1.4 and for CD8+ T-cells from 0.7 to 0.9 were reported by Radojcic and Crompton, who compared the induction of apoptosis in human peripheral lymphocytes following irradiation with 300 kVp X-rays or 138 MeV protons [R18].

147. In other studies [K2, K3] that also directly compared the effects of these two forms of radiation at the same dose (dose: 3 Gy; proton dose rate: 0.4 Gy/min and 1 Gy/min; photon dose rate: 0.3 Gy/min), CD19+ B-cells were the most radiosensitive, although recovery back to normal levels was observed by day 15. T-cell (CD3+) and CD4+ helper T-cell recovery was evident by day 29, while CD8+ cytotoxic T-cell counts remained significantly below normal. Plasma TGF-β1 was elevated on day 7 in the ^{60}Co-irradiated but not in the proton-irradiated mice. However, few differences in assay results were seen between animals exposed to protons versus photons.

148. Pecaut et al. [P5] assessed the functional characteristics of mouse leucocytes following whole-body proton irradiation at doses of between 0.5 and 3 Gy (dose rates of 10 mGy/min or 800 mGy/min). Treatment with protons caused a significant dose-dependent increase in spontaneous blastogenesis in the blood and the spleen, suggesting that acute cell death had stabilized by day 4 post-irradiation, and that DNA synthesis and regenerative mechanisms were increasingly stimulated by an increasing magnitude of cell depletion. The authors also observed a greater effect of irradiation by protons than by gamma rays at the same doses. A dose-dependent decrease in the response to T- and B-cell mitogens was observed, and the splenocyte response to Con A was significantly lower than that of the gamma-irradiated group. The underlying mechanism for this effect is not known. The authors suggest that it may have been due to greater impairment of DNA synthesis in the T-cells capable of responding to Con A. Although the dose rates employed represented a range within which dose-rate-dependent changes in sublethal damage do occur, no dose-rate effects were observed.

149. Although a large number of studies have provided experimental results of long-term effects of radiation on the immune response [C16, V1], there are few data on effects following proton irradiation. Pecaut et al. [P4] studied the immune status of mice exposed to 3 or 4 Gy of 250 MeV protons and sacrificed at day 122 post-irradiation. The main points that emerged are the following:

- There were no effects on thymus, spleen or liver mass;
- There were significant dose-dependent decreases in macrophage, CD3+ CD8+ cytotoxic T-cell, NK cell, platelet and red blood cell populations;
- In contrast, dose-dependent increases were observed in spontaneous, but not in mitogen-induced, blastogenesis;
- Effects of radiation were more evident in leucocytes in the spleen than in the peripheral circulation.

(c) Densely ionizing particles

150. Laboratory studies with mice have provided evidence for acute and chronic effects of high-LET particles on the immune system. Utilizing carbon ions, Erofeeva et al. [E4] and Grigorenko et al. [G12] reported changes in the cell architecture and distribution of lymphoid cells in both the spleen and the thymus that persisted at 60 days post-irradiation. Gridley et al. [G10] evaluated the effects of ^{56}Fe and ^{28}Si irradiation on the lymphoid cells and organs of mice at days 4 and 113 after whole-body exposure. In the short term, dose-dependent decreases were noted in spleen and thymus masses, but there was a greater dependence on dose for thymus mass than for spleen mass. With low-LET radiation, in contrast, the spleen mass is more linearly dependent on dose than is the thymus mass [G10]. This suggests that the response of different lymphoid organs may be dependent on radiation quality. Irradiation resulted in a marked reduction in the number of lymphocytes and granulocytes on day 4

post-irradiation. Interestingly, it was observed that high-LET radiation might shift the nadir of peripheral neutropenia to an earlier point. The radiosensitivities of lymphocyte subpopulations were consistent with those obtained using gamma rays or protons. There were no significant differences in the response to T- and B-cell mitogens or secretion of IL-2 and TNF-α by PHA-stimulated splenocytes. In contrast, proton irradiation induced significant depression in the response to all three mitogens [P5]. Overall, the radiation-induced changes on day 4 were significantly more pronounced in the blood than in the spleen.

151. By day 113 post-irradiation, most of these radiation-induced changes were no longer evident. However, B-lymphocyte numbers and percentages in blood were significantly increased. The percentages of total T-cells and CD8+ T-cells were low in both blood and spleen. According to the authors [G10], these findings suggest that ^{56}Fe irradiation may have compromised cell-mediated or acquired immune responses. These long-term effects seen with 2 Gy ^{56}Fe irradiation were not observed following exposure to 2 Gy ^{28}Si ions. Exposure to ^{28}Si ions did, however, result in increased responsiveness to PHA and LPS, and in lower numbers and percentages of NK cells in both blood and spleen. Thus it appears that exposure to ^{28}Si ions, which have a depth–dose profile considered to be optimal for maximizing high-LET particle effects, may result in chronic immune dysfunction.

152. The above observations differ somewhat from those reported from examination of the long-term effects of exposures to 250 MeV monoenergetic protons, where irradiated animals had significantly increased basal DNA synthesis and no differences in mitogen-induced blastogenesis. These data suggest that different immunomodulatory consequences may be induced by densely ionizing particles.

(d) Radon

153. Data concerning health effects of radon exposure are reviewed in annex E, "Sources-to-effects assessment for radon in homes and workplaces". Only some data concerning the effects of radon exposure on the immune system will be reviewed here. Since previous studies have demonstrated that exposure to radon can trigger genotoxic damage in rat macrophages and deep-lung fibroblasts [K10], it is possible that lymphocytes migrating through the blood or the lymphatic circulatory systems of the lung may be exposed to alpha particles from radon progeny.

154. The effect of radon on the immune response was studied by Nagarkatti et al. [N1]. Mice were exposed 18 h/d for 10 (or 25) days to a concentration of radon and its progeny giving a total cumulative exposure of 1,000 (or 2,500) working level months (WLMs)[1]. A marked decrease in the total cellularity of most lymphoid organs (such as the thymus, spleen, peripheral lymphoid nodes and lung-associated lymph nodes) was observed compared with controls. The percentage of T-cells increased while that of non-T-cells decreased in peripheral lymphoid organs at both doses. It was interesting to note that in the thymus at 2,500 WLMs, a dramatic decrease in the number of CD4+ CD8+ T-cells and an increase in CD4– CD8– T-cells were observed.

155. The exact mechanism by which radon affects T-cell differentiation in the thymus is not clear. Radon exposure led to differential expression of CD44 in lung-associated lymph nodes. CD44 is an adhesion/homing receptor involved in lymphocyte and macrophage homing to lymphoid and other organs. These data suggest that lymphocytes and macrophages may migrate in different patterns to other organs. In the lung-associated lymph nodes, where one would predict the largest number of damaged cells to be present, there was a significant decrease in T-cell responsiveness to mitogens, while the B-cell response was not affected. This may be due to the fact that in lung-associated lymph nodes there may be a marked loss of macrophages, the accessory functions of which are essential for T-cells to respond to mitogens. Interestingly, radon exposure caused an increase in the T- and B-cell responsiveness to mitogens in the spleen and peripheral lymph nodes.

3. Remarks concerning the influence of dose rate and radiation quality

156. Overall, the data presented in the preceding paragraphs show that both high and low dose rates of sublethal gamma radiation induce significant depression in the majority of the parameters evaluated at early times after irradiation. The changes observed were highly dependent on total dose but not on dose rate.

157. The RBE of neutrons differs depending on the end point considered. The level of lymphocyte apoptosis is almost equal for neutron and photon exposures, whereas immune organ weight and cellularity are reduced to a greater extent by neutron irradiation.

158. The response of mononuclear cells following proton irradiation is highly dependent on the total dose but not on dose rate, at least in the range of doses and dose rates analysed. Some cell types exhibited differences in the response to proton versus photon radiation, proton irradiation giving consistently lower values. Significant depressions in some lymphocyte subpopulations were observed after long-

[1] A working level (WL) is a unit of concentration in air of the potential alpha energy released from the decay of radon and its daughter products. The WL is defined as any combination of the short-lived radon daughters in one litre of air that results in the ultimate release of 1.3 × 105 MeV of potential alpha energy. Exposure of a worker to this concentration for 170 hours (a working month) results in an exposure of one working level month (WLM). However, while the cumulative exposure expressed in working level months provides an estimate of the exposure to radon and its decay products (primarily of the bronchial epithelial tissues of the lung), it does not provide a direct measure of the dose to lymphoid tissues. (See annex E for further discussion of the dose due to radon and its decay products.)

term evaluation. Increased spontaneous blastogenesis is a relatively persistent phenomenon throughout short-term to long-term evaluations.

159. Lymphoid cells and tissues are markedly affected by high-LET radiation at relatively low doses, and some rearrangements persist long after exposure. Depression in the percentage of cytotoxic T-cells, and enhancement in the total number of lymphocytes and B-cells, were observed at long times post-irradiation. Increased basal DNA synthesis is a persistent phenomenon.

160. Radon exposure induces marked changes in thymus subpopulations and alters the expression of CD44, a molecule involved in the migration and homing of immune cells. A significant decrease has been observed in T-cell responsiveness to mitogens in lung-associated lymph nodes.

E. Radiosensitivity of lymphocyte subsets

1. General considerations

161. The dose required to induce a defined percentage of cell death in a given cell population defines the level of radiosensitivity of that cell population [N19]. Although the sensitivity of different lymphocyte subsets to ionizing radiation has been extensively studied, there are still controversial results in the literature. The radiosensitivity of lymphocytes is related to the population under study. For B-lymphocytes it depends on their degree of differentiation, and for each subset of T-lymphocytes it depends on their state of activation. Activated lymphocytes have long been known to be more resistant to ionizing radiation than their resting (non-activated) counterparts [A13]. The radiosensitivity of lymphocyte subsets is higher when irradiation is performed on sorted purified lymphocyte subp opulations rather than on unsorted peripheral blood lymphocytes irradiated as a whole [M29].

162. Radiation-induced apoptosis in lymphocytes is triggered by two pathways: the mitochondrial pathway (intrinsic pathway) and the death receptor pathway (extrinsic pathway). Apoptosis via both pathways is mediated by the activation of a series of cysteine proteases, the caspases. Although both pathways of apoptosis involve activation of common effector or executioner caspases, they differ in the activation of apical or initiator caspases. The first one, through Bax protein, induces cell death by acting on mitochondria and accounts for the differential radiosensitivity among lymphocyte subpopulations. The second one is mediated by plasma membrane signals via interaction of the tumour necrosis factor receptor (TNFR) with its ligand (tumour-necrosis-factor-related apoptosis-inducing ligand—TRAIL) and does not seem to be related to intrinsic radiosensitivity of lymphocyte subsets [M29]. Interactions between TRAIL and ceramide signalling pathways in regulating apoptotic death have been reported [L25]. Apoptosis of lymphocytes through the death receptor pathway also occurs in physiological conditions. Activation-induced cell death is a form of apoptosis in which activation of T-cells occurs through proper engagement of TCRs by specific antigen bound to an HLA molecule, and influenced by antigen concentration and co-stimulatory signals. Activation-induced cell death plays an essential role in both central and peripheral deletion (clonal deletion) events involved in self tolerance and homeostasis [Z4].

2. Review of published data

163. In 30 patients who were treated with 12 Gy (fractionated) of WBI, it has been reported that T- and B-lymphocytes showed a sharp radiation-induced decrease, with B-lymphocytes being the most sensitive population [C11]. CD3+ CD4+ CD45RO+ (memory helper T-cells), CD3+ CD4+ CD45RA+ (naive helper T-cells), CD4+ and CD8+ cells appeared equally sensitive. The CD34+ cell subset (progenitor/stem cells) remained basically unchanged, and the CD3– CD56+ CD16+ (an NK cell subset) was relatively radioresistant compared with the other lymphocyte subsets. This study provides evidence that T- and B-cell subsets seem to be highly radiosensitive in vivo, while progenitor/stem cells and NK cells seem to be more radioresistant. Similar findings were reported in eight patients undergoing external beam radiotherapy to the pelvis [L21]. NK cells were the most radioresistant and B-cells the most radiosensitive lymphocytes. No significant differences between helper T-cells and suppressor/cytotoxic T-cells were observed.

164. Data concerning the radiosensitivity of CD4+ helper–inducer and CD8+ cytotoxic T-cells are controversial. Several factors (e.g. the radiation dose, the end point selected and the time period over which this end point is evaluated) could modify the findings. The tendency to spontaneous and radiation-induced apoptosis of lymphocyte subpopulations differs among individuals. In addition, age and sex are factors that may influence the apoptotic response [S8]. Nakamura et al. did not find any difference in the radiosensitivity of CD4+ and CD8+ cells [N4]. By using an in vitro lymphocyte colony assay, they demonstrated that D_{10} (the dose required to reduce the surviving fraction to 10%) was similar for these two types of cell: 3.13 ± 0.10 Gy (mean ± SD) for CD4+, 3.34 ± 0.50 Gy (mean ± SD) for CD8+ and 3.14 ± 0.17 Gy (mean ± SD) for unsorted cells.

165. Several studies have considered B-cells to be more radiosensitive than T-cells [P12, R5, S8, V1, W14]. However, taking into account the spontaneous apoptosis of each different lymphocyte subpopulation, Wilkins et al. [W4] arrived at a different conclusion. Using the modified neutral comet assay (MNCA) they demonstrated that, following 1 Gy of low-LET radiation, CD8+ T-cells had the highest radiation-induced apoptotic fraction, followed by CD4+ T-cells.

166. Crompton and Ozsahin [C17], using a method based on assessment of DNA internucleosomal degradation through flow cytometry, demonstrated that CD8+ T-cells

were the most radiosensitive population, followed by CD19+ B-cells. For interpreting these results, it should be taken into account that the frequency of radiation-induced apoptosis was calculated by subtracting the fraction of apoptotic cells at 0 Gy (spontaneous apoptosis) from the fraction induced by the radiation treatment of samples. Although the results were identical to those reported by Stewart et al. [S30], it is important to keep in mind that the latter studies were performed using high-dose-rate irradiation.

167. Reports concerning the effects of ionizing radiation on NK cells have been contradictory. As NK cells exhibit so large a degree of interindividual variability, it is difficult to reach firm conclusions concerning the effect of ionizing radiation on this lymphocyte subpopulation. Seki et al. [S11] reported that NK cells were the most radiosensitive in vitro and that CD8 cells and B-cells showed lower susceptibility to radiation, whereas CD4+ cells were relatively radioresistant. On the other hand, Mori and Desaintes reported that, among peripheral blood lymphocyte subpopulations, NK cells are more highly resistant in vitro to ionizing radiation than are T- and B-lymphocytes [M28]. In exploring the potential radioprotective effect of different types of cytokine on radiation-induced apoptosis, it was shown that IL-2 inhibited the apoptosis of NK and that IL-2, IL-4 and IL-7 were able to rescue both CD4+ and CD8+ T-cells from radiation-induced cell death [S11]. The viability of B-cells in culture was maintained by the presence of IL-4 but not by other cytokines. The authors speculated that the protective effect by each cytokine might be attributed in part to an enhancement of cellular induction of the expression of the bcl-2 protein family. However, while overexpression of bcl-2 leads to the inhibition of cell death [V5], it is important to remember that there exist in the immune system apoptotic pathways unaffected by bcl-2 expression, such as the CD-95-mediated pathway [S31].

168. Fuggetta et al. have shown impaired NK activity in vitro after gamma irradiation (20 Gy) [F6]. IFN-β (200 IU/mL) was able to completely reverse this inhibitory radiation-induced effect, but was not able to modify the number of CD16+ and CD56+ cells that died by apoptosis after irradiation. Concerning in vivo radiation exposure, no changes in NK cell activity were found in 1,341 atomic bombing survivors residing in Hiroshima [B5].

169. The molecular basis of the differential radiation sensitivity among lymphocyte subpopulations remains unclear [M29]. It has been proposed that the higher radiosensitivity of B-lymphocytes is due to a lower activity of DNA-dependent protein kinase (DNA-PK) in these cells [M14]. Also, the radiosensitivity of lymphocyte subsets has been related to intrinsic differences in basal expression level of specific genes, particularly those related to *Bcl-2* family genes such as *Bax*, *Bcl-2* and *Bcl-X* [I11].

170. However, a recent study using microarray analysis demonstrated that basal gene expression does not account for differential lymphocyte radiosensitivity. Mori et al. [M29] investigated the profile of gene expression in peripheral lymphocytes 8 hours after in vitro X-ray irradiation. Cell suspensions of magnetically purified lymphocyte subpopulations were irradiated with a dose of 1 Gy at a dose rate of 0.3 Gy/min. A total of 18,433 unique sequences were screened for their transcriptional response to ionizing radiation. The authors found 102 genes whose expression was modulated by radiation exposure: 75 were up-regulated and 27 were down-regulated. The most strongly activated genes belonged to apoptosis, cell cycle and DNA repair functional classes, with a clear predominance of the p53 pathway. The authors reported no difference in the basal level of expression of the proapoptotic genes among lymphocyte subsets. In contrast, their levels of activation following exposure to X-rays differed among cell subtypes. For example, *Bax* expression levels increased in lymphocytes in the following order: NK cells < CD4+ T-cells < CD8+ T-cells < CD19+ B-cells, reflecting their differential radiosensitivity [M29]. On the other hand, the expression level of the *TNFR* gene in irradiated cells did not differ among these lymphocyte subsets.

3. Remarks concerning the radiosensitivity of lymphocyte subsets

171. The data presented in the preceding paragraphs indicate that the radiosensitivity of lymphocyte subsets, in terms of the radiation dose required to induce a defined percentage of cell death, depends on several factors:

- Lymphocyte subset under study;
- Irradiation performed on resting or activated lymphocytes;
- Degree of differentiation;
- Irradiation performed on sorted or unsorted subpopulations;
- Influence of cytokines;
- Spontaneous apoptosis exhibited by the subset under study;
- Age and sex of the donors

172. B-lymphocytes (CD19+) seem to be the most radiosensitive subset, both in vivo and in vitro. However, when estimation of radiation-induced apoptosis is performed by subtracting spontaneous apoptosis, CD8+ T-cells exhibit higher radiosensitivity. Most of the data show no difference in radiosensitivity between CD4+ and CD8+ T-cells. While NK cells are relatively radioresistant in vivo, particularly the CD56+ CD16+ NK subset, data concerning in vitro irradiations are more controversial. The level of radiation-induced activation of apoptosis-related genes differs among these subsets, probably reflecting their differential radiosensitivity, as follows: NK cells < CD4+ T-cells < CD8+ T-cells < CD19+ B-cells.

F. Alterations of the immune response after prenatal irradiation

173. Radiation-induced impairment of both humoral antibody and cell-mediated responses have been reported in numerous experimental and epidemiological studies. However, very few data are yet available concerning radiation effects on the immune system following in utero exposure. These data mainly concern numerical or structural changes, and thus they cannot be directly related to effects on the immune function. The developing haematopoietic system is very sensitive to ionizing radiation. The mammalian embryonic haematopoietic system becomes functional in the yolk sac during organogenesis; in parallel, additional haematopoietic activity takes place in the aorta, gonads and mesonephros. Later the function shifts to the foetal liver and spleen, and finally to the bone marrow [T11, U16]. Foetal liver haematopoiesis is first detectable at about the sixth week of gestation in humans. Although the spleen has largely ceased haematopoiesis in humans by the time of birth, it may regain haematopoietic function in abnormal situations [H27].

1. Animal data

174. Grande and Bueren irradiated mice at different stages of development with a single dose of 500 mGy of X-rays. Mice irradiated on days 13 and 17 post-conception showed a significant reduction in the proportion of femoral bone marrow granulocyte–macrophage colony-forming units (CFU-GM) one year after irradiation. This effect was manifested neither in mice irradiated on day 4 post-conception nor in those irradiated in early post-natal life (2 days, 8 days and 12 weeks old) [G6]. The effects of ionizing radiation on the embryo/foetus may differ according to the developmental stage at which the exposure takes place. In this study, the impairment in the CFU-GM population was observed when mice were irradiated after midgestation. As seen in figure VI, this is the period of haematopoetic progenitor cell expansion (day 13) and bone marrow and thymus colonization (day 17) in rodents. This study indicates that, for most stages of development in the mouse, a single acute dose of X-irradiation of 500 mGy is below the threshold dose capable of inducing deterministic effects in the mouse haematopoietic system, although it reveals the induction of a significant impairment in the CFU-GM population when irradiation is given at the late stages of embryonic development.

175. Haematopoiesis is the product of two components: the haematopoietic tissue and the regulatory stromal microenvironment in which it resides. The role of both components in radiation-induced haematopoietic effects remains controversial. Yang et al. [Y3] proposed that irradiation at midterm gestation damages the developing regulatory microenvironment but not the haematopoietic stem cell population that it hosts. To demonstrate this, the authors used an experimental model of prenatal irradiation in combination with cross-transplantation experiments. They found that 1.8 Gy of gamma irradiation given to mice at midgestation caused a 40% reduction in the haematopoietic stem cell population (CFU-S), which persisted up to at least 6 months of age. Spleen colony formation after sublethal doses of gamma rays reflected this reduced complement of endogenous stem cells. The regulatory haematopoietic microenvironment, measured as fibroblastoid colony-forming cells (CFU-F), was similarly depleted. The quality of the stem cell population in the offspring of irradiated mothers was not affected, since normal growth of the CFU-S population was observed after transplantation into standard recipients. In contrast, when used as recipients of a bone marrow transplant from either normal or irradiated offspring, the offspring of irradiated mothers were unable to support normal growth. Compared with normal offspring, there were 70% fewer CFU-F in the femur 1 month after bone marrow transplantation when the offspring of irradiated mothers were used as transplant recipients. This confirmed a reduced capacity to host normal stem cells and also indicated that CFU-F in the transplant were unable to compensate for the poor microenvironment in irradiated offspring hosts.

176. The developing haematopoietic tissues are very sensitive to ^{239}Pu contamination. Mason et al. [M1] studied postnatal haematopoietic function in the offspring of pregnant mice injected with 30 kBq/kg of ^{239}Pu at 13 days of gestation. The maximum dose (10–14 mGy) was absorbed in the liver. A long-term deficit in the number of haematopoietic colony-forming cells (CFU-S) in the spleen, liver and bone marrow was observed. The development of the stromal microenvironment was also deficient, suggesting sublethal damage in those cells destined to become the precursors of the supportive haematopoietic microenvironment.

177. Platteau et al. [P9] looked for medium-term effects in the immune system of rats following 0–2 Gy prenatal or early post-natal WBI. They did not find changes in the histology of the spleen, profiles of lymphocyte subpopulations or serum immunoglobulin levels. At an age of 10 weeks, rats were immunized with a T-dependent or a T-independent dinitrophenylated carrier antigen. T-dependent response was higher in rats irradiated between days 6 and 20 of gestation; however, this increase was significant only for IgM and IgG1 responses.

178. Nold et al. [N14] demonstrated that dogs irradiated prenatally with 1.5 Gy (^{60}Co gamma irradiation) on day 35 of gestation exhibited lower primary humoral antibody responses to a T-dependent antigen (sheep red blood cells), with a decrease in helper T-lymphocyte subpopulations in peripheral blood. Moreover, they found defects in epitheliostromal development of the thymus and concluded that the observed immune alterations could be related to radiation-induced prenatal thymic injury.

179. Thymocytes are either negatively selected as potentially autoreactive and deleted, or positively selected to become mature cells. In addition to the signal mediated by the T-cell receptor (TCR), other signalling pathways also regulate this developmental selection. It has been demonstrated [N12] that the CD28 receptor, which plays a role in

enhancing the survival and expansion of peripheral T-cells, is also involved in negative selection in the thymus. Developing thymocytes of CD28-deficient and wild-type mice displayed similar radiosensitivity in terms of apoptosis, but negative selection was significantly reduced in the former, thus suggesting that CD28 receptor involvement in thymus development is not related to regulation of cell survival.

180. Miller and Benjamin [M6] have studied radiation-induced alterations in prenatal thymic development in the beagle dog. They found that injury to both thymic cortices and medullas was greater following exposures earlier in gestation. Damage to medullas was relatively more severe than in cortices following exposure at any given age. The degree of reduction of medullar volume reflected thymic epithelial injury, which is surprising since the thymic epithelium is considered in the adult to be radioresistant. Such injury may have serious post-natal consequences, as normal differentiation of T-cell subpopulations is dependent upon the integrity of the thymic microenvironment. Damage to the thymic microenvironment could result in immunodeficiencies and defects in immunological regulation.

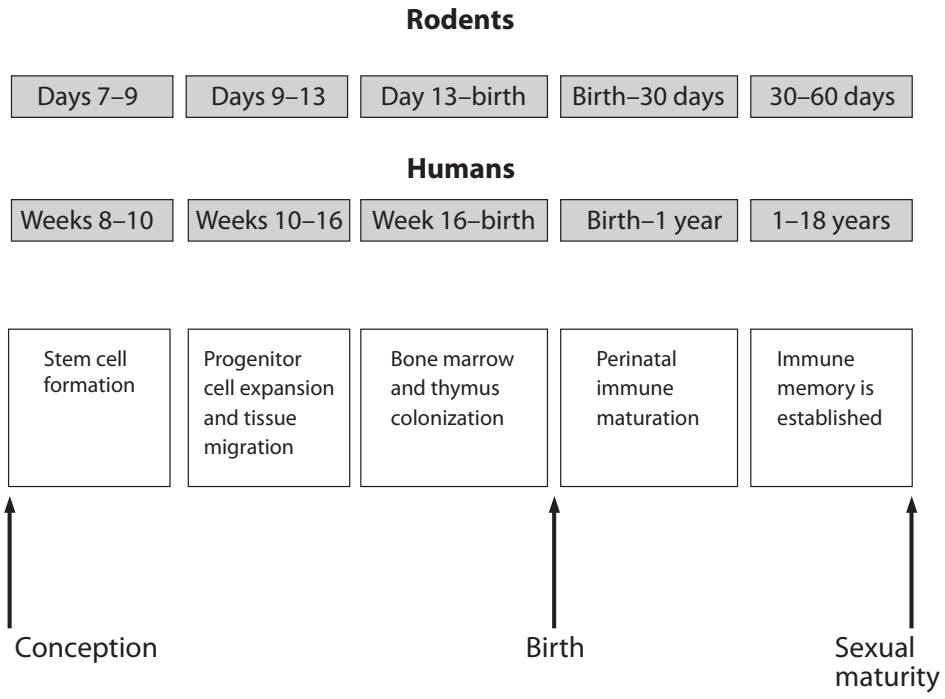
2. Human data

181. Human foetuses are thought to be highly sensitive to ionizing radiation. However, human data concerning the effects of prenatal irradiation on the immune system are scarce. In humans, haematopoietic activity begins in the liver by six weeks of gestation and continues until one week of post-natal life. The spleen also plays a major role in haematopoiesis in the growing foetus. Post-natal, the bone marrow becomes the main haematopoietic organ; the spleen and liver have ceased their function by the time of birth or soon after.

182. During the intrauterine ontogenesis of the immune system, apoptosis plays a central role in thymus development. A distinction should be made between developmental and radiation-induced lymphocyte apoptosis. While apoptosis during T-cell maturation is p53-independent, p53 protein is a critical mediator of apoptosis in response to genotoxic agents such as ionizing radiation. Immature cortical thymocytes are characterized by a double-positive immunophenotype, CD4+ CD8+ TCR^{low}. The interaction between immature thymocytes and thymic reticuloendothelial cells induces positive selection of T-lymphocytes that are not activated by self HLA molecules, thus promoting tolerance induction. T-lymphocytes that do not undergo positive selection are killed by apoptosis. Immature thymocytes also undergo negative selection when thymic stromal cells eliminate self-reactive T-lymphocytes by apoptosis [K1].

183. As seen in animals, the effects of ionizing radiation on the human embryo/foetus may differ according to the developmental stage at which the exposure takes place. The definition of critical windows in the development of the immune system may allow identification of periods of special vulnerability. On the basis of known developmental changes occurring within the immune system, Dietert et al. proposed a relative timeline for critical windows in the developing human immune system [D5]. A parallel approach for the development of rodent and human immune systems is shown in figure VII.

Figure VII. Critical windows of toxic exposure for the developing immune system in rodents and humans.
Gestation: 21 days for rodents, 38 weeks for humans (adapted from reference [D5]).



184. Prenatal events can affect the immune response at birth and, depending on gestational timing, can result in widely varied outcomes. The potential outcomes of toxic exposures to the developing immune system may derive from suppression, perturbation or up-regulation of the immune response, leading to immunodeficiencies, autoimmunity or hypersensitivity reactions, respectively. Moreover, prenatal damage to the thymus would have a more profound effect than post-maturational damage. Similarly, prenatal destruction of abundant pluripotent stem cells may have a more harmful outcome than destruction of single lineages or differentiated cells, which predominate in adults [H14, H27].

185. Ohtaki et al. evaluated the frequency of stable aberrations (translocations) in blood samples from 150 survivors of the atomic bombings at Hiroshima and Nagasaki who were exposed in utero to doses of ≥5 mSv and from 181 controls (dose < 5 mSv). Foetal doses were approximated by the dose to the maternal uterus. The mean age of subjects at the time of sampling was 40 ± 0.7 years. The investigators did not find a dose response but did find a small increase in translocation frequency at doses of <100 mSv, with a peak response at ~30 mSv, at which the net increase in translocation frequency was ~0.4%. There was no evidence for an effect of gestational age in the estimated coefficients. These findings were explained with the hypothesis that the human foetal lymphoid progenitor pool comprises two subpopulations with different radiosensitivity. A major fraction exhibits radiation resistance. The other subpopulation, small in number, was considered sensitive to the induction of both translocations and cell killing, and diminished rapidly following doses of >50 mSv [O9].

3. Remarks concerning the effects of prenatal irradiation

186. On the basis of the preceding paragraphs, the following remarks may be made concerning the effects of prenatal irradiation on the immune system:

- The developing immune system is very sensitive to ionizing radiation;
- There are critical windows of special vulnerability of the prenatal immune system;
- The effects differ according to the developmental stage at which the irradiation takes place; for example, a dose of 500 mGy given to mice in early pregnancy has no effect, whereas the same dose given after midgestation results in significant depletion of bone marrow precursors 1 year later;
- Developing haematopoietic tissues are very sensitive to ^{239}Pu contamination: even doses of around 10 mGy are capable of inducing a long-term haematopoietic deficit;
- Radiation-induced impairment of the developing stromal microenvironment accounts, at least in part, for these long-lasting effects;
- Prenatal thymic injury is greater following irradiation in early pregnancy;
- Prenatal radiation-induced damage in the thymus is more severe for the medulla than for the cortex;
- Human foetal lymphoid progenitors include a major subpopulation that is relatively radioresistant and a minor subpopulation that is very radiosensitive to the induction of both translocations and cell killing.

G. Immune dysfunction and hypersensitivity to ionizing radiation

187. As seen, DNA recombination processes not only account for immune diversification but also constitute a major checkpoint in the development of lymphocytes [D26]. Defects in V(D)J recombination lead to immunodeficiency, and a deregulation of this process may result in higher sensitivity to genotoxic agents and increase the risk of malignancies.

188. The understanding of the mechanisms involved in the development of human pathologies exhibiting immune dysfunction and higher radiosensitivity may be relevant in the context of the effects of ionizing radiation on the immune system. There exist a number of human genetic disorders characterized by chromosomal instability that are associated with a higher incidence of cancer. Both the chromosomal instabilities and the neoplastic outcomes are related to abnormalities of DNA metabolism, DNA repair, cell cycle regulation or control of apoptosis. Among them there are several disorders associated with defects in the immune system and increased susceptibility to radiation, including UV and ionizing radiation [B9]. These observations are especially relevant because they are at the crossroads of basic cellular mechanisms, combining immunodeficiency, increased rate of cancer, and defects in DNA repair and pathways known to be associated with hypersensitivity to radiation. Other pathological conditions involving immune dysfunction, such as autoimmune diseases and acquired immunodeficiency syndrome (AIDS), could be also associated with higher radiosensitivity. Because of the double condition of hypersensitivity to radiation and immunodeficiency, the radiation effects on the immune system may be more severe in these patients.

1. Human genetic disorders

189. Abnormal DNA repair and cell death regulation may result in higher vulnerability to ionizing radiation. Several human genetic disorders associated with defective DNA repair present functional abnormalities in the immune system. Some of them exhibit increased susceptibility to radiation. If individuals with such disorders undergo radiotherapy, they may develop adverse side effects in normal tissues, such as acute effects appearing during or shortly after treatment, or late effects developed months or years later. Some of these genetic disorders are reviewed below and are summarized in table 10.

Table 10 Human genetic disorders with immune system defects and increased susceptibility to radiation

Disease	*Defective mechanism*[a]	*Mutated gene*	*Sensitivity to*[b]	
			UV	*Ionizing radiation*
Xeroderma pigmentosum	NER (+/– TCR)	*XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-F/ERCC1, XP-G*	(+++)	?
Trichothiodystrophy	NER/TCR	TFIIH-related *XP-D* and *XP-B*	(++)	?
Xeroderma pigmentosum variant	TLS	*hRAD30*	(+)	(+)
Ataxia telangiectasia homozygote	DSB cell signalling	*ATM (–/–)*		(++++)
Ataxia telangiectasia heterozygote	DSB cell signalling	*ATM (+/–)*		(+)
Ataxia-telangiectasia-like disorder	DSB cell signalling	*hMRE11*		(++++)
Nijmegen breakage syndrome	DSB cell signalling	*NBS1 (nibrin)*		(++++)
Fanconi anaemia	Cross-linking repair and HR	*FANC-A, FANC-B, FANC-C, FANC-D1/BRCA2, FANC-D2, FANC-E, FANC-F, FANC-G*		(++)
Bloom's syndrome	HR/TLS	*BLM* RecQ helicase	(+)	?
Wiedemann–Rautenstrauch syndrome	Neonatal progeria	*WRS*		?
Hutchinson–Gilford syndrome	Progeria infantum	*HGPS* (lamin A)	(++)	(++)
Cockayne syndrome	NER/TCR (youthful progeria)	*CS-A* and *CS-B*	(++)	(?)
Werner's syndrome	HR/TLS (adult progeria)	*WRN* RecQ helicase	(+)	(+)
Severe combined immunodeficiency (SCID)	V(D)J/NHEJ	*Artemis*		(+++) (SCID with increased radiosensitivity)
Immunodeficiency with microcephaly	NHEJ	*Ligase IV Cernunnos/XLF*		(++++)
Seckel syndrome	DSB cell signalling	*ATR*		(+++)
Dyskeratosis congenita	Telomere metabolism	*Dyskerin/hTR*		(++)
Hyper-IgM syndromes	Class switch recombination	*Uracil-N-glycosylase (UNG)*		(++)

[a] NER = nucleotide excision repair; TCR = transcription-coupled repair; DSB = double-strand break; HR = homologous recombination; TLS = translesion synthesis; NHEJ = non-homologous end joining.

[b] Increasing number of + symbols means increasing degree of radiosensitivity. Query symbol (?) means probably radiosensitive but radiosensitivity not demonstrated.

190. *Xeroderma pigmentosum* (XP) is an autosomal recessive disease with defective nucleotide excision repair (NER) characterized by severe sensitivity to all types of UV radiation. The NER pathway recognizes and removes UV-induced DNA lesions. XP is characterized by cutaneous and ocular abnormalities, predominantly on sites exposed to sunlight, and in some cases by neurological features resulting from progressive neuronal loss. Expression in skin includes disposition for sunburn, pigmentary abnormalities, telangiectasia, dryness, scarring, and susceptibility to multiple benign and malignant neoplasms, including basal and squamous cell carcinomas and melanomas [L7]. A variety of immune abnormalities have been described in XP patients: decreased delayed-type hypersensitivity reactions, decreased T-cell proliferative responses to mitogens, decreased CD4/CD8 ratio and impairment of NK cell activity [G19, L6, N21, N22, W15]. XP is categorized in at least eight genetic subtypes labelled A to G, plus the F/ERCC1 group. While both XP-A and XP-C can be attributed entirely to defects in NER, the other complementation groups involve defects in other DNA repair mechanisms [L6]. Some XP patients in complementation group E (XP-E) lack UV-damaged-DNA-binding protein (UV-DDB), which is present in healthy individuals and in patients in other XP complementation groups. UV-DDB protein is composed of two subunits, DDB1 and DDB2. In vivo, UV-DDB protein plays an important role

in the p53-dependent response of mammalian cells to DNA damage. When cells are exposed to UV, the resulting accumulation of p53 activates DDB2 transcription, which leads to increased levels of UV-DDB. A linear dose response for activation of *DDB2* and *XPC* genes has been demonstrated following exposure of peripheral blood lymphocytes to doses of gamma rays as low as 0.2 Gy [A21].

191. The cells of *xeroderma pigmentosum variant* (XP-V) patients are only slightly more sensitive than normal cells to the killing action of UV radiation. The XP-V cells are NER-proficient but present a deficit in translesion DNA synthesis, with an increased probability of being blocked at a DNA lesion [C31]. Defects in translesion synthesis (TLS) due to mutations in the damage-specific polymerase hRAD30 (polymerase η) are responsible for the UV-hypermutability of XP-V cells leading to skin cancers [K38]. It has been proposed that sunlight carcinogenesis in XP-V patients may be associated with increased genomic rearrangements that result from double-strand breaks and rejoining in cells of the skin in which *p53* is inactivated by UV-induced mutations [L12].

192. *Trichothiodystrophy* (TTD) refers to several autosomal recessive diseases whose diagnostic hallmark is short, brittle hair low in sulphur and cysteine because of impaired synthesis of high-sulphur matrix protein [L7, S38]. The clinical symptoms associated with TTD, such as ichthyotic skin, neurodegeneration and developmental disorders, represent a variable range of abnormalities in organs derived from the ectoderm [L7]. The majority of TTD patients exhibit photosensitivity because their NER pathway does not remove UV-induced DNA lesions efficiently. Although their deficiencies result mainly from mutations in either XP-B or XP-D, TTD patients do not present skin cancer susceptibility [C27, S38] and also do not exhibit hypersensitivity to ionizing radiation. Although some abnormal immune parameters have been found in TTD patients [C19, N22, R10], immune deficiency is not a common feature of TTD. However, the finding of additional TTD patients combining features of XP and Cockayne syndrome indicates that there may be considerable clinical heterogeneity with phenotypic overlap.

193. *Bloom's syndrome* belongs to a family of hereditary diseases caused by defects in genes belonging to the RecQ family of DNA helicases, which play a role in regulating homologous recombination. Bloom's syndrome patients, who exhibit a mutation in the *BLM* gene, are hypersensitive to UV and exhibit abnormal replication patterns following DNA damage. They present sun-induced rash, immunodeficiency, subfertility and cancer predisposition [K4]. One of the defining features of cells from Bloom's syndrome individuals is chromosomal instability, characterized by chromosomal breaks, deletions and rearrangements, as well as elevated levels of sister chromatid exchange.

194. *Ataxia telangiectasia* (AT) is an autosomal recessive multisystem disorder with early-onset cerebellar ataxia, conjunctival and cutaneous telangiectasia, and immunodeficiency as its main clinical features. AT patients are prone to cancer development and are unusually sensitive to ionizing radiation. They carry a mutation in a gene involved in DNA damage signalling [J9], called the ataxia-telangiectasia-mutated (ATM) gene. Only homozygous AT patients present the typical clinical features described above. Homozygous AT is the most common cerebellar ataxia in children under 5 years of age, with a prevalence of 1 in 100,000 live births [S48]. Progressive cerebellar ataxia becomes apparent as early as the first year of life. Oculomotor abnormalities may also be seen. Telangiectases appear after onset of the neurological syndrome and are progressive.

195. AT affects both TCR and Ig genes, thus explaining the immunodeficiency observed in AT patients. Moderate cellular and humoral immunodeficiency, with low levels of certain Ig classes, in conjunction with difficulties in swallowing, lead to frequent pulmonary infections in AT patients. Peripheral lymphocytes from AT homozygotes show elevated chromosomal rearrangements, which preferentially involve chromosomal breakpoints within *TCR* genes, mainly at 14q11 (*TCR*-α and -δ), 7q14 (*TCR*-γ) and 7q35 (*TCR*-β) [T2]. Telomere shortening and fusions, with normal telomerase activity, have been observed in peripheral blood lymphocytes of these patients [T2]. AT patients show progeroid features, such as strands of grey hair or keratoses, which have been related to accelerated telomere shortening [P2]. Endocrine defects typically result in gonadal abnormalities and retardation in somatic growth. Some patients develop insulin-resistant diabetes, which has been attributed to antibodies directed against insulin receptors [S48]. Many homozygous AT patients develop cancer, mostly lymphoid malignancies of both B-cell and T-cell origin, including Hodgkin's lymphoma, non-Hodgkin's lymphoma and leukaemias [B8].

196. Solid tumours, mainly breast and stomach cancer, occur more commonly as the AT patient matures, and are being seen in greater numbers as these patients are living longer [H23]. Although plausible, the hypothesis of a higher risk of radiogenic cancer in homozygous AT patients is not supported by solid data. In contrast, the hypersensitivity to deterministic effects from ionizing radiation, around three to fourfold compared with the general population, is well established. Indeed, the treatment of cancer in AT patients with conventional doses of ionizing radiation may induce extremely high radiotoxicity, resulting in life-threatening sequelae. The frequency of heterozygous *ATM* mutations among the general population is around 1%. Heterozygous carriers are neurologically normal. Although some authors have not found any evidence of abnormal radiotoxicity in heterozygous AT patients [W12], other epidemiological studies have suggested that heterozygous AT women have a predisposition to breast cancer and may exhibit higher clinical radiosensitivity [A24].

197. *Ataxia-telangiectasia-like disorder* (ATLD) is due to mutations in the *hMre11a* gene, involved in DNA double-strand-break repair. ATLD shares many clinical features with AT. While abnormalities in B-lymphocytes have not been

reported in ATLD, T-lymphocytes show an increased level of translocations involving the TCR. Both lymphocytes and fibroblasts from these patients exhibit higher radiosensitivity in vitro [T2].

198. *Nijmegen breakage syndrome* (NBS) is a rare autosomal recessive condition characterized by chromosomal instability, growth retardation, microcephaly, characteristic facial features, radiosensitivity, immunodeficiency, gonadal dysgenesis and cancer predisposition. A high proportion of NBS patients develop lymphoid malignancies, most of B-cell origin. Fibroblasts and lymphocytes from NBS patients are 3–5 times more sensitive to ionizing radiation and radiomimetic drugs than normal cells, and display radioresistant DNA synthesis due to an inability to retard S-phase progression after exposure to ionizing radiation. NBS also involves inversions and translocations in peripheral T-cells affecting the genes for the heavy chain of immunoglobulins and the TCR, thus accounting for the immunodeficiency characteristic of this syndrome [D6]. NBS patients carry a mutation in the *NBS1* gene, which codes for a protein called NBS-1/Nibrin, involved in DNA double-strand-break repair.

199. *Fanconi anaemia* (FA) is a recessive disorder characterized by bone marrow failure, multiple congenital abnormalities and predisposition to cancer, particularly to acute myeloid leukaemia and squamous cell carcinoma [R8, T2]. Although FA is considered a genetically and clinically heterogeneous disorder, the genes mutated in FA are all involved in recruiting and organizing other proteins to cope with DNA damage and participate in the regulation of homologous recombination. FA patients are unusually sensitive to a variety of clastogens, most prominently DNA cross-linking agents such as mitomycin C. Moderate to high sensitivity to ionizing radiation and other oxidative stress inducers has been reported in FA patients [D10]. Djuzenova et al. [D8] observed high initial and residual DNA damage rates and elevated repair half-time in peripheral blood mononuclear cells from FA patients exposed to X-rays.

200. *Human progeria syndromes* are rare disorders characterized by premature ageing. Some of these syndromes are associated with defects in DNA repair pathways and with a higher susceptibility to DNA damage agents, such as ionizing radiation. According to the age at the onset of symptoms, human progeria syndromes may be classified as neonatal, infantum, youth and adult progeria.

201. *Neonatal progeria (Wiedemann–Rautenstrauch syndrome)* is a rare autosomal recessive disorder usually lethal by 7 months. It is characterized by a triangular, aged-looking face with prominent veins and sparse scalp hair, and nearly total absence of subcutaneous fat. These features are apparent at birth and therefore differ from congenital generalized lipodystrophy.

202. *Progeria infantum (Hutchinson–Gilford progeria syndrome—HGPS)* is a rare genetic disorder characterized by prominent scalp veins, the absence of hair, maxillary hypoplasia, delayed tooth eruption, facial cyanosis, insulin-dependent diabetes, accelerated ageing and early death, frequently from coronary artery disease. HGPS is caused by mutations in the *lamin A* gene, placing this syndrome within the group of "laminopathies" [B29]. The main functions of A-type lamins include high-order chromatin organization, nuclear structure maintenance and gene expression control. Hypersensitivity to gamma radiation has been described in these patients [W7]. Concerning immune dysfunction, a decreased CD4/CD8 ratio and decreased T-cell response have been associated with HGPS [H19].

203. *Youth progeria (Cockayne syndrome—CS)* patients show many developmental defects, including mental retardation, microcephaly, long limbs, birdlike face, pigmented retinopathy, gait defects and sun sensitivity due to a failure of RNA synthesis to recover following UV irradiation [L16], but without an increased incidence of skin cancer. Two complementation groups (A and B) have been identified in CS depending on which gene is mutated (*CS-A* or *CS-B*); these genes encode proteins involved in the transcription-coupled repair pathway of NER [L16]. Cells from CS patients accumulate oxidative-induced DNA lesions after exposure to ionizing radiation [T16]. Decreased T-cell proliferative response and decreased serum levels of thymic hormone have been observed in these patients [B22].

204. *Adult progeria (Werner's syndrome—WS)* patients display age-related disorders, including greying and thinning of the hair, bilateral cataracts, type II diabetes, atherosclerosis, osteoporosis and increased incidence of sarcomas. The incidence of epithelial cancer and mesenchymal sarcoma is 10 times that of the general population. The incidence of WS is extremely high in Japan compared with other countries [Y6]. WS patients carry a mutation in the *WRN* gene, which encodes a protein belonging to the RecQ family of DNA helicases. WS cells exhibit genomic instability, premature senescence, defects in telomere metabolism, and hypersensitivity to DNA cross-linkers and to ionizing radiation [C25, M17]. The WRN protein is recruited in the radiation-induced DNA double-strand breaks and collocalizes with the Mre11 and γH2AX complex via binding to NBS1. *NBS1* is the gene mutated in the Nijmejen breakage syndrome, thus suggesting a functional link between these two genetic syndromes with partially overlapping phenotypes [C24, C25]. Immune dysfunction has been found in WS patients [G29, G30, N16].

205. *Severe combined immune deficiency* (SCID) is the most severe inherited immunological disorder and is caused by a variety of molecular defects, all affecting at least the T-lymphocyte population [F16]. Human SCID is characterized by the absence of the T- and B-lymphocytes owing to a defect in V(D)J rearrangement of TCR and Ig genes [D26]. Mutations in the *Artemis* gene, which encodes a protein essential for V(D)J recombination and DNA double-strand-break repair, cause a variant of human SCID with increased radiosensitivity (RS-SCID) [L28, M19]. Hypomorphic *Artemis* mutations are associated with the development of B-cell lymphomas [M13].

206. *Immunodeficiency with microcephaly* patients, presenting with various degrees of immunodeficiency and growth delay, display mutations in the *DNA ligase IV* gene, which encodes a protein essential for DNA repair through non-homologous end-joining (NHEJ) and V(D)J recombination [B33, O2]. This clinical phenotype closely resembles Nijmegen breakage syndrome, and cells from the patients show pronounced radiosensitivity [O2]. A new NHEJ factor, Cernunnos, was identified through the survey of similar patients with SCID and microcephaly [B34]. This resemblance is not surprising, given the interactions of Cernunnos and DNA ligase IV in a same protein complex [A26].

207. *Hyper-IgM syndromes* are primary immunodeficiencies characterized by normal or increased serum IgM levels contrasting with low or absent IgG IgA, and IgE. They are caused by a defect in the class switch recombination [D31]. A subset of these patients display defects in DNA repair genes such as *Uracil-N-glycosylase* [I12].

208. *Seckel syndrome* shares features with the above disorders involving impaired DNA damage repair and elevated radiosensitivity. Seckel syndrome patients have a mutation in *ATR*, the gene encoding ataxia telangiectasia and Rad3-related protein, resulting in failed checkpoint arrest following exposure to genotoxic agents [A9, O3].

209. *Dyskeratosis congenita* (DC) is a rare syndrome caused by defective telomere maintenance characterized by atrophy and reticular pigmentation of the skin, nail dystrophy, leucoplasia of mucosa membranes, hypotricosis, dysphagia, skeletal abnormalities, mental retardation, bone marrow failure and predisposition to malignancies [C29]. The major X-linked form of DC is due to mutations in a nucleolar protein, dyskerin, found in the telomerase complex [D23]. An autosomal dominant form is due to mutations in the RNA component of telomerase (hTR) [S46]. Patients with this form of the disease are more severely affected in later generations that carry the mutations, possibly owing to the inheritance of shortened telomeres, disguising the inherited nature of the disease, which in some cases is classified as aplastic anaemia. Increased sensitivity of DC cells to ionizing radiation and alkylating agents has been described [D24]. It was demonstrated that fibroblasts from DC patients are highly susceptible to X-irradiation-induced chromatid breakage [D18], and hypersensitivity to radiation therapy has been described in these patients [C20]. A severe infantile variant of X-linked DC called Hoyeraal–Hreidarsson syndrome exhibits increased in vitro sensitivity to radiation and alkylating agents in circulating lymphocytes and fibroblasts. Accelerated telomere shortening was also reported in these patients [M24]. Severe B-lymphopenia with decreased IgM levels and moderate T-lymphopenia were observed in autosomal dominant form, probably as a consequence of replicative failure and premature senescence of lymphocytes, supporting a role of telomerase in immune homeostasis [K41].

2. Autoimmune diseases and radiation response

210. DNA damage and its defective repair could be important in the pathogenesis of autoimmune diseases. Impaired response to oxidative stress has been postulated as another condition found in autoimmune diseases [H17, K51]. Bhusate et al. demonstrated that the spontaneous number of DNA strand breaks in peripheral blood mononuclear cells from patients with rheumatoid arthritis is significantly increased compared with those from healthy donors [B24]. Neutrophils from systemic lupus erythematosus (SLE) patients display increased spontaneous DNA damage and defective repair of oxidative DNA damage [M20]. Higher sensitivity to oxidative stress has been also described in lymphocytes from patients with SLE, vasculitis and Behcet's disease [B20].

211. McCurdy et al. studied DNA repair of peripheral blood lymphocytes from patients with autoimmune diseases, irradiated with 1.5 Gy. By using single-cell alkaline gel electrophoresis (comet assay) they observed that lymphocytes from patients with systemic lupus erythematosus, juvenile rheumatoid arthritis and systemic sclerosis exhibited delayed repair of radiation-induced DNA damage compared with lymphocytes from healthy donors [M21]. These findings were in accordance with a previous study by Harris et al., who evaluated the lymphocyte responsiveness to mitogens (Con A) following irradiation and found that lymphocytes from patients with rheumatoid arthritis, systemic lupus erythematosus and polymyositis were more radiosensitive than those from healthy volunteers or patients with conditions not associated with autoimmunity [H6].

212. Cossu et al. evaluated the proliferative responsiveness following irradiation with doses of between 0 and 10 Gy in peripheral blood lymphocytes from patients affected by systemic lupus erythematosus in the active and remissive phases. They compared their results with those found in a group of normal subjects. Patients in the clinically active phase of the disease exhibited a decreased responsiveness of the total lymphocyte population as well as of the subpopulations of T- and B-cells. Responsiveness from patients in the remissive phase was similar to that of normal subjects. They concluded that the lymphocytes of patients affected by this autoimmune disease in the active phase are more radiosensitive than those of patients in the remissive phase of these diseases [C15]. Greater toxicity from radiation therapy has been described in patients with collagen diseases, such as systemic lupus erythematosus, polymyositis, dermatomyositis and scleroderma, manifested as significant acute toxicity and severe late effects [M12].

3. Ionizing radiation and acquired immune deficiency syndrome

213. A higher incidence of several cancers, e.g. Kaposi's sarcoma, non-Hodgkin's lymphoma, conjunctival squamous carcinoma and Hodgkin's disease, has been observed in patients with acquired immunodeficiency syndrome (AIDS).

Since there is accumulating evidence that AIDS patients exhibit higher radiosensitivity, the effects of ionizing radiation on the immune system may be relevant in medical practice when radiotherapy is administrated to AIDS patients with cancer [S53].

214. Enhancement of the human immunodeficiency virus type 1 (HIV-1) by heterologous viral, chemical and physical agents has been shown both in vitro and in vivo. Exposure of AIDS patients to ionizing radiation (for example during radiation therapy) could play a role in activating HIV-1 in vivo. Faure et al. demonstrated in lymphoid cell lines that HIV long-terminal-repeat-directed gene expression is activated by X-irradiation in a dose- and time-dependent manner [F2].

215. Stress agents such as ionizing radiation could directly activate HIV-1 virus replication or reporter gene expression. The kappa B regulatory elements seem to be involved in this up-regulation [F1]. Antioxidant drugs inhibit this effect, which suggests that reactive oxygen species (ROS) are involved. Taher et al. found that ionizing radiation, at both low and high doses, stimulates HIV gene expression to a lesser extent and with different kinetics than does UV radiation [T1]. While UV activation was completely blocked by p38 MAP kinase inhibition, activation by ionizing radiation was reversed by MAP kinase/MEK inhibition. This finding suggests that ionizing radiation modulates HIV gene expression by this latter signalling pathway.

216. Integrase-induced host DNA damage associated with HIV-1 infection elicits DNA damage signalling. Waninger et al. have established that the DNA-PK is involved in HIV-1 replication, suggesting that the NHEJ pathway, involved in repair of radiation-induced DNA double-strand breakage, is required to support efficient retroviral infection [W8]. In addition to DNA-PK, Lau et al. have recently presented evidence that ATM kinase activity has an important role in HIV-1 infection. By using a specific ATM inhibitor (KU-55933), they demonstrated that ATM is required for HIV-1 replication in NHEJ-proficient cells [D14, L24]. A direct correlation exists between apoptosis and HIV gene expression in T-cells in response to ionizing radiation exposure. Caspases seem to be involved in ionizing-radiation-induced activation of HIV, since caspase inhibitors abolish both apoptosis and HIV gene expression [O1]. However, triggering of apoptosis is not sufficient to induce HIV gene expression, since in other cell types (e.g. carcinoma cells), ionizing radiation induces apoptosis but does not enhance HIV expression.

217. Higher morbidity associated with radiation therapy has been reported in HIV-1+ patients [S25], although the mechanisms involved in this response remain controversial. It has been postulated that the pattern of immune dysregulation present in these patients could play a role in their abnormal response to ionizing radiation. Cells infected with HIV-1 exhibit down-regulation of the DNA repair gene *DNA-PKcs* and other cell-cycle-related genes, suggesting that impairment of DNA repair and dysregulation of cell cycle checkpoints may be involved in the higher radiosensitivity of AIDS patients [S53]. Decreased levels of major endogenous antioxidant systems have been seen in HIV-1 disease, including lower levels of glutathione and decreased levels of superoxide dismutase and catalase [B13]. The state of chronic immune activation secondary to HIV-1 infection and the frequent secondary or opportunistic infections (as well as reactions to other antigenic products) lead to a constant state of oxidative stress. ROS generated by ionizing radiation might trigger additional oxidative stress in these patients.

4. Remarks concerning human pathologies with immune dysfunction and hypersensitivity to ionizing radiation

218. As described in the preceding paragraphs, the identification of human pathologies in which immunodeficiency is associated with higher radiosensitivity is relevant, because the effects of ionizing radiation on the immune system may be more severe in these patients. Table 10 summarized the main human genetic disorders in which immune dysfunction is associated with hypersensitivity to ionizing radiation. The mutated genes and the defective mechanisms that account for these disorders are also presented in this table. The data indicate that ataxia telangiectasia, ataxia telangiectasia-like-disorder, Nijmegen breakage syndrome, severe combined immune deficiency, ligase IV and Cerunnos deficiencies, and Seckel syndrome are the disorders exhibiting a very high radiosensitivity. To a lesser extent, higher radiosensitivity has been proven for xeroderma pigmentosum variant, Fanconi anaemia, progeria infantum and adult, and dyskeratosis congenita. Concerning the other conditions described, their probable hypersensitivity to ionizing radiation has not yet been demonstrated.

219. Defective DNA damage repair and impaired response to oxidative stress seem to be involved in the pathogenesis of autoimmune diseases. Lymphocytes of patients in the active phase are more radiosensitive than those of patients in the remissive phase of these diseases.

220. Concerning AIDS, ionizing radiation activates human immunodeficiency virus (HIV-1) replication. Higher radiotoxicity is observed in AIDS patients, probably owing not only to immune dysregulation but also to impairment of DNA repair combined with a chronic state of oxidative stress.

H. Summary

221. This section has summarized the influence of dose, dose rate and radiation quality on radiation effects on the immune system. Data concerning the radiosensitivity of lymphocyte subsets and the immune effects of prenatal irradiation were reviewed. Human immune pathologies associated with radiosensitivity were summarized.

222. Activation of the immune function by low-dose irradiation has been mainly demonstrated in splenic and

thymic lymphocytes from some strains of animals. Low-dose enhancement of the immune response is mainly seen in T-lymphocytes, particularly in CD8+ T-cells. This effect, which has not yet been demonstrated in humans, seems to take place within a very narrow dose range. While a dose of 20 mGy (acute exposure) induces immunoenhancement in rodents, changing the dose to 200 mGy induces a shift towards immunosuppression. The same dose (200 mGy) administered using a protracted schedule exhibits immunoenhancing properties. However, this effect may vary according to the age and strain of the animal. The suppressive effect of low-dose irradiation on tumour growth has been reported in animal models. An adaptive response to radiation in the immune system seems to be related to a reduction of apoptosis and an enhanced capacity to repair DNA damage. Even at very low doses, tritium incorporation appears to have a higher RBE. The long-term impact of low doses on the immune functions related to human health remains to be determined.

223. Concerning high doses, minimal depression of leucocyte counts may be observed following acute WBI in the range 0.5–1 Gy. Acute radiation syndrome occurs after acute whole-body or significant partial-body exposure of >1 Gy. The kinetics and severity of immunosuppression depend on the whole-body dose. In non-uniform exposures, temporal blood cell parameters are more relevant for dose estimation than the magnitude of cytopenia. Reconstitution of innate immunity is more rapid than that of acquired immunity. Humoral recovery precedes T-cell reconstitution, which includes both thymus-dependent and thymus-independent pathways.

224. Radiation-induced changes in immune parameters seem to be more dependent on total dose than on dose rate. The RBE of high-LET radiation differs, depending on the end point considered. Some cell types exhibited differences in the response to proton versus photon radiation, proton irradiation inducing more significant immunosuppression. Lymphoid cells and tissues are markedly affected by high-LET radiation at relatively low doses in terms of cell depletion and organ size, whereas neutron RBE is close to unity for the induction of lymphocyte apoptosis. Radon exposure induces marked changes in thymus subpopulations and alters molecules involved in the migration and homing of immune cells.

225. Although the radiosensitivity of lymphocyte subsets depends on several factors related to the experimental conditions, the data reviewed indicate that B-lymphocytes are the most radiosensitive subset and that NK cells are more radioresistant.

226. There are critical windows of special vulnerability for prenatal irradiation of the immune system, and the effects differ according to the developmental stage at which the irradiation takes place. Radiation-induced damage to the developing thymus and haematopoietic stromal microenvironment account, at least in part, for the observed effects.

227. The identification of human pathologies in which immunodeficiency is associated with higher radiosensitivity is relevant, because the effects of ionizing radiation on the immune system may be more severe in patients with these disorders. Delayed repair of radiation-induced DNA damage and increased lymphocyte radiosensitivity have been found in patients with autoimmune diseases. AIDS patients exhibit higher radiotoxicity. Ionizing radiation activates HIV-1 replication, and bystander effects involving reactive oxygen species seem to be involved in this activation.

III. POSSIBLE MECHANISMS INVOLVED AND IMPLICATIONS

228. In order to better understand the findings described in section IV (Epidemiological studies), this section summarizes some mechanisms that are probably involved in radiation-induced alterations of the immune system. Mechanisms of cancer development and the possible roles of the immune system are also discussed.

229. The pathways activated by ionizing radiation that have been investigated in the greatest depth are those dealing with the responses to DNA damage. These pathways involve sensor molecules linked to primary downstream components that integrate and process the signals. As a result of this response to DNA damage, the cell cycle arrests, DNA repair takes place and the cell may survive or die. However, not all the effects of ionizing radiation on the immune system can be ascribed to cytotoxicity. An additional outcome of radiation exposure, which is not often considered, concerns the production of "danger signals" that may also influence the classical cellular responses normally related to DNA damage [M2]. The following possible mechanisms involved in radiation-induced alterations of the immune system will be summarized:

- Lymphocyte apoptosis;
- TCR mutations;
- Modification of Th1 and Th2 balance;
- Bystander effects and genomic instability;
- Shift towards an inflammatory profile;
- Acceleration of immunological ageing;
- Modification of antigen presentation;
- Autoimmune reactions;
- Perturbation of the immunological homeostasis;
- Other possible mechanisms involved.

A. Lymphocyte apoptosis

1. Review of published data

230. Apoptosis plays a crucial role within the immune system, in particular in negative regulation, and is a key mechanism in the response to ionizing radiation. Lymphocytes die by apoptosis immediately after exposure (interphase death) [J5] or by reproductive cell death [M22]. The apoptotic process can be divided into three phases: a death-stimulus-dependent induction phase; an effector phase during which the "decision to die" is taken; and a degradation phase, during which the morphological and biochemical features of apoptosis become apparent [K20].

231. There are at least two pathways for radiation-induced apoptosis: one is mediated by mitochondrial factors and is p53-dependent, and the other is mediated by cell surface receptors (Fas/CD95) [S16]. The latter pathway may be acidic-sphingomyelinase-dependent and ceramide-mediated [K46]. ROS generated by ionizing radiation result in oxidative damage to the cell membrane, and lymphocytes are known to be sensitive to oxidative stress because of their high mitotic potential and the content of polyunsaturated fatty acids in their cell membranes [S51]. In recent years, evidence has accumulated suggesting that the damage to the cell membrane contributes to radiation cell killing [R14]. It has been demonstrated that activation of membrane-bound sphingomyelinase after irradiation produces ceramide, which strongly induces expression of Fas ligand (FasL/CD95L), cleavage of caspases and apoptosis [D22]. These data demonstrate that ceramide links cellular stress responses induced by gamma irradiation or anticancer drugs to the Fas pathway of apoptosis.

232. Radiation-induced apoptosis in lymphocytes may be initiated by the Fas/FasL system. Radiation induces the synthesis of the death ligand FasL. Ligand-mediated cross-linking of the Fas receptor initiates signalling pathways to apoptosis. Anti-Fas or anti-FasL antibodies reduced the level of radiation-mediated cell killing [B3]. Furthermore, ionizing radiation up-regulates the surface expression of Fas, thus increasing the sensitivity of those cells to FasL. The Fas receptor stimulates a variety of molecules, including several members of the caspase family and the acidic sphingomyelinase. Brenner et al. [B10] described a signalling cascade from the Fas receptor via caspases to acidic sphingomyelinase, release of ceramide and activation of Jun N-terminal kinase (JNK) and p38-K kinases (phospho-p38 mitogen-activated protein kinase). Fas-mediated apoptosis could be prevented in CD4+ or CD8+ T-cells by several protease antagonists, suggesting the involvement of the interleukin-1β-converting-enzyme-related cysteine protease in CD4+ T-cell death, and of both a CPP32-related cysteine protease and a calpaine protease in CD8+ T-cell death triggered by Fas [E5].

233. In contrast, Ogawa et al. [O4] demonstrated that peripheral blood mononuclear cells irradiated with 5 or 10 Gy of gamma radiation showed positivity to apoptosis markers but displayed no increase in surface Fas expression or caspase-3 activity relative to non-irradiated

cells, suggesting a Fas-independent mechanism. A Fas-independent mechanism was also reported by Kuida et al. [K35] in thymocytes from ICE mice that were sensitive to apoptosis induced by dexamethasone or ionizing radiation but resistant to apoptosis induced by Fas antibody. In addition, TCR stimulation of CD8+ T-cells led to a different Fas-independent death process [E5].

234. A p53-dependent, mitochondria-mediated apoptotic pathway was proposed. The p53 molecule is a key modulator of radiation-induced apoptosis in several cell types [L22]; however, it has also been shown that ionizing radiation can induce apoptosis in human lymphocytes independently of p53 status. Radiation-induced up-regulation of FasL protein defines a p53-independent pathway to apoptosis, since apoptosis induced by FasL does not require p53. In fact, radiation-induced p53-dependent and p53-independent apoptotic pathways are both present in different cell types of the immune system. Seki et al. [S10] demonstrated that p53 protein was inducible in TCR $\alpha\beta$ T-cells (CD4 and CD8 cells) and B-cells, but not in TCR $\gamma\delta$ T- and NK cells after gamma irradiation. Cycloheximide was able to inhibit radiation-induced cell death in TCR $\alpha\beta$ T-cells and B-cells, indicating a requirement for protein synthesis, including p53 protein.

235. Mitochondria serve to integrate and amplify upstream cell death signals, clarifying the cellular reaction to a go/no-go response. These signals include proteins, reactive species and divalent cations. The protein signals include the proapoptotic members of the Bcl-2 family, such as Bax, Bad and Bak. Anti-apoptotic members of the Bcl-2 family include bcl-2 and bcl-x_L. Reactive compounds include reactive oxygen species (ROS) and reactive nitrogen species (RNS). If the stress is sufficient, mitochondria respond by releasing a series of protein factors. These factors include cytochrome c and Smac/DIABLO, which act to initiate and facilitate the downstream stages of the caspase-dependent cascade. Other factors released include apoptosis-inducing factors and endonuclease G, which then initiate the downstream stages of the caspase-independent cascade [K20, S29].

236. Cui et al. [C13, C18] found an increased expression of Bax protein in thymic lymphocytes of mice 3 hours after exposure to lethal doses of gamma radiation. On the other hand, the expression of bcl-2 and bcl-x_L proteins was reduced at 3 hours after irradiation, reaching their lowest level at 24 hours. However, a Fas-mediated pathway unaffected by bcl-2 has been described [S31].

237. The role of ROS in radiation-induced apoptosis of human peripheral T-cells was studied by Ogawa et al. [O5]. They found that ROS formation occurred immediately after irradiation, continued for several hours and resulted in oxidative DNA damage. Early (13 hours post-irradiation) and late (23 hours post-irradiation) apoptotic changes were correlated. Therefore the origin of the hyperradiosensitivity of T-lymphocytes seems to be the high production of ROS in the mitochondrial DNA following irradiation. Ogawa et al. recently described the possible existence of a new apoptotic cascade involving early lysosomal membrane destabilization. Therefore possible involvement of lysosomal protease leakage caused by hydroxyl radical formation in lysosomes (possibly resulting in mitochondrial membrane dysfunction) is considered to play an important role in radiation-induced T-cell apoptosis [O6]. Moreover, in a recent study, Sharma et al. [S51] investigated the immunomodulatory effect of chlorophyllin (CHL), a water-soluble mixture of salts of chlorophyll that had earlier been shown to reduce the level of intracellular ROS. CHL significantly inhibited apoptosis in Con-A-stimulated spleen cells from BALB/c mice, whereas the expression of anti-apoptotic genes *bcl-2* and *bcl-xL* was up-regulated in lymphocytes of CHL-treated mice compared to controls.

238. Down-regulation of mitochondrial transmembrane potential by inhibitors of electron transport and adenosine triphosphate (ATP) synthesis prevented stress-induced p53 protein accumulation and eliminated p53-dependent apoptosis in a wild-type p53 leukaemia cell line (MOLT-3) and in normal T-lymphocytes, identifying mitochondrial activity and ROS levels as critical intracellular determinants of the p53 activity [K7]. On the other hand, Chen et al. [C8] demonstrated that nitric oxide, a key RNS, protects thymocytes from gamma-irradiation-induced apoptosis. The mechanism may involve inhibition of p53 up-regulation and reduction of mitochondrial damage, with subsequent inhibition of downstream caspase activation.

239. The apoptotic response of lymphocytes may differ following low-dose irradiation. Data recently communicated by Shankar and Sainis in whole-body gamma-irradiated mice (cumulative dose 200 mGy at 40 mGy/day) demonstrated that such very low doses enhanced the mitogen responsiveness to Con A of spleen lymphocytes, reduced expression of p53 and down-regulated apoptosis. This anti-apoptotic effect was associated with a higher expression of cyclin D1 and proliferating cell nuclear antigen (PCNA), two critical proteins for cell cycle transition and cell proliferation. Interestingly, these authors also demonstrated that down-regulation of apoptosis was not mediated by the Fas/FasL pathway but rather through the mitochondrial pathway [S16]. These findings suggest that those apparently opposite effects of ionizing radiation on some immune responses observed following moderate to high doses compared with low doses, e.g. immunosuppression versus immunostimulation, may be related to signal transduction pathways involving cell cycle regulatory proteins such as p53, cyclins and PCNA.

2. Remarks concerning lymphocyte apoptosis

240. The preceding paragraphs indicate that apoptosis plays a crucial role within the immune system, in particular in negative regulation, and is also a key mechanism in the response to ionizing radiation. It is accepted that there are at least two pathways for radiation-induced apoptosis: DNA-damage-mediated and p53-dependent, and ceramide-mediated.

241. Radiation-induced apoptosis in lymphocytes may be initiated by the Fas/FasL system. Radiation induces the synthesis of the death ligand FasL and up-regulates the surface expression of the Fas receptor, which stimulates the release of ceramides and members of the caspase family. Radiation-induced up-regulation of FasL protein is a p53-independent pathway to apoptosis.

242. A p53-dependent, mitochondria-mediated apoptotic pathway has been proposed. Radiation-induced p53 apoptosis and p53-independent pathways are both present in different cell types of the immune system. Mitochondria integrate and amplify upstream cell death signals. These signals include proteins, reactive species and divalent cations. The proteins include proapoptotic and anti-apoptotic members of the Bcl-2 family, and mitochondria respond by releasing protein factors that initiate the caspase-dependent cascade. Reactive compounds include ROS and RNS, and divalent cations are mainly Ca^{2+} and Mg^{2+}.

243. The apoptotic response may differ following low-dose irradiation. Protracted exposure to radiations at low dose rate reduces the expression of p53 and down-regulates apoptosis. This effect was associated with a higher expression of cyclin D1 and PCNA, two critical proteins for cell cycle transition and cell proliferation. These findings suggest that the apparently opposite effects observed in the immune system at high and low doses might be related to signalling pathways involving cell cycle regulatory proteins such as p53, cyclins and PCNA.

B. TCR mutations

1. Review of published data

244. It has been well established that ionizing radiation induces somatic mutations in a dose-dependent manner both in vivo and in vitro. Radiation-induced mutations in TCR genes could result in the phenotypic expression of TCR-defective T-cells. Since the TCR/CD3 complex is involved in the first step of a variety of other T-cell-dependent immune functions, loss or alteration of TCR expression in surviving cells may contribute to radiation-induced impairment of the T-cell response [K34].

245. A considerable volume of literature is available describing the defective expression of TCR gene α or β. A marginally significant ($p = 0.051$) dose-related increase in the TCR mutation frequency has been detected among survivors of the atomic bombings in Japan. The presence of TCR mutation is dependent on the in vivo selection that occurred soon after exposure, and TCR mutants were almost completely eliminated in vivo during the first decades following the exposure to radiation from the atomic bombings. The half-life of TCR mutants is quite short, between two and three years. Similar results were found in patients treated with Thorotrast and with ^{131}I [A7, K34, U19]. An increased frequency of TCR mutations was found among persons exposed to effluents of the Techa River in the Russian Federation [A17].

246. On the other hand, a positive correlation has been found between TCR mutation frequencies and dicentric chromosome frequencies in lymphocytes from patients who had previously received a full course of radiation therapy for gynaecological disorders. By comparing mutation frequencies of hypoxanthine-guanine phosphoribosyltransferase (HPRT) and TCR using flow cytometry, it was possible to demonstrate that the frequency of TCR mutants correlated well with that of dicentric chromosomes [I4].

247. Although the TCR in vivo somatic mutation assay has been proposed as a sensitive indicator of ionizing radiation exposure, this assay cannot be applied immediately, since the mutant phenotype may require as long as several months to express. Ishioka et al. [I3], using IL-2 after PHA pulse stimulation, demonstrated a dose-dependent increase of mutation frequency in CD4+ cells during the first seven days post-irradiation.

248. In 2002, Smirnova et al. described the mutation frequency of TCR genes in lymphocytes from 165 individuals exposed to ionizing radiation [S24]. The cohort was divided into three groups depending on the type of irradiation and time elapsed since exposure: group 1, analysis performed 16–40 years after acute irradiation; group 2, 13 years after acute irradiation; and group 3, 9–13 years after prolonged irradiation. Elevated frequencies of TCR mutant cells were detected in all three groups: 36%, 25% and 15% in the first, second and third group, respectively. The same authors have recently found elevated frequencies of TCR mutant cells in nuclear chemical plant workers chronically exposed to low doses of external and internal irradiation [S44]. In both studies, the frequency of mutant cells was significantly higher than in control groups.

249. Although several papers have provided strong evidence for mutation of the TCR, the question concerning the lifespan of human memory cells in the absence of TCR signalling remained open. The long-term kinetics of TCR/CD3 mutation between CD4+ CD45RA+ naive and CD4+ CD45RA– memory T-cell fractions was studied in cancer patients receiving radiotherapy [U17]. Both the proportion and the number of mutant cells decayed exponentially with time following radiotherapy. The results indicated that the lifespan of mature CD4+ T-cells is limited regardless of their memory or naive phenotype, suggesting that continued cell receptor signalling is required for lifetime maintenance of human memory cells.

250. Moreover, in vivo models to establish the spontaneous and radiation-induced TCR mutation frequency showed that the TCR mutation frequency dose response fitted a linear–quadratic or quadratic function. The general trend was that radiation-induced TCR mutation frequency started to increase 3 days after WBI with X-rays, reached a peak at 2–3 weeks and then gradually decreased, with a half-life

of about 2 weeks. The coefficients of the quadratic term in BALB/c mice were significantly higher than for C57BL/6 or C3H/He mice, suggesting that genetic factors may control the susceptibility of somatic genes to both spontaneous and radiation-induced mutagenesis [U18].

251. Studies involving TCR mutation frequencies in p53-deficient mice have clarified the important role of the p53 protein in the repair of radiation-induced mutagenic damage to the immune system. In p53 (-/-) mice, the TCR mutation frequency did not decline significantly with time. It was concluded that complete repair of mutagenic damage in irradiated tissues requires the integration of DNA repair and p53-dependent apoptotic mechanisms [K8, S32].

252. In order to explore the ability of ionizing radiation to induce rearrangements in TCR leading to TCR $\beta\gamma$ variants (hybrids) in human lymphocytes, peripheral blood lymphocytes from healthy donors were exposed in vitro to 3 Gy of either X- or gamma rays. The TCR $\beta\gamma$ frequency was not significantly different in irradiated versus control samples up to 55 days after PHA stimulation, suggesting that low-LET radiation is not able to induce this type of TCR rearrangement in vitro [M4].

2. Remarks concerning TCR mutations

253. Some remarks may be made on the basis of the information presented in the preceding paragraphs. Radiation-induced mutations in TCR genes could result in the phenotypic expression of TCR-defective T-cells and thus contribute to radiation-induced impairment of the T-cell response. A significantly increased frequency of TCR mutation has been detected among survivors of the atomic bombings, in patients treated with Thorotrast and ^{131}I, and among the Techa River cohort. It must be emphasized that the presence of mutation is dependent on the in vivo selection, and that the half-life of TCR mutants is between two and three years. Elevated frequencies of TCR mutant cells were also observed in chronically exposed workers at nuclear plants.

254. In vivo models to establish the spontaneous and radiation-induced TCR mutation frequency showed that the dose–response function fitted a linear–quadratic or quadratic function. The coefficients were dependent on the strain of mouse studied, suggesting that genetic factors may control the susceptibility of somatic genes to both spontaneous and radiation-induced mutagenesis. Likewise, in a p53 (-/-) mouse model it was observed that the TCR mutation frequency did not decline significantly with time, leading to the conclusion that repair of mutagenic damage in irradiated tissues requires the integration of DNA repair and p53-dependent apoptotic mechanisms.

255. To date, there are no definitive data demonstrating that low levels of TCR mutation frequency have induced immunodeficiency.

C. Modification of Th1/Th2 balance

1. Review of published data

256. As described earlier, two distinct functional cytokine secretion patterns, designated Th1 and Th2, have been defined for helper T-cells. While Th1 cytokines promote cell-mediated immunity, Th2 cytokines favour humoral immunity, providing B-cell assistance for antibody production. Controversial results have been published concerning the effects of ionizing radiation on the Th1/Th2 balance and its impact on human health. Th1 and Th2 helper T-cells are cross-regulatory in vitro, and the balance of these cells in vivo determines the character of the cell-mediated immune and inflammatory response [N10]. An imbalance between Th1 and Th2 may be responsible both for the progression of several diseases and their resultant complications. Patients with advanced cancer often have impaired cell-mediated immunity associated with a switch from Th1 to Th2. While organ-specific autoimmune diseases have been related to an overreactive Th1 pathway, the Th2 pathway may underlie allergy and systemic autoimmune diseases [G13, K11]. On the other hand, shifting from one cytokine pattern to another may be highly beneficial in certain physiological conditions; for instance, IL-10 (a Th2-type cytokine) may play a role in pregnancy-associated immune tolerance through the establishment of a Th2 cytokine bias at the maternal–foetal interface [S20].

257. The impairment of cell-mediated immunity associated with the increase in the B-cell component and humoral immunity observed in atomic bombing survivors led to the hypothesis that radiation exposure could induce an imbalance towards a Th2 profile. The observed increase in percentage of CD4– CD8– $\alpha\beta$+ double-negative T-cells, known to produce primarily Th2-type cytokines, supported the idea that ionizing radiation could induce a shift from a Th1 to a Th2 response [K23]. Reduced IL-2 production in response to Con A in survivors was reported to be caused by a reduced number of naive CD4+ T-cells [K31]. However, a dose-dependent increase in TNF-α and IFN-γ production was observed in atomic bombing survivors, suggesting that Th2 does not dominate over Th1 [N3].

258. Interaction between cytokines and their receptors leads to the activation of multiple signalling molecules, including the family of "signal transducer and activator of transcription" (STAT) proteins. Different STAT proteins are capable of regulating the activity of diverse types of cytokine. It has been reported that mice with a disrupted *STAT* gene have impaired IL-12 responsiveness of NK and T-cells, a lack of Th1 responsiveness and enhanced Th2 function [K5]. Gamma radiation has been shown to reduce STAT1 phosphorylation. In contrast, mRNA levels for IL-5 were only slightly increased by gamma radiation compared with non-irradiated samples, suggesting that ionizing radiation induces a polarized Th2 response by interfering with STAT signals, thereby causing suppression of the Th1 response [H4].

259. In addition to the STAT protein family, the transcription factor NF-κB is one of the key regulators of the genes implicated in the immune inflammatory response. In a rat abdominal gamma irradiation model, Linard et al. [L26] have shown that genesis of the inflammatory process involved the translocation/activation of the NF-κB/Rel p65 subunit in the intestine. This activation was inhibited by a specific NF-κB inhibitor (caffeic acid phenethyl ester) that contributed to a reduction in the expression of TNF-α, IL-6 and IL-6 receptors. The cytokine signalling is under negative feedback regulation by intracellular proteins such as the suppressor of cytokine signalling (SOCS) gene. The differentiation into Th1 and Th2 is accompanied by a preferential expression of distinct SOCS (SOCS1 is highly expressed by Th1, whereas Th2 expresses high levels of SOCS3). Reports indicated that Th1 responses are likely to be negatively regulated by SOCS3 in vivo, rather than Th2 responses being attenuated by SOCS1 [I7]. Irradiation induced an intestinal overexpression of SOCS3 and a repression of SOCS1 in the first week. The inhibition of NF-κB activation reduced the SOCS3 expression [L27]. These data suggested that the polarized Th2 response induced by ionizing radiation involved the transcription factor NF-κB.

260. Bass et al. [B2] studied the ratio of Th1 and Th2 clones in the spleens of mice 4–6 weeks following total lymphoid irradiation by high-dose X-rays (5.5 and 8.5 Gy). The Th1/Th2 ratio was 1/0.6 in control mice, whereas the ratio in total lymphoid irradiated mice was approximately 1:7, supporting a conclusion that radiation exposure enhances Th2-type cytokine production [B2]. In contrast, it has been shown that low-dose WBI with 0.075 Gy X-rays is able to generate changes in IL-12 p35/p40 mRNA and IL-12 p70 protein levels, while IL-10 decreased significantly in splenocytes, and mRNA levels for both IL-12 p35 and p40 subunits increased in macrophages following WBI. The suppression of IL-10 expression and stimulation of IL-12 expression were interpreted as representing a shift of the immune response in favour of Th1 differentiation [L19].

261. The prevalence of a radiation-induced Th2 response has been verified in irradiated lung. Enhanced lymphocyte reactivity, dominated by Th2 cells, was shown in radiation-induced pneumonitis and subsequent pulmonary fibrosis. The kinetics was studied in T-cell lymphocytes isolated from lungs irradiated with 20 Gy. A selective increase of CD4+ T-cells was observed, peaking four weeks after irradiation of the lungs. When the rats were depleted in CD4+ T-cells, post-irradiation thickening of the parenchyma was significantly reduced, as determined by morphometric analysis. The CD4+ cell subtype of the T-lymphocyte population was analysed by measuring different cytokine mRNAs by RT-PCR (reverse transcription polymerase chain reaction). It was found that IL-4 mRNA was selectively increased in the CD4+ cells isolated from irradiated lungs, which indicates a lymphocyte reactivity by Th2 cells. The authors suggested a critical role for Th2 CD4+ cells in the pathogenesis of radiation-induced pneumonitis preceding lung fibrosis [W3].

262. Pharmacological improvement of the impaired Th1 function after irradiation has been used as an indirect way to investigate the radiation-induced enhancement of Th2 response. Ginsan (an acidic polysaccharide from *Panex ginseng*) is able to induce proliferation of lymphokine-activated killer cells, to increase the mitogen activity in different systems and to induce the production of several cytokines (such as IL-1, IL-6, IFN-γ and IL-12) that are required for haematopoietic recovery. In an experimental model of WBI, ginsan was injected in vivo and its action was evaluated by measuring its effect on CFU-S bone marrow cells and cytokine generation. Ginsan was shown to enhance Th1 function while interfering with the radiation-induced Th2 response [S27].

263. Interestingly, the cytokines characteristically expressed in Th1 cells seem to be regulated by cell-mediated suppression. The induction of TNF-β mRNA in lymphoid cells is greatly enhanced by gamma radiation. However, the level of TNF-β mRNA expressed in response to radiation and other stimuli, whether by mitogen or antigen, is strongly reduced by concomitant activation of suppressive cell subsets. Removal of CD8+ or CD11b+ cells leads to a substantial induction of TNF-β mRNA in the depleted cell population; this induction precedes the appearance of suppressive cell activity, allowing for temporary suppression. TNF-β, as well as other Th1 cytokines such as IFN-γ and IL-2, is suppressed by CD8+ or CD11b+ cells [A1].

264. IL-4 and IL-5 synthesis in lymph node cells primed by keyhole limpet haemocyanin (KLH) was greatly diminished after irradiation. In addition, the capacity of irradiated KLH-primed lymph node cells to induce IgG, IgM and IgE synthesis in hapten-primed cells was studied. Irradiation was not able to modify IgG synthesis in these cells, but their capacity to induce IgE was significantly reduced. Thus irradiation greatly inhibited the capacity of Th2 clones, but only minimally inhibited the capacity of Th1 clones, to induce IgG synthesis in primed B-cells. By adding IL-4 and IL-5, the capacity of Th2 cells to produce IgE was completely restored [D2].

2. Remarks concerning modification of Th1/Th2 balance

265. As discussed in the preceding paragraphs, two distinct functional cytokine secretion patterns have been defined for helper T-cells: Th1 and Th2. While Th1 cytokines promote cell-mediated immunity, Th2 cytokines favour humoral immunity. The balance of Th1 and Th2 helper cells in vivo determines the character of cell-mediated immunity and inflammatory response, the imbalance being responsible for the progression of several diseases and their resultant complications.

266. The results concerning the effects of ionizing radiation on Th1/Th2 balance are controversial. In survivors of the atomic bombings, the impairment of cell-mediated immunity associated with the increase in the B-cell component and humoral immunity suggests an imbalance towards a

Th2 profile induced by the radiation exposure. The observed increase in the percentage of CD4– CD8– αβ+ T-cells, known to produce mainly Th2-type cytokines, supports the hypothesis of a shift from Th1 to Th2. Nevertheless, a dose-dependent increase in TNF-α and IFN-γ secretion suggests that Th2 does not dominate over Th1.

267. The prevalence of a radiation-induced Th2 response has been verified in experimental studies, for example of the spleens of mice irradiated with high-dose X-rays and the lungs of rats irradiated with 20 Gy, where it was found that Th2CD4+ cells might play a critical role in the pathogenesis of radiation-induced pneumonitis. In contrast, after low-dose WBI, 0.075 Gy, the changes observed might contribute to a shift in favour of Th1 differentiation.

268. STAT proteins are key molecules in the regulation of the activity of different types of cytokine, and mice with disrupted STAT genes have a lack of Th1 responsiveness and enhanced Th2 function. It has been shown that radiation reduces STAT phosphorylation, inducing suppression of Th1 response. The transcription factor NFκB is another key regulator of the genes implicated in the immune inflammatory response, and the action of specific NFκB inhibitors after irradiation in a rat model contributed to a reduction of Th2 cytokine expression. In addition, the cytokines expressed in Th1 cells seem to be regulated by cell-mediated suppression.

D. Bystander effects and genomic instability

1. Review of published data

269. Bystander effects and genomic instability are two general mechanisms possibly involved in the effects of ionizing radiation on the immune system. The general features of these two mechanisms are reviewed in annex C, "Non targeted and delayed effects of exposure to ionizing radiation". Thus this section reviews only observations of these non-targeted and delayed effects that relate to the immune system.

270. Irradiation has been shown to induce leukaemic transformation of non-irradiated stem cells transplanted into syngeneic mice [D13]. These findings may reflect the altered characteristics of the stem cell microenvironment after irradiation, since irradiated haematopoietic stromal cells release mutagenic ROS, produce different sets of adhesion molecules and growth factors, and alter the overall growth and phenotypic characteristics of co-cultured non-irradiated stem cells [G7].

271. Haematopoietic tissues exposed to ionizing radiation have been shown to exhibit increased macrophage activation [L8]. Activated macrophages are able to induce apoptosis in neighbouring cells [B12, D12] and produce gene mutations [W2], DNA base modifications [D7], DNA strand breaks [S14] and cytogenetic damage [W1] in neighbouring cells. These various end points have all been demonstrated as non-targeted effects of ionizing radiation. Many properties of activated macrophages are consistent with in vitro studies that implicate free radical generation in non-targeted radiation effects [N5], and with other studies in which oxidative processes and nitric oxide have been implicated as having roles in the mechanisms [C12, G4, L11]. Nitric oxide can be either proapoptotic or anti-apoptotic, can either down-regulate or up-regulate p53 activity [B11], and can be either pro-inflammatory or weakly anti-inflammatory [G13, N6], depending on context.

272. A stimulatory bystander effect can be induced in immune cells by low-dose irradiation. When a mouse macrophage cell line (J774A.1) was exposed to a low dose (0.075 Gy) and co-cultured with a non-irradiated mouse T-lymphocyte cell line (EL-4), the irradiated macrophages exerted a stimulatory effect on the EL-4 cells, as shown by increased proliferation. At a high dose (2 Gy), irradiated J774A-1 cells exerted an inhibitory effect on the proliferation of the non-irradiated EL-4 cells. Preliminary mechanistic studies show that changes in CD48 expression and nitric oxide production by the J774A.1 cells after high- and low-dose irradiation might be important factors underlying the differential bystander effects elicited by different doses of radiation [L13].

273. There are few data on bystander effects in whole animals. However, prior to the recent interest in non-targeted effects, there were numerous reports that a transferable clastogenic activity capable of causing chromosome breaks in non-irradiated lymphocytes was present in the plasma of patients after radiotherapy, though with considerable inter-individual variation in both production and response [M15]. Clastogenic factors in plasma have also been obtained from atomic bombing survivors and Chernobyl liquidators [P3, W6], and from patients with a variety of chromosomal instability syndromes and inflammatory disorders. The chromosome-damaging effects of clastogenic factors are mediated by the superoxide anion. Their clastogenic activity may be related to the formation of lipid peroxidation products and cytotoxic cytokines, which are possible agents for mediating radiation-induced bystander effects. A body of clinical and experimental radiotherapy data exists concerning the "abscopal effects" of radiation, where responses are noted in unrelated organs or tissues that had not been irradiated [C2]. However, it is far from clear whether bystander killing contributes to the curative potential of radiotherapy and whether inducible instability is an important component of late adverse effects.

274. Xu et al. demonstrated that low doses of radiation (0.25–10 mGy) stimulate expression of IL-2 receptors (CD25) on the surface of peripheral blood lymphocytes taken from normal human donors [X1]. Clastogenic factors can also stimulate CD25 surface expression in non-irradiated cells, suggesting that this radiation-stimulated surface expression is a bystander effect resulting from the secretion into the medium of a soluble factor from the irradiated cells.

Stimulation of CD25 expression by ionizing radiation shows a triggered-type response rather than being proportional to dose.

275. Persistent subclinical inflammation has recently been reported among survivors of the atomic bombings in Japan [N7], and it was suggested that radiation-induced enhancement of inflammatory reactions might contribute, as an epigenetic and/or bystander effect, to the development of several radiation-induced disorders.

276. Thymic stromal cell cultures are able to support T-cell precursor proliferation and differentiation in the presence of IL-7 and stem cell factor. By exposing thymic stromal cell cultures to 10 Gy of gamma irradiation before the seeding of T-cell precursors, it was possible to demonstrate a reduction of these T-cell precursors without changes in their differentiation. The effect could be reproduced by the addition of supernatants from irradiated stromal cell cultures on to sham-irradiated cultures, which suggests that gamma irradiation induces the production of soluble factors by thymic stromal cells, which in turn modify their ability to support proliferation of T-cell precursors [B4].

277. Radiation-induced overexpression of IL-7 from thymic stromal cells is key to understanding the radiation-induced differentiation of CD8+ TCR γδ T-cells. When double-negative foetal thymocytes were co-cultured with foetal thymus irradiated with 25 Gy of low-LET radiation in the absence of direct contact or mitogen stimulation, induction of TCR γδ T-cells was observed, reaching 50% after 4 days of co-culture. Supernatants of the irradiated foetal thymus were also able to induce the differentiation from double-negative thymocytes to CD8+ TCR γδ T-cells after 3 days of culture, suggesting a radiation-induced production of soluble factors by thymic cells. It was possible using RT-PCR to detect an increased expression of IL-7 mRNA in the foetal thymus 24 hours after irradiation, and antibodies against IL-7 inhibited the radiation-induced differentiation [T7].

278. Shankar et al. studied bystander effects and adaptive response induced by gamma radiation in murine lymphocytes, using irradiated conditioned medium (ICM) from lymphocytes exposed to 0.1 Gy, 0.5 Gy and 1 Gy. They found that ICM enhanced the proliferation response of non-irradiated lymphocytes to Con A, with increased expression of IL-2 receptor and cyclin D, two proteins that drive progression through the cell cycle. ICM also enhanced intracellular ROS content and nitric oxide generation in non-irradiated lymphocytes. Apoptosis was significantly lower in lymphocytes exposed to a challenge dose of 1 Gy when they were preincubated with ICM. The results of these authors suggest that soluble factors released by irradiated lymphocytes trigger signalling pathways that result in increased response to mitogens and resistance to radiation exposure in non-irradiated lymphocytes [S50].

2. Remarks concerning bystander effects and genomic instability

279. Some remarks may be made on the basis of the data presented above. Delayed effects and genomic instability in the immune system have been demonstrated in vitro and in vivo after exposure to ionizing radiation. Chromosomal instability in haematopoietic cells can be induced by a bystander-type mechanism, providing a link between these two untargeted effects, as well as other radiation responses that are consistent with the microenvironment contributing to cell damage as a consequence of an inflammatory response to radiation-induced injury.

280. Activated macrophages after irradiation are able to induce apoptosis, gene mutations, DNA base modifications, DNA strand breaks and chromosome-damaging effects in neighbouring cells. Intercellular signalling and free radical generation are implicated in these non-targeted effects of ionizing radiation. Nitric oxide emerges as a key mediator in the bystander effects elicited by high- and low-dose irradiation. Likewise, the superoxide anion has been described as a mediator of clastogenic factors in chromosome-damaging effects.

281. Long-lasting inflammation has been reported among the atomic bombing survivors, and it was suggested that radiation-induced enhancement of inflammatory reactions might contribute as an epigenetic and/or bystander effect to the development of several disorders. However, the potential impact of such delayed effects in humans is not known.

E. Shift towards an inflammatory profile

1. Review of published data

282. Ionizing radiation may induce a persistent inflammatory status that could increase the risks of both cancer and non-cancer diseases [N3]. Higher risks of hepatic, cardiovascular and thyroid pathologies have been observed among atomic bombing survivors, and several authors have investigated the relationship between these chronic diseases and impairment of the immune system.

283. One of the main functions of cytokines is to mediate interactions between the immune and the inflammatory response. Chronic immune inflammatory diseases might be caused in part by dysregulation of cytokine production [B14]. TNF-α, IFN-γ, IL-6 and IL-10 coordinate the inflammatory response [H10]. The stimulus for production of acute phase proteins in response to tissue injury is likely to be mediated by these inflammatory cytokines. Thus the cytokine profile as well as the level of acute phase proteins may be useful markers of inflammation.

284. Exposure to high doses of ionizing radiation, such as those delivered during radiotherapy or in cases of accidental irradiation, can induce fibrosis in many tissues as a late effect.

Formation of the fibrotic tissue requires chronic activation of several cell types, including myofibroblasts, that secrete the collagenous matrix. The origin of the chronic activation of these cells is still a matter of debate. TGF-β1 has been proposed as a master switch for the fibrotic programme [M18]. This cytokine can be secreted by the inflammatory cells that chronically invade the irradiated tissue and locally by the myofibroblasts. Antioxidant treatment that reduces established fibrotic tissues in patients can act on both of these cell populations [D19, D20].

285. Neriishi et al. [N7] investigated the status of several inflammation parameters in atomic bombing survivors. They demonstrated a positive association between radiation dose and erythrocyte sedimentation rate, total leucocyte counts, alpha-1 and alpha-2 globulin, and sialic acid.

286. Hayashi et al. [H8] found increased plasma levels of C-reactive protein (CRP) and IL-6 in atomic bombing survivors. This increase was significantly related to radiation dose, by about 30% Gy^{-1} for CRP and 10% Gy^{-1} for IL-6, and was associated with a decrease in the percentage of peripheral CD4+ T-cells.

287. Inflammatory parameters were analysed in blood samples from atomic bombing survivors, including 180 non-exposed subjects and 90 subjects from each of the following dose groups: low dose (0.0005–0.7 Gy), medium dose (0.7–1.5 Gy) and high dose (>1.5 Gy). The levels of TNF-α, IFN-γ and IL-10 were significantly increased with radiation dose, as was the erythrocyte sedimentation rate. There was a radiation-dose-dependent increase in plasma levels of IL-6 and CRP. IL-6 stimulates the synthesis of acute phase proteins (such as CRP) involved in complement activation. A dose-dependent increase was also observed in total immunoglobulin levels. While the levels of IgA and IgM increased significantly with radiation dose, those of IgG and IgE did not [H10]. Increased serum levels of TNF-α [S13] and IgM [K21, S13] with higher functional complement activity [K21] were reported in Chernobyl emergency and clean-up workers. Higher serum levels of both IgG and IgM were found in children resident in contaminated areas around the Chernobyl nuclear power plant [K15, T5].

288. These observations provide evidence of a persistent inflammatory profile as a long-term effect after radiation exposure. Preclinical inflammatory status is linked in some way to a decrease in CD4+ T-cells, suggesting that radiation-associated immunological changes may cause long-lasting inflammation [K36]. This T-cell impairment may generate age-associated chronic inflammation, which may be responsible for an increased risk of various lifestyle-associated diseases, such as atherosclerosis, coronary heart disease, diabetes and several cancers [K36, N3].

2. Remarks concerning the shift towards an inflammatory profile

289. As reviewed in the paragraphs above, a persistent inflammatory status induced by ionizing radiation has been associated with impairment of the immune system and with cancer and non-cancer diseases. Since TNF-α, IFN-γ, IL-6 and IL-10 coordinate the inflammatory response, immune inflammatory diseases might be attributed in part to dysregulation of cytokine production.

290. Significant dose-dependent increases of TNF-α, IFN-γ and IL-10 in parallel with the erythrocyte sedimentation rate as a biomarker of inflammation were observed from blood analysis of atomic bombing survivors. Total immunoglobulin levels were also enhanced in a dose-dependent manner. On the other hand, increased serum levels of TNF-α and IgM with higher functional complement activity were reported in Chernobyl emergency and clean-up workers.

291. Preclinical inflammatory status may be linked to impairment of cellular immunity, e.g. a decrease in CD4+ T-cells observed after radiation exposure, suggesting that radiation-associated immunological changes could account for long-lasting inflammation.

F. Acceleration of immunological ageing

1. Review of published data

292. It has been proposed that acceleration of immunological ageing may be associated with radiation effects in humans. Immunosenescence has been described in section II above. As illustrated in table 11, comparison of the main features of immunosenescence with the experimental and epidemiological findings concerning radiation effects on the immune system supports this hypothesis.

Table 11 Comparison between the main features of normal immunosenescence and observed radiation effects on the immune system

Parameter	*Normal ageing*	*Irradiation*
Renewal capacity of stem cells and haematopoietic progenitor cells	Decreased [H1, L1]	Decreased [G5]
Total CD4+ T-cells	Decreased, about 4% per 10 years [K29]	Decreased [C7, C9, G9, K21, K33, P5, S8, T6, Y4], about 2% Gy^{-1} [K29] Increased [T9, V9]

Parameter	*Normal ageing*	*Irradiation*
CD4+ naive T-cells	Decreased [H16], about 7.5% per 10 years [K36]	Decreased [K25, K29, K37, Y2], about 4.5% Gy^{-1} [K36]
CD4+ memory T-cells	No significant changes [K36]	No significant changes [K36, Y2]
CD4+ naive/memory T-cell ratio	Decreased [H1, L5, V2, V4]	Decreased [K24, K25, K37, Y2]
Total CD8+ T-cells	No significant changes [K36]	No significant changes [K36]
CD8+ naive T-cells	Decreased, more than 40% per 10 years [K36]	Decreased [Y2], about 7.7% Gy^{-1} [K36]
CD8+ memory T-cells	Increased, about 7.3% per 10 years [K36]	Increased [Y2], about 5.6% Gy^{-1} [K36]
CD8+ naive/memory T-cell ratio	Decreased [U21]	Decreased [Y2]
Double-negative CD4– CD8– $\alpha\beta$+ T-cells	Increased [H15]	Increased [A6, K37, N1]
Available TCR repertoire	Reduced [H1, V2, V4]	Reduced [K25]
CD8+ CD28– (effector) T-cells	Increased proportion of CD8+ CD28– T-cells [E2, H16]	CD28 expression: up-regulation (low doses) and down-regulation (high doses) [L14]
T-cell responsiveness to mitogens	Lower [B6, B15, V2]	High doses: lower [A4, A5, K26, K31, P5] Low doses: higher [J6, L17, L18, N20, P11, S16, S17, Y1]
Thymus mass and cellularity	Decreased [H16]	Decreased [G8, N1, P6]
B-lymphocytes	Decreased, about 7.3% per 10 years [K36]; less ability to generate antibody responses [B15, F3, H1, L5]	Increased [K29, Y1], about 8.5% Gy^{-1} [K36] Decreased [G9, K24, P6] Hyporesponsiveness to LPS [C10, P6]
Lymphocyte oxidative status	Oxidative stress [V2]	Oxidative stress [C10]
IL-2 production	Decreased [E1, H16]	High doses: decreased [A2, B5, G8, K31] Low doses: increased [J8, L18]
TNF-α release	Increased [B6, E1], about 15% per 10 years [N3]	Increased [S13], about 7% Gy^{-1} [H10, N3]
IL-10	Increased, about 8% per 10 years [N3]	Increased, about 6% Gy^{-1} [H10, N3]
IL-6 release	Increased [B6, E1], about 24% per 10 years [H10, N3]	Increased [H5], about 13% Gy^{-1} [H10, N3]
C-reactive protein level	Increased, about 25% per 10 years [H10, N3]	Increased [H5], about 39% Gy^{-1} [H10, N3]
IgA	Increased, about 5% per 10 years [H10, N3]	Increased [T5], about 8% Gy^{-1} [H10, N3] Increased in females [F4] No change [K21]
IgG	–	Decreased [K21] Increased [K15, T5] No change [F4, H10]
IgM	–	Increased [F4, H10, K15, K21, T5]
IgE	–	Increased [K15] No change [F4, H10]
Total Ig	Increased, about 3% per 10 years [H10]	Increased [K14], about 3% Gy^{-1} [H10]
Erythrocyte sedimentation rate	Increased, about 15% per 10 years [H10, N3]	Increased [N7], about 17% Gy^{-1} [H10, N3]
Th1/Th2 imbalance	Shift from Th1 to Th2 [R1, S7]	Shift to Th2 [A1, B2, H4, K23, S27, T5, W3] Shift to Th1 [D2, L19, N3]

Parameter	*Normal ageing*	*Irradiation*
Production of autoantibodies	Increased [H13]	Increased [T6, Y1, Y4] No change [F4]
Neutrophils	Impaired function [G25]	Impaired function [K21]
Monocytes/macrophages	Impaired function [B15, H1]	Impaired function [G18, K33] Macrophage activation [C21, I6, L8, N24, P11] Increased proportion of peripheral monocytes [S13]
Absolute number of NK cells	Increased [B15], about 20% per 10 years [K36]	Increased [K24, K33] No significant change [K29, K36]
NK cell function	Impaired [H1]	Impaired [K15] Enhanced [C21, K18, Y1] No significant change [B5, P6]
CD3+ CD56+ CD16+ (NKT) cells	Increased [M9]	Increased [K33]
Immunoproteasome function	Impaired [M8]	Impaired [L10]

293. Kusunoki et al. [K29] tested the hypothesis that ionizing radiation may accelerate ageing by using the proportion of CD4+ T-cells as an index. They reported that the normal age-related decrease of CD4+ T-cells was about 4% per 10 years and that the radiation-induced decrease of CD4+ T-cells was about 2% Gy^{-1}. These findings suggest that exposure to 1 Gy is equivalent to about a 5-year increase in age.

294. In 442 atomic bombing survivors without a history of cancer or inflammatory diseases, Nakachi's group [H10, N3] found that both age and radiation exposure were associated with increases in selected plasma inflammation markers. Their findings indicated that the radiation effect might be estimated in terms of acceleration of ageing by applying a multivariate model (table 12). The per cent increments in TNF-α, IL-10, IL-6, CRP, IgA and erythrocyte sedimentation rate observed in atomic bombing survivors ranged from 6% Gy^{-1} to 39% Gy^{-1}, corresponding on average to an increase in age of 10 a/Gy.

Table 12 Multivariable model of the effects of age at time of irradiation and of radiation dose on inflammatory biomarkers and ageing[a]

Adapted from reference [N3]

Variable[b]	*Change in per cent (95% CI)*					
	TNF-α	*IL-10*	*IL-6*	*CRP*	*ESR*	*IgA*
Age at time of irradiation (per cent per 10 years)	15 (9, 20)	8 (4, 13)	24 (19, 30)	25 (13, 38)	15 (9, 20)	5 (2, 9)
Radiation dose (% Gy^{-1})	7 (1, 15)	6 (0, 12)	13 (6, 20)	39 (20, 62)	17 (9, 24)	8 (3, 13)
Estimated ageing effect of radiation (a Gy^{-1})	5 (0, 10)	6 (–1, 14)[c]	5 (2, 8)	14 (4, 24)	11 (5, 17)	15 (1, 29)

[a] Subjects were a total of 442 atomic bombing survivors who did not have a history of cancer or inflammatory-associated disease (e.g. chronic bronchitis, collagen disease, arthritis, myocardial infarction).

[b] TNF-α = tumour necrosis factor α; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

[c] Estimated by the δ-method.

2. Remarks concerning the acceleration of immunological ageing

295. The preceding paragraphs indicate that comparison of the main features of immunosenescence with the epidemiological and experimental findings on radiation effects on the immune system supports the hypothesis that acceleration of immunological ageing may be involved in radiation effects in humans. No demonstrable relationship with cancer or inflammatory diseases in atomic bombing survivors has been found.

G. Modification of antigen presentation

1. Review of published data

296. "Danger" signals affect antigen-presenting cells (APCs), and the dendritic cells are the most powerful. Functional antigen presentation by dendritic cells requires that the cells mature under the influence of different danger signals. However, in addition to the maturation of dendritic cells being essential for lymphocyte activation, immature dendritic cells themselves maintain a state of immunological tolerance. Cells dying by necrosis are more potent at inducing dendritic cell maturation than cells dying by apoptosis (the latter may even block maturation). However, cells that have undergone apoptosis in response to stressors such as heat or ionizing radiation may be more effective for inducing dendritic cell maturation than cells that have undergone natural physiological apoptosis.

297. Although dendritic cells are relatively radioresistant, ionizing radiation affects their functions. Liao et al. demonstrated that non-cytotoxic effects of ionizing radiation might account for the impairment of antigen processing and presentation following irradiation of dendritic cells. Cell viability was not significantly affected over a 24 h culture period following 10 Gy of gamma irradiation at 4.5 Gy/min. However, immunoproteasome activity was down-regulated in irradiated cells by 50–60% [L10].

298. It has been reported that ionizing radiation may cause a generalized decrease in proteasome functions in mammalian cells [M3]. Immunoproteasomes are responsible for the processing of antigens for presentation by the class I HLA pathway. Alterations of immunoproteasome function have been proposed as a signal of immunosenescence [M8]. Proteasomes are direct redox-sensitive targets for the action of ionizing radiation [M2]. Using microarray analysis, Snyder [S26] has studied gene expression profiles of cells exhibiting radiation-induced genomic instability. Two of the genes that were underexpressed belonged to the proteasome/ubiquitin pathway. These findings suggest that ionizing radiation could impair immune function by altering antigen processing at the immunoproteasome level.

299. Findings concerning the effect of ionizing radiation on HLA molecules are controversial. Liao et al. did not find significant changes in the expression of class I and class II HLA molecules on dendritic cells following gamma irradiation with a single dose of 10 Gy [L10]. Reits et al. found that cell surface expression of class I HLA molecules increased in a radiation-dose-dependent manner on murine colon adenocarcinoma cells [R19]. Radiation-induced enhancement in the expression of class I HLA molecules has been reported by Hauser et al. in a murine melanoma cell line following fractionated gamma irradiation (50 Gy in 25 fractions) [H7]. In contrast, a decrease in the expression of class I HLA molecules was found in a human melanoma cell line following gamma irradiation with a single dose of 20 Gy [G35]. These discrepancies may be due to the pathways activated by ionizing radiation in different cell types as well as to different dose protraction and total doses.

300. HLA mutant lymphocytes are induced by radiation exposure and eliminated by NK cells [K30]. Autologous NK cells are responsible for the elimination of mutant lymphocytes that have lost the ability to express self class I HLA molecules in vivo [K27], and therefore may explain why it has not been possible to detect increased frequencies of HLA mutants in blood samples from atomic bombing survivors.

2. Remarks concerning modification of antigen presentation

301. The data reviewed indicate that dendritic cells, the most powerful APCs, are relatively radioresistant, but that radiation may affect their function. Down-regulation of immunoproteasome activity has been reported. Immunoproteasomes are responsible for the processing of antigens for presentation by the class I HLA pathway; consequently, ionizing radiation could impair immune function by modification of antigen processing at the immunoproteasome level. This was also proposed as a sign of immunosenescence.

302. Concerning the effects of ionizing radiation on HLA molecules, controversial results are presented. Non-significant changes were found in the expression of class I and class II HLA molecules following irradiation of dendritic cells. Enhancement in the expression of class I HLA molecules after fractionated irradiation of a murine melanoma cell line, as well as down-regulation of their expression following acute irradiation of a human melanoma cell line, have been reported. Different dose protraction and total doses, as well as different cell types, may account for the discrepancies.

H. Autoimmune reactions

1. Review of published data

303. Ionizing radiation can produce functional alteration the immune system and break self tolerance. Autoimmune diseases are characterized by the activity of autoreactive lymphocytes that produce antibodies targeting self tissues or organs for destruction. Autoimmunity may be seen as a case of "mistaken identity", in which the immune system mistakes part of the body for a foreign invader. One explanation for why the immune system attacks self tissues in some people is molecular mimicry, which means that a part of a molecule of a given protein closely resembles a part of another, totally different protein. It has been shown that peptides in various infectious agents resemble parts of various self proteins. Therefore, if these protein fragments are presented to T-cells, the activated immune system will not only attack all foreign invaders with the same pattern but could also attack a very similar pattern in a self protein [O10]. The significant dose-dependent impairment of the immune system demonstrated in irradiated populations [K14, K21, K29, K31, V9] could lead to many of these persons being less responsive to

infectious agents. These individuals are at a higher risk for viral or microbial infection, and are therefore more prone to developing autoimmune diseases by molecular mimicry.

304. It was not proved that autoimmune reactions were involved in the pathogenesis of thyroid diseases in atomic bombing survivors [I8]. However, the immune system has been demonstrated to be involved in the pathogenesis of thyroid diseases in victims of the Chernobyl accident. These apparently contradictory results may be due to the different thyroid exposure conditions (acute external irradiation versus internal exposure from radioiodine). It is possible that thyroid damage induced by internal exposure from radioiodine sets into motion antigenic mechanisms that lead to autoimmune responses. Epidemiological data from children exposed to radioiodine during the Chernobyl accident indicate signs of autoimmune thyroid disorder and impaired NK-related elimination of tumour cells, both of which may be contributing to the promotion of thyroid neoplasia in this population.

305. Sakaguchi et al. [S6] demonstrated that high-dose fractionated total lymphoid irradiation (42.5 Gy in 17 fractions) caused various organ-specific autoimmune diseases in mice. Irradiation of the target organs alone failed to elicit the autoimmunity, and shielding the organs from irradiation failed to prevent it, suggesting that radiation-induced tissue damage is not the primary cause of the autoimmune disease. A significant decrease in mature thymocytes and peripheral T-cells was observed for one month post-irradiation. These findings suggest that high-dose fractionated total lymphoid irradiation can cause autoimmune disease by affecting the T-cell immune system (rather than the target self antigens), presumably by altering T-cell-dependent control of self-reactive T-cells.

306. In a further paper, Sakaguchi [S3] demonstrated that elimination of CD4+ CD25+ T-regulatory cells (Treg) led to the development of various organ-specific autoimmune diseases in mice. Reconstitution of the Treg population prevented the development of autoimmunity. Moreover, elimination or reduction of the Treg population by environmental agents also induced autoimmune diseases in normal mice, suggesting that radiation-induced CD4+ T-cell alterations may cause autoimmunity without altering the antigenicity of the host organs concerned [K23].

2. Remarks concerning autoimmune reactions

307. As discussed in the preceding paragraphs, self tolerance breaking may be among the alterations on the immune system induced by radiation. No radiation-induced autoimmune reactions were involved in the pathogenesis of thyroid diseases observed in atomic bombing survivors. In contrast, the immune system was involved in the pathogenesis of thyroid diseases in people exposed to radiation from the Chernobyl accident. This may be due to the different thyroid exposure conditions. Experimental data obtained from fractionated total lymphoid irradiation showed that the development of autoimmunity was related to the alteration of T-cell-dependent control of self-reactive T-cells.

I. Perturbation of immunological homeostasis

1. Review of published data

308. Immunological homeostasis is the mechanism by which the immune system responds to foreign antigens (e.g. an infectious organism) and then returns to its original state, although retaining memory cells that will protect the host against subsequent infection by the same organism. This homeostasis is achieved in the T-cell system by the balance between renewal and death of naive and memory T-cells. The ability to maintain both naive and memory T-cell pools, which declines with age, is critical for immune function. An immunostatic mechanism exists that controls the proportions of immune cell types, the production of cytokines and the level of expression of functional immune cell molecules.

309. Exposure to ionizing radiation is thought to affect T-cell homeostasis. Both experimental and epidemiological data have demonstrated that ionizing radiation may perturb T-cell homeostasis by reducing the ability of the immune system to produce new naive T-cells and by disturbing the regulation and maintenance of memory T-cell pools. A diverse pool of naive T-cells is necessary to produce immune responses to new antigens. Exposed atomic bombing survivors showed lower numbers of naive CD4+ and CD8+ T-cells. Although memory T-cell pools were either normal (CD4+) or even larger (CD8+) in size [Y2], their TCR repertoire was significantly reduced with radiation dose [K25], probably due to clonal expansion of memory T-cells. Alterations in T-cell subpopulations were also described in Chernobyl clean-up workers [T6, Y4].

310. Radiation-induced perturbation of T-cell homeostasis may have important health implications. The reduction of the naive T-cell pool may lead to reduced ability of the host to defend against new pathogens, and the clonal expansion of memory T-cells associated with TCR repertoire deviation may compromise the ability of the host to control recurrent and latent infections [K36].

2. Remarks concerning perturbation of immunological homeostasis

311. The data reviewed indicate that immunological homeostasis is the mechanism by which the immune system responds to foreign antigens and returns to its original state, but retaining memory cells to protect against subsequent infection by the same agent. This homeostasis is achieved by the balance between renewal and death of naive and memory T-cells. Experimental and epidemiological data have demonstrated that radiation alters T-cell homeostasis by reducing the ability of the immune system to produce new

naive T-cells and by disturbing the maintenance of memory T-cells. The perturbation of T-cell homeostasis may have health implications in terms of decreased defences against new pathogens and compromised ability to control recurrent and latent infections.

J. Other possible mechanisms involved

1. Review of published data

312. Liu et al. [L16] proposed that catecholamines mediate the increase in proliferative reactivity of splenic and thymic lymphocytes that has been observed in mice irradiated at a low dose (75 mGy). In a similar model, they demonstrated the involvement of intracellular calcium and protein kinase C in the facilitation of signal transduction in lymphocytes and suggested that this facilitation was implicated in the mechanism of immune enhancement after low-dose irradiation [L17].

313. Modulation of oxidative status has been postulated as another mechanism for radiation-induced immune stimulation following low-dose irradiation. Kojima et al. [K16] found an increase of immune function in mouse splenocytes that correlated with endogenous glutathione (GSH) accumulation within the first six hours after irradiation. This effect was enhanced by exogenous addition of precursors of GSH synthesis and was completely blocked by inhibition of GSH synthesis, suggesting that low-dose exposure to ionizing radiation enhances immune function through the induction of GSH [K16, K17, K18].

314. Indirect effects of local radiotherapy on tumour cells outside the radiation field have been reported in many malignancies [A14, C2, E3, K13, N11, O7, R2, S15, U20]. It has been proposed that this phenomenon, originally described as the "abscopal effect", may be related to radiation-induced effects on the immune system. Several factors could influence this effect, such as the immunological state of the tumour-bearing host and the immunogenicity of the tumour cells, as well as the schedule and overall dose of radiotherapy.

315. In an experimental mammary carcinoma model, Demaría et al. [D3] demonstrated that the growth of tumours outside the radiotherapy field was impaired by the combination of radiotherapy and Flt3 ligand, a haematopoietic cytokine that has been shown to facilitate the expansion of dendritic cells and the generation of an antitumour immune response. Importantly, in this experimental model Flt3 ligand alone had no effect on the growth of primary or secondary tumours, indicating that ionizing radiation was involved in the abscopal phenomenon. In addition, this abscopal effect was absent in nude mice (i.e. in mice lacking T-cells). In contrast to immunocompetent mice, the addition of Flt3 ligand did not result in any abscopal effect in nude mice. These findings indicate that the abscopal effect triggered by local irradiation was T-cell dependent [D3].

316. In a recent paper, Van der Meeren et al. [V3] investigated the radiation-induced inflammatory response after total-abdominal or whole-body irradiation of mice at a dose of 15 Gy. A comparison with WBI was used to take into account haematopoietic involvement in the inflammatory process. The authors found a systemic inflammatory reaction after both abdominal irradiation and WBI, with an increased cytokine and chemokine production at the intestinal and lung levels, indicating a possible abscopal effect of radiation. They postulated that the effects observed in the lungs after irradiation of the abdominopelvic region may be caused by circulating inflammatory mediators due to the gut inflammatory response.

317. One question that remains unanswered is whether the immunogenetic background may be involved in disease risks of irradiated subjects. There are large individual variations in the level of immunological parameters and inflammatory markers. Only some individuals with reduced immune function and/or elevated inflammatory biomarkers develop particular diseases [K36]. Thus it may be postulated that individual immunogenetic background may determine individual susceptibility to certain diseases. One particularly important genetic factor that can affect host immune response appears to be the *HLA* gene. Higher risks of type 2 diabetes were found between heavily exposed atomic bombing survivors with different class II HLA *DQA1* and *DRB1* alleles [H12], suggesting that certain class II *HLA* genes regulate one or more components of the immune system that are related with the risk of diabetes development in irradiated people.

318. Ionizing radiation exhibits immunomodulatory properties. The "danger" model of immunity describes antigen-specific cellular immunity engendered by an inflammatory milieu, where an important role is played by dendritic cells. Ionizing radiation may create an inflammatory setting via induction of apoptosis, necrosis, cell surface molecules and secretory molecules. Radiation may influence the expression of immunomodulatory surface molecules (MHC, co-stimulatory molecules, adhesion molecules, death receptors, heat shock proteins) as well as secretory molecules (cytokines, inflammatory mediators), in both tumour and normal cells. Experimental data indicate possible radiation-mediated modulation of tumour antigen-specific immunity [F5]. Radiation-mediated immunomodulation currently remains unquantified and poorly understood. A major research effort will be required to better elucidate the mechanisms involved.

2. Remarks concerning other possible mechanisms involved

319. Other possible mechanisms involved are:

- Involvement of catecholamines in the proliferative activity of splenic and thymic lymphocytes, as well as intracellular calcium and protein kinase C in the signalling of immunoenhancement after low-dose irradiation;

- Modulation of oxidative status following low-dose irradiation;
- Indirect effects of local radiotherapy, outside the radiation field, described originally as "abscopal effects";
- Possible involvement of immunogenetic background in disease risks of irradiated subjects.

K. Immune mechanisms and cancer

1. General considerations

320. That cancer may result as a stochastic effect from exposure to ionizing radiations has been well known for a long time, and mechanisms of radiation carcinogenesis have been extensively reviewed [U2, U4, U5, U6]. In the context of this annex, it is most important to understand the mechanisms of cancer development and especially to investigate the possible roles of the immune system.

321. Cancer is a multifactorial disease for which a genetic susceptibility and environmental factors—chemical, physical or viral—can be responsible. It is worth noting that some specific cancers are usually linked to specific exposures to environmental or infectious factors [N3, W18]. Some evidence has been recorded of a possible connection between haematological malignancies and exposure to ionizing radiation, although nitrates, pesticides, HTLV1 or Epstein–Barr virus infections, and immunodeficiency are other possible risk factors [D27]. The increased cancer incidence observed in organ transplant recipients has been related to immunosuppressive treatments that must be maintained to prevent and treat acute rejection [A11, A30, O11].

322. Cancers are not merely autonomous masses of mutant cells, but are composed of multiple cell types such as fibroblast and epithelial cells, cells that form blood and lymphatic vasculature, specialized mesenchymal cells that are unique to each tissue environment, and indeed innate and acquired immune cells [C35]. While tissue homeostasis is maintained by collaborative interactions between these diverse cell types, cancer development is enhanced when mutant cells neutralize homeostatic growth constraints and hijack the normal physiological processes to favour their own survival. Furthermore, tumours can develop an angiogenic phenotype which gives them a potential of growth and metastatic migration [F14].

323. It is recognized that each stage of cancer development can be exquisitely susceptible to modulation by immune cells. In essence, there is a complex relationship between immune cells and developing tumours with the following paradox: full activation of immune cells in response to the tumour may result in eradication of malignant cells and conversely an inefficient immune response may leave tumour cells with the possibility to grow, whereas chronic activation of various types of innate immune cell in or around premalignant tissues may actually promote tumour development. In cancers, the abundance of infiltrating lymphocytes, which are the predominant cells involved in the acquired response, correlates with a favourable prognosis. On the other hand, an abundance of infiltrating innate immune cells, such as macrophages, mast cells and neutrophils, correlates with increased angiogenesis and/or a poor prognosis [D17], although clusters of macrophages around tumours are often associated with tumour regression [G27].

324. Therefore, with regard to the role of the immune system in cancer development, it could be inferred that ionizing radiation might modify cancer risk not only by acting as a carcinogen per se but also by modulating host immune response. Genotoxic stress and stalled DNA replication forks induce the expression of ligands for the NKG2D receptor found in NK and certain T-cells, cell types that are able to attack tumour cells [G36]. This activation depends on proteins involved in DNA damage-sensing pathways and cell cycle regulation. This might explain how DNA damage response participates in altering the immune response to the presence of potentially dangerous cells.

2. Immune surveillance theory

325. The fact that so many persons die each year of cancer suggests that their immune response to tumour cells is inefficient. Indeed the immune system may respond only if novel antigens are expressed on the cell surface by tumour cells and are subsequently recognized as non-self neoantigens [L29]. A number of alterations occur in the cell during tumorigenesis: depression of some genes, expression of others or alteration of genes via mutations. Thus genetic changes related with carcinogenesis may result in the expression of "aberrant" molecules by transformed cells (reappearance of embryonic proteins not expressed in adult life, expression of unique antigens not expressed by normal cells). However, the prevalence of an antigen expression can vary, meaning that not all tumours of a particular type may express the antigen at all. Many antigens have heterogeneous expression, with the result that the proportion of cells that express the antigen within each tumour may vary from patient to patient. Indeed, antigen expression depends on the status of the cellular machinery; for example, antigen expression would be reduced in the case of proteasome deficiency [D28]. It is worth noting that most tumours induced by physical, chemical or viral agents express neoantigens [S52], while in contrast, spontaneously occurring tumours are only often weakly immunogenic or are non-immunogenic.

326. In the immune surveillance theory of cancer, tumours can develop only when cancer cells can escape from the immune surveillance either by reducing the expression of tumour antigens or by modifying immune recognition and activation [Z3]. As an example, the down-regulation of the synthesis of class I HLA molecules in tumours and metastases is a potential mechanism by which cancer cells can escape from class I HLA restricted lysis by cytotoxic T-cells.

In addition, the expression of the non-classical class I HLA molecule HLA-G on the cell surfaces of various cancers (melanomas, kidney cancers, breast cancers) inhibits both the cytotoxic activity of NK lymphocytes and the antigen-specific cytotoxic lymphocyte response [R22].

327. In support of the immune surveillance theory is the fact that the incidence of cancers is significantly increased in immunodepressed patients whatever the cause of their immunodepression. This is well documented in patients with AIDS due to viruses of the HIV family. These patients, who present with an unusually high frequency of Kaposi sarcoma, are also more sensitive to radiation. Unlike the classical Kaposi sarcoma, where radiation therapy is associated with minimal toxicity, radiotherapy treatment of this tumour in AIDS patients is associated with very high morbidity [C32, H30, R11].

328. A higher incidence of cancers is also observed in transplant patients who receive immunosuppressive treatments [A11, O11]. This higher incidence concerns not only tumours associated with latent infections [N3], but also tumours commonly observed in the general population, such as digestive, respiratory, endocrine and breast cancers. The increased incidence of these tumours can be associated with the immunosuppressive treatment [L3], although some confounding factors such as tobacco and alcohol certainly play a role [C28, F13, H26].

329. The immune system can produce significant antitumour effects, for example after allogenic bone marrow grafting. T-cells from the donor recognize the tumour as non-self and develop impressive antitumour effects (graft-against-tumour effects).

330. Natural antibodies are not very efficient at destroying tumour cells. However, it has been suggested that the immune system may eliminate tumour cells that carry tumour-specific antigens, leaving room for the tumour to grow cells with a low level of these antigens or with antigens that differ only very slightly from those of normal cells [G27]. On the other hand, monoclonal antibodies can be successful in controlling tumours when they have a high affinity for the tumour and can be used in large quantities. The most successful examples are the treatments of B-lymphoma with anti-CD20 monoclonal antibodies and of some breast cancers with anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibodies.

331. Against the immune surveillance theory, it has been observed that the relative risk for some common non-virus-associated solid tumours of epithelial origin (breast, prostate and bladder) is decreased in some immune-suppressed patients [F12, G17]. This finding has not yet been completely explained, although it has been suggested that immunosuppressive drugs could have a direct antitumour effect.

332. In order to test the validity of the immune surveillance theory, experiments can be carried out in animals. This is the case in nude mice with no thymus, which do not develop more cancers than normal mice though this would be expected given the lack of T-cells. On the other hand, it has been observed in some tumour grafting experiments that the tumour does not develop after injection of intermediate doses of cancer cells, while tumour development is observed after injection of low and high doses of cancer cells; this finding is difficult to reconcile with the immune surveillance theory.

3. Immune response against tumours

333. Cancer immune surveillance involves innate and acquired responses. Innate responses for transformed cells are associated mainly with NK cells, and the function is balanced between activating and inhibitory receptors. This balance significantly influences the efficacy of the immune response and consequently of tumour progression [S12]. Activating NK receptors may respond to stress-inducible proteins overexpressed by tumour cells. Conversely, the lost or down-regulated expression of class I HLA molecules in transformed cells (“missing self”) may suppress the inhibitory signalling in other NK receptors. NK cells can protect against experimental tumour growth, in part by producing mediators with anti-angiogenic properties [H22, S45]. NK cell deficiency as observed in the Chediak–Higashi syndrome in humans and in Beige mice results in some circumstances in an excess of cancers.

334. Tumour cells may express HLA-G, a non-classical class I HLA molecule involved in immunotolerance. The expression of HLA-G by malignant cells prevents their elimination, and constitutes a newly described mechanism by which tumour cells may evade immune surveillance [C4]. Through the interaction with specific inhibitory receptors, HLA-G can protect tumour cells lacking classical class I HLA expression from cytotoxicity mediated by NK and T-cells [S12]. Through the inhibition of MICA signals, HLA-G may lead to tumour escape from immune surveillance [R9].

335. Genotoxic stress and stalled DNA replication forks induce the expression of ligands for the NKG2D receptor found in NK and certain T-cells, cell types that are able to attack tumour cells [G36]. This activation depends on proteins involved in DNA damage-sensing pathways and cell cycle regulation. This may explain how DNA damage response participates in altering the immune response to potentially dangerous cells.

336. Activated macrophages can play a significant role in the immune response against tumours. Their antitumour activity is probably linked to lytic enzymes and the generation of free radicals. Furthermore, macrophages produce TNF-α, a cytotoxin with a powerful antitumour activity.

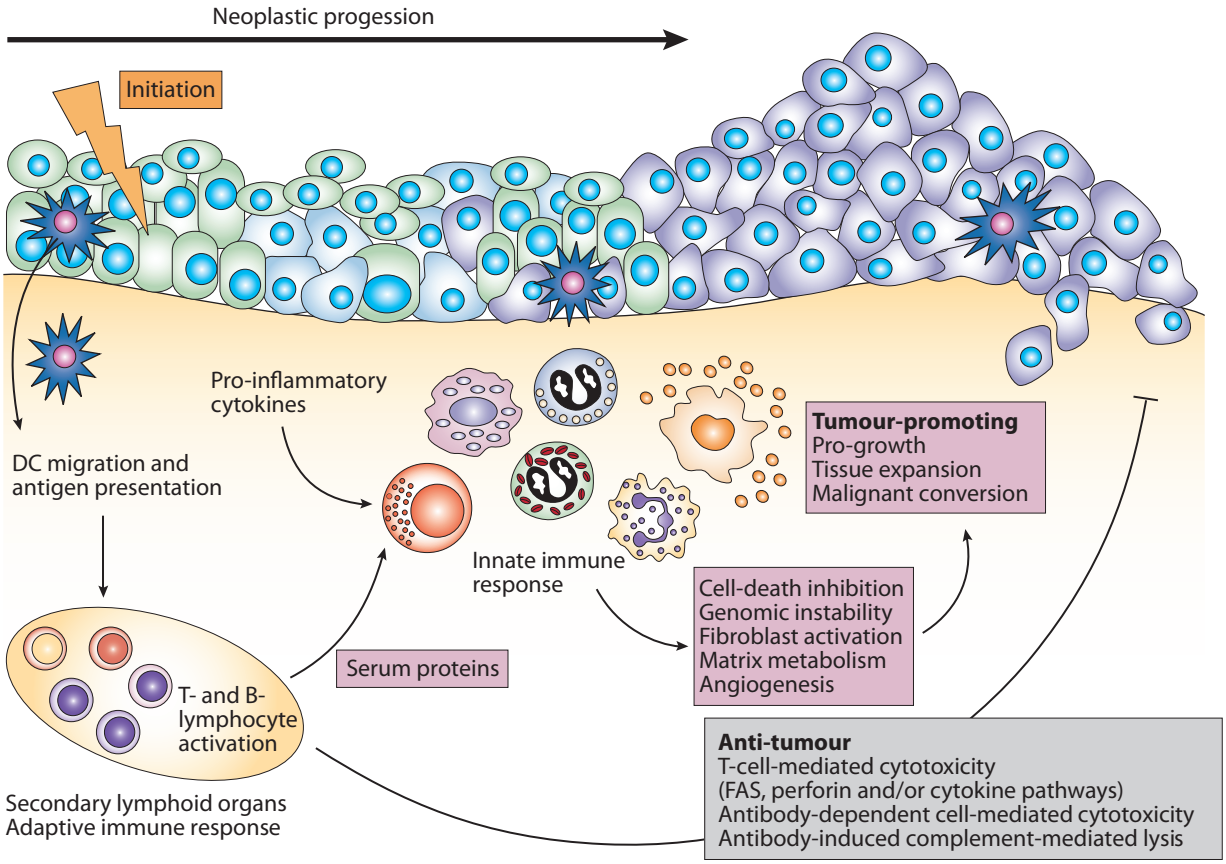
337. Acquired immunity involves the recognition by T-cells of the products of mutated genes, oncogenic virus

products or normal proteins aberrantly expressed. Moreover, interactions between T- and B-cells mediated by cytokines, as well as minor T-cell subsets such as NKT cells and γδ T-cells, may act in eliminating transformed cells.

4. Immunologic promotion of tumours

338. Exposure to ionizing radiation may impair the immune system and, on some occasions, result in low-grade chronic inflammation. It has been proposed that persistent inflammation could play a role in promoting the proliferation of initiated cells through the generation of ROS, the production of inflammatory cytokines and the induction of genetic instability. During chronic inflammatory processes there is an excess production of free radicals, which deregulate cellular homeostasis and can drive normal cells to malignancy [B1]. Recent findings have provided evidence suggesting that persistent inflammation involving repeated infection could be a key step in carcinogenesis. Indeed, the long-term inhibition of chronic inflammation by aspirin and selective cyclooxygenase-2 (COX-2) inhibitors in patients with premalignant disease, or who are predisposed to cancer development, has significantly reduced cancer risk [D15]. Figure VIII shows a model proposed by de Visser et al. [D17] for explaining innate and acquired immune cell functions during inflammation-associated cancer.

Figure VIII. Model of innate and acquired immune cell functions during inflammation-associated cancer.
Tumour antigens are presented by dendritic cells (DCs) to activate acquired immune responses, which may result in both antitumour (direct) effects and tumour-promoting effects (via innate immune response) [D17].



339. Chronically activated innate immune cells can also contribute indirectly to cancer development through suppression of antitumour escape from immune surveillance. For example, myeloid suppressor GR+CD11b+ cells, e.g. a subset of innate immune cells, induce T-lymphocyte dysfunction by direct cell–cell contact and by production of immunosuppressive mediators, and therefore actively inhibit antitumour acquired immunity [G16, S42, Z3].

340. Immunological activation of tumour growth by tumour cells has been observed in experimental animals. In many circumstances, attempts to protect animals against tumour growth by active immunization by tumour-specific antigens or by passive immunization by specific tumour antibodies have, surprisingly, yielded tumour growth. This is interpreted as being due to the existence of blocking factors. either the antibody itself, which after binding the antigen hides it from

cytotoxic T-cells, or the antibody–antigen complex, which inhibits the antibody-dependent cell-mediated cytotoxicity by binding to the Fc receptors on the surface of NK cells or macrophages and blocking their activity.

5. Remarks concerning immune mechanisms and cancer

341. On the basis of the preceding paragraphs, some remarks may be made concerning immune mechanisms and cancer. A complex relationship giving rise to a paradox is observed between immune cells and developing tumours: full activation of immune cells in response to the tumour may result in the elimination of tumour cells, whereas an inefficient immune response allows their growth. In addition, chronic activation of various types of innate immune cells in or around premalignant tissues may actually promote tumour development.

342. The immune surveillance theory suggests that tumours can develop only when cancer cells can escape from the immune surveillance either by reducing the tumour antigens or by modifying the immune response to them. A large body of evidence in favour of the immune surveillance theory comes from immunodepressed patients, who present a higher incidence of cancer. Evidence has also been seen in transplant patients who receive an immunosuppressive treatment. In contrast to these observations, it has been reported that the relative risk for some common non-virus-associated solid tumours of epithelial origin is decreased in some immunodepressed patients. An antitumour effect of the immunosuppressive drugs was suggested in these cases.

343. The capability of the immune system to develop antitumour effects involves innate and acquired responses. The innate response is mainly associated with NK cells. Its function is balanced between activating and inhibitory receptors. NK cell deficiency as observed in human and mice syndromes results in some circumstances in an excess of cancers. A newly described mechanism by which malignant cells avoid their elimination is through the expression of HLA-G, a non-classical class I HLA molecule that can protect tumour cells lacking classical class I HLA expression from cytotoxicity mediated by NK and T-cells. Similarly, activated macrophages play an antitumour role linked to lytic enzymes and the generation of free radicals along with the release of cytokines with a powerful antitumour activity.

344. Acquired immunity involves the recognition by T-cells of the products of mutated genes, oncogenic virus products and normal proteins aberrantly expressed.

345. Finally, long-lasting inflammation could play a role in promoting the proliferation of initiated cells through the generation of free radicals, the release of inflammatory cytokines and the induction of genetic instability, because of alterations of immune cells.

L. Summary

346. There are many mechanisms potentially involved in radiation-induced alterations of the immune system:

- Radiation-induced apoptosis is a key mechanism, well established for blood-circulating white cells, mostly lymphocytes.
- Mutations of TCR genes is a radiation-dose-dependent mechanism which can produce defective TCRs and alter the discrimination between “self” and “non-self”.
- There is still some controversy regarding the functional cytokine secretion pattern of helper T-cells following exposure to ionizing radiation, although it is likely that the homeostatic balance between Th1 pattern (cell-mediated immunity) and Th2 pattern (humoral immunity) is shifted towards a pro-inflammatory profile.
- Delayed effects, e.g. bystander effects and genomic instability, have been demonstrated after exposure of the immune system to ionizing radiation. However, the potential impact of such delayed effects in humans is not known.
- Inflammation resulting from the effects of ionizing radiation can be observed at the microscopic level and involves immune cells within and around tumours, but inflammation may be large enough to produce significant alterations of parameters in blood samples. Inflammation may be associated with chronic diseases.
- There is a vast literature showing that immune cells after exposure to ionizing radiation show abnormalities that are quite similar to those observed in normal ageing. These observations at the biological level have so far not been linked to diseases.
- Alterations of the process of antigen presentation at the level of the immunoproteasome have been demonstrated and are possibly a signal of immunosenescence after exposure to ionizing radiation.
- Ionizing radiation can contribute to a disturbance of self tolerance and consequently can pave the way towards autoimmunity.
- Finally, the immunological response against foreign antigens implies T-cell homeostasis, which is disturbed after exposure to ionizing radiation.

347. Besides apoptosis, which is a key mechanism within the immune system, it is rather difficult to classify the other mechanisms according to their importance after exposure to ionizing radiation. It likely that these mechanisms are interlinked, e.g. microscopic inflammation, propagated by a type of bystander mechanism and contributing to the ageing of tissues and promotion of cancer. Three hypotheses to further explore the mechanisms involved in the effects of

ionizing radiation on the immune system and their impact in human health have been postulated by the Radiation Effects Research Foundation (RERF) (see section IV):

- Ionizing radiation may accelerate immunological ageing by perturbing T-cell homeostasis;
- Ionizing radiation may induce long-lasting inflammation that may lead to disease development;
- Individual immunogenetic background may determine individual susceptibility to succumbing to disease.

348. Immune surveillance is different for the various cancer entities and has not been reported for all of them. The immune surveillance theory of cancer development remains controversial. Although the immune system has the capability to develop impressive antitumour effects, it is not very clear that cancer results from a deficiency of the immune system, and tumours can be promoted through low-level chronic inflammation because of alterations of immune cells. The potential effects of low doses of ionizing radiation on the critical balance existing in the immunological network (promoting or suppressing antitumour immunological response arising in the tumour microenvironment) has been insufficiently studied.

IV. EPIDEMIOLOGICAL STUDIES

A. Atomic bombing survivors

1. General considerations

349. Epidemiological studies of the survivors of the atomic bombings in Japan are currently the most important single source of radiation risk estimates for humans. Most of the information on the health effects of ionizing radiation available to date comes from long-term studies of survivors of the atomic bombings in Hiroshima and Nagasaki. Almost sixty years after exposure to radiation, survivors of the atomic bombings still exhibit increased risks of developing solid tumours. Immune mechanisms have consistently been associated with either resistance to or development of numerous tumours. Also, an association between non-cancer mortality and radiation dose has been observed among survivors, cardiovascular, thyroid and liver diseases being the more frequently reported causes. Long-lasting inflammation may be considered an important contributory factor for the development of some of these diseases [K36]. Knowledge of the impact of radiation on the immune system is therefore critical to assessing radiation-induced effects on the long-term health of survivors [K23, K36].

2. Short-term effects

350. It has been estimated that around 114,000 people in Hiroshima (an additional 20,000 military personnel not included) and 74,000 people in Nagasaki died before the end of 1945, in total about 210,000 deaths, as a direct result of the bombings. These deaths are referred to as “acute deaths”. The short-term effects of the bombings have been extensively described, and include thermal, mechanical and radiation injuries (in particular radiation-induced bone marrow depletion).

351. At the time of the bombings, the haematopoietic systems of survivors underwent a level of damage for which the severity and persistence were dose-dependent. The most serious effects were those related to radiation-induced cell death resulting in the development of symptoms of acute radiation syndrome. Several months after exposure, the haematolymphoid function of many survivors had almost completely recovered [O8]. Studies initiated soon after the bombings showed little evident dose-dependent effects on the immune system [A6]. However, even several decades later, it was still possible to detect long-term alterations in the immune system of exposed survivors.

3. Long-term effects

352. In an early paper investigating T-cell immunity among the atomic bombing survivors, Akiyama et al. [A5] looked at the responsiveness of peripheral blood lymphocytes to allogenic antigens in mixed lymphocyte cultures from 139 atomic bombing survivors. This study revealed a significant decrease in mixed lymphocyte culture response with increasing radiation dose. The decline was most marked in the survivors who were more than 15 years old at the time of the initial exposure. These results were interpreted as an impaired thymic function.

353. In 1983 Akiyama et al. [A4] described functional defects in T-cell response to mitogens such as PHA. IL-2 is known to have different actions regarding T-cell proliferation and T-cell development. CD4+ T-cells are those primarily responsible for producing IL-2 in response to mitogens such as concanavalin A (Con A). Decreased production of IL-2 has been implicated as a potential factor in radiation-induced impaired immunity [B5]. To elucidate the biological significance of the T-cell abnormalities observed in atomic bombing survivors long after exposure, Kusunoki et al. investigated the percentage of T-cells capable of responding to PHA or Con A or that could produce IL-2. The study used a limiting dilution assay method to evaluate the responsiveness of the T-cell population to these mitogens. The subjects in this study were 251 atomic bombing survivors exposed to <0.005 Gy and 159 survivors exposed to >1.5 Gy. The percentage of CD2+ cells (activated T-lymphocytes) capable of proliferating in response to PHA in the presence of exogenous IL-2 did not differ substantially between distally and more heavily exposed survivors. In contrast, T-cell capacity in response to Con A was lower in the more exposed individuals. Moreover, heavily exposed survivors possessed fewer T-cells with the capability of producing IL-2. It was concluded that peripheral blood samples from heavily exposed survivors contained significantly fewer IL-2-producing CD4+ T-cells than did similar samples from those distally exposed to radiation from the atomic bombings. Radiation might have a long-lasting negative effect on the capacity of the CD4+ T-cell populations involved in IL-2 production [K31].

354. A decreased proportion of mature CD3+ T-cells was found in peripheral blood lymphocytes among the atomic bombing survivors exposed to >1.5 Gy, particularly in the proportion of the CD4+ CD45RA+ naive T-cell subset. The frequency of a rare T-lymphocyte subpopulation bearing CD3 surface antigen and TCR (α and β chains), but lacking

both CD4 and CD8 (double-negative CD4– CD8– αβ+), was studied in 409 atomic bombing survivors (160 who had been exposed to ≥1.5 Gy and 249 controls). The frequency of CD4–CD8– αβ+ T-cells was significantly elevated in individuals exposed to >1.5 Gy [K37]. The authors interpreted this finding as a result of altered differentiation and development of T-cells. This rare T-cell population may be differentiated through a pathway different from that of conventional CD4+ or CD8+ αβ+ T-cells [K32].

355. Alterations of T-cell population subsets were confirmed in further studies. The proportions of subsets of T-, B- and NK cells in peripheral blood lymphocytes of atomic bombing survivors were studied by flow cytometry analysis [K29]. Blood samples from 159 survivors estimated to have received >1.5 Gy and from 252 controls were evaluated using multiple combinations of monoclonal antibodies to lymphocyte differentiation antigens. The findings revealed that the proportion of CD4+ T-cells was decreased significantly in the heavily exposed survivors and that a similar tendency was apparent for the CD4+ CD45RA+ naive T-cell subset. No significant changes were found in the proportion of CD8+ T-cell subsets between exposed individuals and controls. Also, a dose-dependent increase in the frequency of PHA-stimulated lymphocytes bearing chromosome aberrations was reported in 1975 [A15].

356. The high sensitivity of CD4+ CD45RA+ naive T-cells to ionizing radiation in long-term studies appears to involve functional defects in T-cell response or demonstrable impairment in CD4+ T-cell immunity. In T-cells from 723 atomic bombing survivors, almost uniformly distributed with respect to age, sex and dose, the ability of T-cells to proliferate in vitro was tested after a challenge by each of the *Staphylococcus aureus* toxins SEB, SEC-2, SEC-3, SEE and TSST-1. The results revealed that the proliferative responses of the T-cells of the atomic bombing survivors became progressively weaker as the radiation dose increased, and that they did so in a manner that correlated with the decrease in the percentage of CD4+ CD45RA+ (naive) T-cells, but not with that of CD4+ CD45RA– (memory) T-cells. These findings indicated that irradiation from the atomic bombings led to impairment of the ability of exposed individuals to maintain their naive T-cell pools, explaining why they responded poorly to toxins encoded by common pathogens [K26].

357. A dose-dependent increase in the relative risk of myocardial infarction has been observed in RERF's Adult Health Study cohort of atomic bombing survivors [K43]. The effects of ionizing radiation on conditions other than cancer have previously been reviewed in the UNSCEAR 1982 [U8] and 1993 [U5] Reports. This subject is now extensively reviewed in annex B, "Epidemiological evaluation of cardiovascular disease and other non-cancer diseases following radiation exposure". However, some data will be discussed here concerning the hypothesis of a causal relationship between immune dysfunction and myocardial infarction in atomic bombing survivors.

358. The T-cells of survivors with a history of myocardial infarction responded poorly to *Staphylococcus aureus* toxins, and these individuals had proportionally fewer CD4+ CD45RA+ (naive) T-cell populations than survivors with no myocardial infarction in their history [K26]. It had previously been reported that among 1,006 survivors uniformly distributed with respect to age, sex and dose, 18 persons had a history of myocardial infarction; the proportion of CD4+ cells was significantly decreased with increased dose and history of this disease. Further, the prevalence of myocardial infarction was significantly greater in those individuals who had a lower proportion of CD4+ helper T-cells [K28].

359. Kusunoki et al. suggested that myocardial infarction in atomic bombing survivors may be due at least in part to their having diminished ability to mount an immune response against certain infections that may be implicated in the aetiology of cardiovascular disease [K36]. However, this inference may be premature. Blood samples for determination of the proportion of CD4+ and CD8+ T-cells were taken between 1992 and 1995, while histories of myocardial infarction were recorded from 1958 to 1990. Although Kusunoki et al. assumed that measurements of T-cell subsets before disease onset would have shown similar values to those obtained in their study, in order to demonstrate a causal relationship they had to carry out a prospective study to record newly diagnosed cases and compare the incidence between subjects exhibiting low and normal CD4+ T-cell proportions. Changes in the proportion of CD4+ T-cells have been described in patients with acute myocardial infarction and post-myocardial-infarction syndrome without antecedents of radiation exposure [A19, B26, K53, T15]. Although some of these changes were exhibited only temporarily, long-term effects may also exist. It is thus possible that CD4+ T-cell deficiencies in survivors with myocardial infarction history are, at least in part, of a fundamental nature rather than being totally attributable to radiation exposure.

360. Kusunoki's observation is reminiscent of an earlier report by Roberts-Thomson et al. [R7], who examined the number of positive delayed-type hypersensitivity reactions and the number of deaths among study participants aged over 80 years. They found that those who manifested fewer than two positive reactions had significantly greater mortality over the two-year period of study than those who showed two to five positive reactions. Bronchopneumonia, cerebrovascular accidents and cardiac failure were the most commonly recorded causes of death; no deaths were attributed to cancer, suggesting that reduced T-cell-mediated immune responsiveness resulting from earlier exposure to radiation could cause diseases other than cancer related to old age by as yet unknown mechanisms.

361. No dose–response relationship was found between anti-hepatitis-C-virus (HCV) seropositivity and radiation dose in atomic bombing survivors. The relative risk of chronic liver diseases among anti-HCV-positive individuals was marginally increased with radiation dose. The authors interpreted their findings with the hypothesis that radiation

exposure may accelerate the progress of chronic liver disease associated with HCV infection [F8]. The rates of seropositivity for hepatitis B surface antigen (HBsAg), which indicates current hepatitis B virus (HBV) infections, and anti-HBV core antibody, which indicates either cured or current infections, increased with radiation dose among 6,121 atomic bombing survivors. However, no relationship was observed between radiation and anti-HBV surface antibody, indicating cured infection, suggesting a lower likelihood of clearance after HBV infection among those who were more likely to have been infected with HBV as adults after irradiation from the atomic bombings [F7]. Taking into account that Th1 responses are able to clear hepatitis virus infections very efficiently, these findings could be interpreted to be the result of a persisting radiation-induced Th1/Th2 imbalance promoting chronic infection [K23].

362. As discussed earlier, a large and diverse TCR repertoire, necessary for recognition of the many antigenic peptides possible, is a critical feature of T-cell populations. Kusunoki et al. [K25] evaluated whether the recovery of CD4+ T-cell populations in atomic bombing survivors was associated with a long-term reduction in the diversity of the TCR repertoire. Using a panel of monoclonal antibodies against 13 TCR V β families, they employed flow cytometry to analyse peripheral blood samples from 710 survivors, distributed almost uniformly with respect to age, sex and dose (controls, <0.0005 Gy; exposed, ≥0.0005 Gy). They defined a parameter referred to as "repertoire deviation", which expressed the extent to which the TCR V β repertoires of T-cells from a given individual deviated from the mean for the whole population. The naive helper T-cell pools (CD4+ CD45RA+) of exposed individuals showed a dose-dependent decline without changes in their TCR V β repertoires. In contrast, the percentages of memory helper T-cells (CD4+ CD45RA–) did not decline, but their TCR V β repertoires were skewed in a dose-dependent manner among individuals who were at least 20 years of age at the time of the bombings.

363. Normally, since fewer new T-cells emerge from the thymus in the elderly, naive T-cell pools gradually become smaller with age. In contrast, memory T-cells that are lost tend to be replaced through clonal expansion. Thus the memory T-cell pool remains almost constant in size but gradually loses the diversity of TCR repertoire with age. Recovery of T-cell populations after radiation-induced depletion involves two different pathways: the production of new T-cells from thymus stem cells and the proliferation of peripheral mature cells that have managed to survive. The observations by Kusunoki et al. [K25] lead to the conclusion that restoration of the peripheral T-cell pools of atomic bombing survivors involved these two different pathways, and suggest that ionizing radiation accelerated the normal processes of immunological ageing.

364. Radiation exposure from the atomic bombings was demonstrated to be associated with long-lasting deficits in both naive CD4+ and CD8+ T-cell populations. Statistically significant dose-dependent decreases in the percentages of naive CD4+ and CD8+ T-cells were found in the peripheral blood lymphocyte populations of 533 Hiroshima atomic bombing survivors. In contrast, while the percentages of memory CD8+ T-cell subsets were found to increase with radiation dose, no changes were observed in the percentages of memory CD4+ T-cell subsets [Y2].

365. The numbers of peripheral blood lymphocytes belonging to different subsets were studied in 1,328 atomic bombing survivors using immunocytochemistry (fluorescent antibodies) [K24]. A decreasing trend in the numbers of CD4+ and CD8+ T-cells and in CD19+ B-cells was observed with increasing age. The CD5 molecule has an important role in T-cell/B-cell interactions [B25]. The number of CD5+ B-lymphocytes was significantly lower in those persons exposed to >1 Gy within the group exposed at the age of 30 years or later. A similar tendency towards decreased numbers of CD4+, CD8+ and CD19+ cells was observed in these older survivors, although the differences were not statistically significant. These results suggest that ageing of the T-cell-related immune system is accelerated in people irradiated at an advanced age. Owing to the age-related decrease in thymic function, subjects who were older at the time of the bombing may have decreased functional capability of the immune system for recovery after radiation injury.

366. In a further paper reporting flow cytometric analyses of the lymphocyte subsets from atomic bombing survivors, Kusunoki et al. [K29] demonstrated a significant increase in the proportion of B-lymphocytes in heavily exposed survivors. The increase was evident in both positive and negative cells for CD5 antigen (a marker of mature B-cells), as well as in both positive and negative cells for CD23 antigen (a marker of stimulated B-cells). The discrepancy between these results and those from earlier studies [K24] may be due to a difference in the measurement method.

367. NK cell numbers were studied in atomic bombing survivors using immunocytochemistry. The NK cell population was found to be increased significantly in the older compared with the younger age group, but there was little dependence on dose [K24]. These results are in good agreement with a further study carried out using flow cytometry that did not find any effect of radiation dose on the proportion of NK cell subsets [K29]. Concerning NK cell activity, no significant changes were observed in a study of 1,341 atomic bombing survivors [B5].

368. Several studies have been carried out since 1968 concerning serum immunoglobulin levels. Data published by Hall et al. [H2] and by King et al. [K12] in 1973 showed no relationship between radiation dose and serum immunoglobulin levels in the cohort of atomic bombing survivors at that time. Fujiwara et al. [F4] determined the levels of autoantibodies and immunoglobulins among 2,061 individuals exposed to radiation from the atomic bombings in Hiroshima and Nagasaki for whom dose estimates ranged from 0 to 5.6 Gy. They found: a significant increase in IgA levels

in females, a significant increase in IgM levels in both sexes, and no changes in the prevalence of antinuclear antibody, antithyroglobulin antibody and antithyroid microsomal antibody, or in levels of IgG and IgE. These results were recently confirmed by Hayashi et al. [H10].

369. In 1994 Nagataki et al. reported an increase in the prevalence of antibody-positive hypothyroidism among atomic bombing survivors in Nagasaki [N2]. In contrast, the Adult Health Study by the Atomic Bomb Casualty Commission/RERF reported a lack of dose–effect relationship on the prevalence of autoantibodies [F4]. Imaizumi et al. have recently re-evaluated these observations and found that the prevalence of hypothyroidism with autoantibodies marginally increased among moderately exposed survivors but not among highly exposed survivors in Nagasaki. They concluded that there is no statistically significant dose response in the prevalence of antithyroid autoantibodies or hypothyroidism with autoantibodies in atomic bombing survivors [I8].

370. In their studies of somatic mutations, Kushiro et al. [K22] used a flow cytometric assay to identify both gene mutation and somatic recombination. In 168 Adult Health Study participants and 58 employees of RERF, the frequency of variant lymphocytes lacking expression of HLA-A2 or HLA-A24 allele products was about 10^{-4} in heterozygous donors and increased with donor age. Possible mutant cells lacking expression of HLA-A2 or HLA-A24 were isolated and clonally propagated. Molecular analysis of 164 clones derived from six donors revealed that 71–100% were defective in expression of the selected HLA-A alleles. On the other hand, 80% of the clones consisted of CD4+ T-cells, and the remainder were CD8+ T-cells. The effect of exposure to radiation from the atomic bombings was not statistically significant. However, while 1 out of 50 mutants in vivo derived showed a possible small deletion at the *HLA*-locus without any chromosomal abnormality, the majority of mutants derived from in vitro X-irradiated peripheral blood mononuclear cells bore important deletions [K22].

371. As already discussed, the *HLA* gene seems to be a particularly important genetic factor that can affect host immune response. Significant differences in type 2 diabetes prevalence were found between heavily exposed (>1.5 Gy) and low-dose or non-exposed Hiroshima atomic bombing survivors with different class II HLA *DQA1* and *DRB1* alleles [H12]. The prevalence was higher for heavily exposed individuals who were less than 20 years old at the time of the bombing and who presented *DQA1*0401* and *DRB1*08* alleles or *DQA1*0301* and *DRB1*09*. These results suggest that certain class II *HLA* genes regulate one or more components of the immune system related with the risk of diabetes development among the younger and more heavily exposed survivors. This was the first report suggesting that the development of a particular disease can be affected by radiation exposure in individuals with different genetic backgrounds.

372. The possibility of various degrees of radiation-associated immune suppression being dependent on HLA type has been addressed. To investigate the possibility of differing frequency distributions of HLA type in the Hiroshima cohorts, HLA-*DQA1* alleles and HLA-DR antigens were typed for 201 survivors exposed to >1.5 Gy, 339 exposed to between 0.005 and 1.5 Gy, and 388 in a distally exposed group (<0.005 Gy). Although no dose-related differences were found, when the subjects were grouped by the presence of a specific allele or antigen, males carrying DQA1*0103 in at least one of their two HLA-*DQA1* loci exhibited frequency distributions that decreased as radiation dose increased [H9].

373. In earlier work, Kusunoki et al. [K27] had demonstrated that mutant lymphocytes lacking expression of class I HLA molecules were eliminated by autologous NK cells. In an attempt to explain the inability to detect any increase in HLA-A2 negative cell number in HLA-A2 heterozygous individuals exposed to irradiation from the atomic bombings, the hypothesis was tested that HLA mutant lymphocytes might well have been induced by radiation exposure but eliminated by strong negative selection associated with their almost inevitable exposure to autologous NK cells [K30]. The results strongly supported the hypothesis that autologous NK cells are responsible for the elimination of mutant lymphocytes that have lost the ability to express self class I HLA molecules in vivo, and therefore might explain why it has not been possible to detect increased frequencies of HLA-A2 mutants in samples from any of the 164 atomic bombing survivors whose HLA-A2 heterozygote status made their lymphocytes suitable for such a test.

374. Kodama et al. wanted to investigate whether radiation exposure had induced chromosomal instability in peripheral lymphocytes. Ordinary cytogenetics was not expected to help them solve this problem, as lymphocyte stable aberrations (mainly translocations) induced at the time of the bombings would not be distinguishable from those that may have arisen later as a result of the instability. Therefore they studied clonally expanded T-cell populations, e.g. cells bearing identical translocations, because they are descendants of a single progenitor cell which acquired aberrations as a result of radiation exposure. By determining the frequency of additional translocations among clonal cells, the authors found that clonally expanded T-cell populations of atomic bombing survivors do not exhibit increased chromosomal instability [K44].

375. It has been argued that chronic low-level inflammatory responses induced by radiation could be a significant risk factor in the well-documented increase of non-cancer disease occurring in atomic bombing survivors. Hayashi et al. investigated the long-term effects of ionizing radiation on the levels of two markers of inflammatory response, C-reactive protein (CRP) and IL-6, in blood samples from 453 participants in an epidemiological cohort of atomic bombing survivors [H8]. Blood lymphocyte subpopulations were identified by flow cytometry, using monoclonal antibodies to CD3, CD4 and CD8. CRP levels were significantly increased, by about 35% Gy^{-1} ($p = 0.0001$). After adjustment

for confounding factors (sex, age, etc.), CRP levels were still increased significantly with dose, by 28% Gy^{-1} ($p = 0.0002$). IL-6 levels also increased with radiation dose, by 9.3% Gy^{-1} ($p = 0.0003$) and, after multiple adjustments, by 9.8% at 1 Gy ($p = 0.0007$). Elevated CRP and IL-6 levels were associated with decreases in the percentages of CD4+ T-cells in the peripheral blood lymphocyte population [H8]. Hayashi et al. recently reported long-term effects of radiation dose on inflammatory markers in atomic bombing survivors. They found that erythrocyte sedimentation rate, IFN-γ, TNF-α and IL-10 increased significantly with radiation dose [H10].

4. Remarks concerning data on survivors of the atomic bombings

376. The preceding review of data concerning atomic bombing survivors shows that short-term effects of radiation on the immune system were expressed mainly as dose-dependent acute bone marrow depletion due to radiation-induced cell death. These effects were reversed over several months, and studies initiated shortly after the bombings showed few dose-dependent effects on the immune system [A6].

377. Studies of the late effects of radiation on the immune system commenced about 20 years after the atomic bombings. The most remarkable late effects of radiation were functional and quantitative abnormalities of T- and B-cells in survivors exposed to high doses (≥1 Gy). The main findings observed up to 1995 have been reviewed and summarized by Akiyama [A6]. With respect to data published thereafter, the most remarkable late effects of radiation on the immune system of atomic bombing survivors are summarized in the following paragraphs.

378. Effects on T-cell immunity:

- Decreased proportion of CD3+ CD4+ TCRαβ + T-cells;
- Decreased proportion of CD3+ CD8+ TCRαβ + T-cells;
- Decreased proportion of CD3+ CD4+ CD45RA+ naive T-cells;
- Non-significant changes in the proportion of CD3+ CD4+ CD45RA– memory T-cells;
- Skewed TCR repertoires of CD3+ CD4+ CD45RA– memory T-cells in individuals exposed in adult life;
- Decreased proportion of CD3+ CD8+ CD45RA+ naive T-cells;
- Increased proportion of CD3+ CD8+ CD45RA– memory T-cells;
- Increased frequency of CD4– and CD8– (double-negative) αβ+ T-cells;
- No change in the proportion of CD3+ TCR γδ+ T-cells;
- Functional defects in T-cell responses to mitogens and alloantigens.

379. Effects on B-cell immunity:

- Significant increase in the proportion of B-cells;
- Increase in serum IgA levels in females;
- Increase in IgM levels in both sexes;
- No changes in IgG and IgE levels.

380. Effects on innate immunity:

- In contrast with acquired immunity, significant dose effects were not observed on the number and function of NK cells. However, some studies showed an increase in the proportion of NK cells in the peripheral blood of atomic bombing survivors.

381. Other findings:

- Increased frequencies of somatic mutations (TCR and HLA) and chromosome aberrations;
- Marginal increase with radiation dose in the prevalence of chronic liver diseases and hepatocellular carcinoma among anti-HCV-positive individuals;
- Decreased cellular immunity and enhanced humoral immunity may have led to long-term imbalance in Th1/Th2 responses, resulting in altered cytokine expression profiles;
- Qualitative and quantitative changes suggesting a radiation-induced acceleration of the normal process of immunological ageing;
- Radiation-associated chronic inflammatory responses;
- Decreased proportion of CD4+ cells with increased dose and history of myocardial infarction, and higher prevalence of myocardial infarction in those survivors who had a lower proportion of CD4+ cells;
- Differences in type 2 diabetes prevalence in heavily exposed individuals with two particular class II HLA *DQA1* and *DRB1* alleles.

B. Chernobyl workers and residents

1. General considerations

382. The accident at the Chernobyl nuclear power plant (NPP) on 26 April 1986 resulted in both acute and long-lasting health effects. Early and late immune system changes were among the key points studied after the accident. Acute radiation effects in victims were extensively described in an appendix to the UNSCEAR 1988 Report [U6]. Further information about the immunological effects of exposure to radiation from the Chernobyl accident as they were known up to

the year 2000 was provided by the Committee in reference [U2]. The Committee is currently updating its assessment of the health consequences of the accident. However, some data concerning immunological issues will be discussed here. A broad spectrum of immune abnormalities had been reported among Chernobyl victims; however, it has not been possible to interpret these results, since it was unclear whether all possible confounding factors (such as heavy metal contamination, infections and diet) had been taken into account.

383. Early changes in immune parameters were characterized by alteration of the amount or function of peripheral lymphocytes and changes in serum immunoglobulins. The data indicate that radiation-induced effects on the immune system remained detectable for a considerable period after the accident. The available data vary widely, according to the characteristics of the populations studied, the dose received and its protraction in time, the mode of exposure (external irradiation and/or internal contamination) and the time elapsed since the accident.

384. Immunological monitoring of persons affected by the Chernobyl accident comprised two main groups:

- Individuals who worked at the NPP during the emergency and/or participated in further clean-up activities;
- Residents of contaminated areas, particularly children living in several settlements around the NPP, who were included in the Children of Chernobyl Project, as requested by the Ukrainian Government.

Different parameters have been evaluated and several phases may be identified in these two groups concerning the temporal behaviour of the immunological effects.

2. Emergency and clean-up workers

385. The workers involved in the recovery and clean-up after the Chernobyl accident were subjected not only to external and internal radiation exposure but also to other non-radiation factors that may have affected their health. As a consequence of the accident, different heavy metals were deposited at the reactor site, with a significant amount being vaporized and distributed in rain clouds. Higher blood concentrations of iron, zinc and lead have been found in workers who took part in cleaning up after the accident [N9]. It could be hypothesized that some of the observed changes in the immune status of clean-up workers might be due to a combined action of ionizing radiation and heavy metal contamination. [B19, G10]. Moreover, these workers suffered strong psychological stress, which may significantly affect the immune system [G21].

386. Yarilin et al. [Y4] evaluated disorders in T-cell subpopulations 5 years after the accident in two groups of workers from the Chernobyl NPP accident, the first being workers without manifestations of acute radiation syndrome (ARS), for which total doses from external irradiation were 0.1–0.5 Gy (group 1), and the second being individuals who survived ARS, for which total doses from external irradiation were 0.5–9 Gy (group 2). Decreases in the percentage and absolute number of CD3+ T-cells were observed in both groups. A decrease in the percentage and absolute number of CD8+ T-cells was observed only in group 1 (which had lower doses). A decrease in the percentage and absolute number of CD4+ T-cells was evident only in heavily exposed people from group 2.

387. Titova et al. found that the mean number of CD8+ T-cells was decreased in personnel working in the 30 km control zone around the NPP [T6]. The absolute number of CD4+ T-cells was also decreased, but their percentage remained higher. Kurjane et al. characterized the immune status of a group of Latvian workers who received external radiation doses of 0.01–0.5 Gy [K21]; this study was performed 10–14 years after the accident. A significant decrease was observed in the number of CD3+ T-cells. This study also demonstrated that both CD4+ and CD8+ T-cells were decreased. A study performed by Kuzmenok et al. [K33] 11–14 years after the accident did not, however, find significant changes in the phenotypic characterization of the main subpopulations of peripheral lymphocytes in a group of Belarussian clean-up workers who received mean doses of 0.15–0.5 Gy.

388. Other lines of evidence point to the role of serum inhibitory factors that down-regulate T-cell surface antigen expression and that could regulate T-cell differentiation in vitro. It has been reported that the decreased percentage of CD4+ helper–inducer T-lymphocytes of recovery operations workers returned to normal values after 3 days of culture without activation, suggesting that the phenotype may be under the control of suppressive soluble factors in the blood of these individuals [K33]. The fact that the percentage of CD4+ T-cells in control samples did not change after culture reinforces this hypothesis.

389. A decline in the number of CD3+ and CD4+ T-cells, with augmentation of the percentages of CD8+ T-cells and CD16+ CD56+/NK cells, was observed after 3 days of culture with the polyclonal activator PHA at optimal concentration (10 $\mu g\ mL^{-1}$). This activation-induced deviation in the maturation of T-cell subpopulations may be a consistent characteristic of impaired T-cell immunity in clean-up workers [K33].

390. The functional status of T-cells plays a key role in the immune regulation of human pathology. The proliferative response of lymphoid cells to mitogens was also altered in recovery operations workers. Kuzmenok et al. [K33] reported a significant decrease of the response of peripheral blood mononuclear cells to PHA and the phorbol ester PMA. Neither the TCR-restricted proliferative response of T-cells nor the level of Con-A-induced proliferation was significantly decreased.

391. The proliferative response of peripheral blood mononuclear cells to exogenous IL-2 was higher in recovery operations workers, which may indicate an up-regulation of the IL-2 receptor CD25 in their cells [K33]. Interleukin-2 (IL-2) is a cytokine responsible for a variety of immune stimulatory and regulatory functions, including activation and stimulation of cytotoxic cells able to recognize and kill human tumour cells, and T-cell proliferation and differentiation. Similar results had previously been obtained by Xu et al. in an experimental model of low-dose (0.25–10 mGy) in vitro irradiation [X1].

392. Changes in cell subpopulations have been reported as evidence of radiation-induced disturbance of the T-cell system [W5]. An increase in peripheral blood CD3+ CD16+ CD56+ NKT cells, a small subpopulation of lymphocytes that exhibits certain characteristics of both T-cells and NK cells and that can be the source of an abnormal pattern of cytokines, was observed in Belarussian clean-up workers [K33].

393. One of the best described effects of the Chernobyl accident on the immune system was the increased level of monocytes in peripheral blood. Senyuk et al. evaluated the long-term effects of ionizing radiation in Chernobyl recovery operations workers [S13]. In many cases, the cumulative gamma radiation dose was >0.5 Gy. The authors found a high number of monocytes with an increase in plasma levels of cytokines IFN-α and TNF.

394. Functional changes have been reported in the monocytes and macrophages of clean-up workers. T-cell activation implies cooperation between the APCs and T-cells. The functions of monocytes as APCs were compared in clean-up workers and control individuals. Impaired function of monocytes as APCs was found in the T-cell proliferation assay. Indeed, allogenic monocytes purified from clean-up workers significantly augmented the proliferative response to mitogens of T-cells from control individuals but inhibited proliferation of T-cells from clean-up workers. In contrast, allogenic monocytes purified from healthy donors marginally augmented the proliferative response to mitogens from both clean-up workers and control individuals [K33].

395. Yarilin et al. evaluated the effect of ionizing radiation on the thymus and its role in radiation-induced T-cell disorders in victims of the Chernobyl NPP accident. A decrease in serum concentration of thymosin alpha-1 and serum thymic activity (STA) level was found with increased titres of autoantibodies to epithelial cells of the thymus [Y4]. This titre was higher in persons with lower levels of thymosin alpha-1. The dynamics of post-irradiation recovery of CD4+ and CD8+ cells were different. It had been suggested previously that preferential CD4+ cell deficiency might result from radiation-induced damage to the thymus [A12]. The good correlation observed by Yarilin et al. between thymic hormone and CD4+ cell levels would confirm this hypothesis [Y4].

396. Yarilin's results are in good agreement with those of Titova et al. [T6], who also found lower levels of thymosin alpha-1 and an increased serum level of antithymic epithelium autoantibodies in personnel from the 30 km control zone around the Chernobyl NPP. The dose-dependent decrease of the serum level of thymosin alpha-1 and STA observed 5 years after the accident indicates late impairment of thymic function, which could be a result of a disturbance of thymic epithelial cell renewal. Thymus dysfunction after low doses could be due to the action of anti-epithelial autoantibodies induced by the release of antigenic material from the cells damaged, even minimally, by ionizing radiation.

397. Thomas et al. [T4] performed a multiple end point study comparing hypoxantine phosphoribosyltransferase (HPRT) mutation frequency with chromosome translocations in peripheral blood lymphocytes of clean-up workers. When adjusted for age, smoking status and year of sampling, the authors demonstrated a significant increase in HPRT mutation frequency in clean-up workers, with a dependence on time elapsed since radiation exposure (a decline of 4.4% per year). They found little difference in the overall deletion spectra. However, they observed a decline in the average size of deletions of clean-up workers as time after exposure increased from 6 to 13 years. Jones et al. found an increase, more than 5 years after the accident, of chromosome translocations and in HPRT mutation frequency in peripheral lymphocytes of clean-up workers with absorbed doses of below 0.25 Gy [J4].

398. Chumak et al. demonstrated an accumulation of autoxidized lipoxygenase products of polyunsaturated fatty acids in the peripheral blood mononuclear cells of 23 clean-up workers with absorbed doses of below 0.3 Gy [C10]. They observed higher levels of free and esterified fatty acids, with a positive correlation between the absolute number of CD4+ T-cells and the amount of 15-hydroxyeicosatetraenoic acid (15-HETE) in the phospholipid fraction. The percentage of CD4+ T-cells was higher in heavily irradiated workers, and the percentage of CD8+ T-cells tended to decrease with dose.

3. Residents of contaminated areas

399. Titov et al. investigated the production of immunoglobulins in children living around the Chernobyl NPP. They found a decrease in B-cell numbers, a transient decrease of IgM and IgG and an increase of IgA levels (in both serum and saliva) during the first months following the accident. Over a six-year period of living in contaminated areas, children exhibited increasing production of IgG and IgM. A correlation was found between the changes in B-system immunity and the levels of ^{137}Cs contamination. There was also a strong correlation between the production of natural (heterophilic) antibodies and dose to the thyroid due to incorporation of ^{131}I (in the range 0.1–1 Gy). Higher accumulation of ^{131}I resulted in decreased titres of these antibodies. High levels of heterophilic antibodies correlated with high levels of IgE. Altered production of subclasses

of IgG associated with the increasing biosynthesis of IgE suggests that T-cells may have been driven towards the Th2 profile [T5]. However, trying to find a causal association between radiation exposure and these changes in Th2 profile would be speculative, because no data about confounding factors such as parasite infections in these children were taken into account.

400. Chernyshov et al. examined peripheral blood lymphocyte subsets eight years after the accident in children living around the Chernobyl NPP [C9]. They evaluated children living in 15 contaminated settlements in Ukraine, with and without recurrent respiratory disease (RRD), and a control group of children living in non-contaminated areas, again with and without RRD. The average dose (internal plus external contributions: 0.57–3.09 mSv) was calculated on the basis of the average density of contamination with ^{137}Cs and ^{90}Sr; no data were included concerning thyroid doses due to ^{131}I incorporation. Lower percentages of CD3+ T-cells were observed in RRD children from contaminated areas than in control RRD children. Lower percentages of CD3+ CD4+ (helper) T-cells were observed in RRD children from contaminated areas than in control RRD children. RRD children from contaminated areas exhibited lower percentages of CD3+ T-cells and CD3+ CD4+ (helper) T-cells than healthy children. Healthy children (no RRD) from contaminated areas had the same mean values for lymphocyte subsets as control healthy children. However, a wider range of percentage levels of CD3+ CD4+ T-cells was found among healthy children from contaminated areas, which allowed them to be divided into three subpopulations: children with very low, normal or very high percentage levels of CD3+ CD4+ T-cells. The other lymphocyte subsets studied did not differ among groups.

401. Children from contaminated areas had a healthy population (non-RRD) without major lymphocytic manifestations of immune disorders and an RRD population that exhibited lower percentages of total T-cells and helper T-cells than RRD controls. This decrease was more marked in RRD children with higher doses [C9]. This dose dependence provided strong evidence for a radiation-induced effect. The question is what accounts for the difference in immune response observed between exposed children who are healthy and those with RRD. It may be that repeated exposure to pathogenic or antigenic stimuli is necessary for the development of radiation-induced immune disturbances. Moreover, individual features (including genetic factors) are involved in the sensitivity of immunocompetent cells to ionizing radiation. It could be proposed that children predisposed to RRD have an impaired immune response and may exhibit greater radiosensitivity. Although this study did not consider thyroid doses, it is well known that the region where the study was performed (northern Ukraine) had a high level of ^{131}I contamination. It could be hypothesized that healthy children from contaminated areas exhibited a higher percentage of CD3+ CD4+ T-cells owing to clinical and subclinical autoimmune disorders related to radioiodine incorporation.

402. The same group of researchers published further results that confirmed previous findings and showed in addition that children with RRD from contaminated areas had higher levels of CD3– CD56+ CD16+ NK cells than did RRD children living in non-contaminated regions [V8]. It is interesting to note that children examined 8–10 years after the accident exhibited a more marked decrease in CD4+ cells than those with the same dose but who were examined at 5 years after the accident. This finding shows that long-term exposure to low doses (i.e. a time effect) rather than low-dose radiation exposure itself (i.e. a dose effect) altered the composition of peripheral blood lymphocyte subsets in children with RRD living in contaminated areas. Although it has been suggested that CD4+ T-cells are relatively radioresistant in vitro [S8], it may be that the combination of repeated exposure to antigenic stimuli together with long-term exposure to low doses of ionizing radiation leads to CD4+ T-cell depletion. Considering that NK cells have a lower in vivo radiosensitivity [C11, L21], their relative increase in children with RRD from contaminated areas could also be an effect of long-term low-dose exposure. The different responses of blood lymphocyte subsets could contribute to the lower risk of developing autoimmune thyroid abnormalities in children with RRD living in contaminated areas.

403. Koike et al. compared NK activity in children living in Gomel, a highly contaminated area, with that of children living in non-contaminated areas [K15]. While children living in non-contaminated areas exhibited a narrow range of NK cell cytotoxicity percentages, a wider range of NK cell cytotoxicity (from 8.9% to 76%) was found in children from contaminated areas. The NK cell cytotoxicity of these children was correlated neither with NK cell number nor with the amount of internal contamination by ^{137}Cs. The authors interpreted these findings as a loss of the normal regulatory mechanisms that maintain a correlation between cytotoxic activity and NK cell number. It seems a rather speculative conclusion. Even if such dysregulation exists, it cannot be attributed to ionizing radiation. The lack of correlation between these abnormalities and the levels of internal contamination suggests that related factors, other than direct internal exposure to ^{137}Cs, may be responsible. Diet and/or environmental exposure to some agent (such as heavy metals) might be proposed. The effect of external ionizing radiation from contaminated ground and the effect from radionuclides other than ^{137}Cs remain to be determined.

404. Mikhalevich et al. investigated cytogenetic and mutational effects in the lymphocytes of children living in a contaminated region of Belarus nine years after the accident [M5]. Their results indicated a doubling of the percentage of micronuclei in the mononucleated lymphocytes of exposed children, while the same parameter studied in binucleated lymphocytes showed no differences. No evidence was found for induction of HPRT mutations.

405. An adaptive response of lymphocytes to radiation has been suggested by a number of in vitro studies indicating that cells can become less susceptible to radiation-induced

damage when a "challenge" exposure to ionizing radiation is preceded by a very low "priming" dose. Padovani et al. [P1] administered a challenge dose of 1.5 Gy to stimulated peripheral lymphocytes of children chronically exposed in contaminated areas around Chernobyl. They did not find any decreased susceptibility for the two end points examined (chromosome and chromatid aberrations). An important consideration relates to the dose rate at which the priming dose is delivered. Assuming a constant intake for 1 year, the average value of committed effective dose equivalent was 450 μSv (range 50–2,000 μSv); this dose rate is lower than that used in most published studies. Another point of concern is that the priming dose in this study took place in resting lymphocytes, and it has been reported that a radio-adaptive response cannot be induced in the G_0 stage of the cell cycle.

4. Radioiodine contamination, immune status and thyroid diseases

406. A higher incidence of goitre has been reported among the Chernobyl clean-up workers. Kurjane et al. [K21] analysed several parameters of the immune system in 385 male Latvian residents who participated in the clean-up work at the Chernobyl site. The results were compared with those from 47 healthy age- and sex-matched controls. This group of clean-up workers received external doses of 10–500 mGy. No data were provided in this paper concerning thyroid doses due to radioiodine incorporation. Workers were exposed during 2–6 months, while working at Chernobyl, and then they lived in uncontaminated territories after they returned to Latvia. The prevalence of non-cancer thyroid diseases as determined in January 2000 was higher among clean-up workers (47 cases among 385 workers; 121/1,000), than in a non-exposed Latvian population (30/100,000), with goitre being the most frequent disorder. Diminished acquired cellular immunity (total CD3+ cells, CD4+ and CD8+ T-cells) was found among the Latvian clean-up workers. The phagocyte activity of neutrophils was significant decreased. Lower levels of IgG and higher levels of IgM, without changes in IgA were also found in this group. The observed decrease in the percentage of CD16+ NK cells contrasts with previous reports suggesting that NK cells display radioresistance. It is important to note that blood lead concentration was six times higher in clean-up workers [K21].

407. Kurjane et al. [K21] also reported that some immune parameters of workers with thyroid diseases differed from those observed in workers without thyroid disease: a lower number of NK cells, higher IgG plasma concentrations and higher activation of the classical pathway of complement.[2] Complement split product C3d was higher in both groups (i.e. with and without thyroid disease) of clean-up workers. Thyroid follicular cells are protected from lysis by locally activated complement. Although the underlying mechanism of the complement activation in clean-up workers is unclear, it may reflect a secondary radiation-induced inflammatory response. The question of whether this mechanism is also involved in the development of thyroid abnormalities among these workers remains unanswered.

408. Considering that the immune system is a vulnerable target for the effect of lead contamination [B19], the results presented for Kurjane et al. should be interpreted cautiously. It has been reported that CD16+ NK cells and CD4+ T-cells are vulnerable targets for the effects of lead [G10]. Elevated blood and urine lead concentrations were found in Kurjane's group of clean-up workers. It could be concluded that even 10–14 years after exposure, a combined impact of both radiological and non-radiological factors could be observed on the immune system of Latvian workers: impairment of phagocytic activity, reduction of cell-mediated immunity parameters and a shift towards an inflammatory profile.

409. Kiseleva et al. reported an increase in serum levels of antithyroglobulin and microsomal fraction autoantibodies, with higher levels of circulating immune complexes in liquidators 11 years after the Chernobyl accident [K14]. Vykhovanets et al. investigated the involvement of autoimmune mechanisms in the development of thyroid abnormalities in the context of radioiodine exposure of children living around the Chernobyl NPP [V9]. The study, which was carried out 8 years after the accident, included children living in 15 contaminated settlements and control children living in non-contaminated areas. Individual absorbed doses to the thyroid due to radioiodine (<1 Gy, 1–2 Gy and >2 Gy) and average doses (internal plus external; range 0.57–3.09 mSv) due to ^{137}Cs and ^{90}Sr were estimated. A positive correlation was found between thyroid ^{131}I dose and serum AbTg levels, content of CD4+ T and CD4+/CD8+ ratio. In contrast, a negative correlation was observed between thyroid ^{131}I dose and CD8+ T-cells and NK cells. The lack of correlation between thyroid-stimulating hormone (TSH) levels and thyroid dose suggests that the higher levels of TSH found among children living in contaminated areas may be due to iodine deprivation in areas of endemic goitre. Children with individual absorbed doses of >2 Gy due to radioiodine have several signs of autoimmune disorder: abnormal thyroid echogenicity, positive sera for AbTg, higher levels of CD4+ T-cells (which play a central role in immune response and are able to increase immunoglobulin production by B-cells), lower levels of CD8+ T-cells and higher CD4+/CD8+ T-cell ratios, a frequent finding in autoimmune thyroid diseases [V9].

410. Two further studies [P10, V12] also reported a higher prevalence of antithyroglobulin or antithyroperoxidase antibodies in children living in contaminated areas; this was already apparent in individuals who were in utero or newborn at the time of the accident. Autoimmune phenomena were limited to an increased prevalence of circulating

[2] Note: These results are presented here as discussed by Kurjane et al. [K21] in the text of their paper. However, the Committee found a lack of correlation between these comments and the values presented by the authors in table 2 of their paper, which seem to be contradictory: a *higher* number of NK cells, *lower* IgG plasma concentrations and *lower* functional activity of the classical pathway of complement in clean-up workers with thyroid diseases. The Committee considered that this discrepancy might be due to a mistake in typing column headings.

thyroid autoantibodies, without evidence of significant thyroid dysfunction. It should be taken into account that children involved in these geographical correlation ("ecological") studies may present a combined effect of iodine deficiency and internal contamination with short-lived iodine isotopes.

411. Koike et al. evaluated the immune status in children with goitre living in the highly contaminated area of Gomel. They found increased serum levels of IgG, IgM and IgE, and depressed NK cell activity [K15]. Although IFN-γ and IL-2 enhanced the cytotoxicity of these NK cells, the response to IFN-γ was still below control values. The other parameters were within normal values.

5. Remarks concerning data on Chernobyl workers and residents

412. Some remarks may be made on the basis of the publications on the Chernobyl accident reviewed above. Even many years after the accident, the impacts of both radiological and non-radiological factors on the immune system were observed in Chernobyl recovery operations (clean-up) workers. Ionizing radiation accelerated the natural ageing of the immune system due to a progressively declining thymic function. The dynamics of post-irradiation recovery are different for CD4+ and CD8+ cells, and it has been suggested that preferential CD4+ cell deficiency may result from radiation-induced damage to the thymus.

413. Short-term as well as long-term effects were detectable in B-cell and T-cell function profiles, as well as in the biosynthesis of immunoglobulins in both serum and saliva, of children living in contaminated areas around the Chernobyl NPP. Some of these changes were dose-dependent and were characterized by phases. Indeed, immune profiles were different several months as opposed to several years after the accident. The NK cell system of these children may have lost the normal regulatory mechanisms that maintain a correlation between cytotoxic activity and NK cell number. Repeated exposure to pathogenic or antigenic stimuli seems to be necessary for the development of radiation-induced immune disturbances. Individual features, including genetic factors, are involved in the sensitivity of immunocompetent cells to ionizing radiation. Illnesses associated with both radiation exposure and genetic factors could be determinants of the immune status after the accident.

414. The immunological effects of exposure to ionizing radiation from the Chernobyl accident were mainly related to changes in the amounts or function of peripheral lymphocytes and serum immunoglobulin levels. These effects were detectable long after the accident. The immune system seems to be involved in the pathogenesis of thyroid diseases in victims of the Chernobyl accident, probably owing to antigenic mechanisms being triggered by radiation-induced thyroid damage, leading to autoimmune responses. Neuroendocrine and other stress-related factors, respiratory diseases, chronic infections, chemical contamination and autoimmune dysbalance could also be factors in some of the immune disorders found in this population.

C. Techa River study

1. General considerations

415. The Techa River study is seen as an excellent opportunity to obtain more reliable risk estimates for a general population exposed over an extended period to low-dose-rate gamma rays. More than 25,000 inhabitants of Techa riverside villages were exposed, predominantly during the early 1950s, to external gamma radiation from fission products associated with discharges of high- and medium-level wastes into the river from the Mayak nuclear facility. In addition, residents incorporated, via drinking water and through the food chain, large activities of short-lived fission products such as ^{89}Sr, and subsequently long-lived activity, especially from ^{90}Sr and ^{137}Cs. Mean and median doses to soft tissue and bone marrow according to the earlier Techa River Dosimetry System (TRDS-1996) and the revised dosimetry system (TRDS-2000) are shown in table 13.

Table 13 Mean and median doses to soft tissue and bone marrow in inhabitants residing near the Techa River [K9]

Dosimetry system	*Soft tissue dose (mGy)*	*Bone marrow dose (mGy)*
TRDS-1996		
Mean	99	405
Median	17	267
TRDS-2000		
Mean	35	353
Median	7	253

2. Epidemiological data

416. In the early period, cases of chronic radiation sickness (CRS; 940 cases in total) were diagnosed. The diagnosis of CRS was based on the occurrence of the following signs: changes in blood parameters (leucopenia, thrombocytopenia, granulocytopenia); nervous system disorders; ostealgia; cardiovascular syndrome; and changes in immunity

(inhibition of innate immunity, autoimmunity). At dose rates in excess of 300–500 mSv/a to red bone marrow, a portion of the irradiated population developed post-irradiation reactions of the haematopoietic and immunological systems [A8]. Some of the residents exposed did not develop CRS, but in the early years they manifested isolated reactions most commonly represented by haematological changes in peripheral blood studies. The dynamics of blood parameters clearly manifested a reduction in the number of cellular elements at the highest dose rates and a subsequent normalization with a decrease in dose rate. The average leucocyte counts for CRS patients persisted at lower than 90% confidence intervals of the reference value for three decades after the beginning of exposure. Only after 1970 did the difference in leucocyte counts between followed-up patients and reference values disappear (figure IX). The dynamics of segmented neutrophils correlated with leucocyte dynamics, indirectly corroborating the decrease in leucocyte counts as associated with the decreased number of granulocytes (figure X) [K19]. According to the data for individuals with CRS, there was an increase in the fractions of myelocytes and metamyelocytes in the bone marrow, corresponding to the occurrence of leucopenia and granulocytopenia in peripheral blood. Such findings can be interpreted as delayed maturation and differentiation of granulocytes at the final stage of cell development. The subjects who were initially exposed in utero or at age 1–2 years showed the greatest changes in the immune system parameters already mentioned. Haematopoietic disturbances developed almost at the same time as the signs of immune insufficiency [K19].

Figure IX. Mean leucocyte counts at different times after the beginning of exposure [K19].

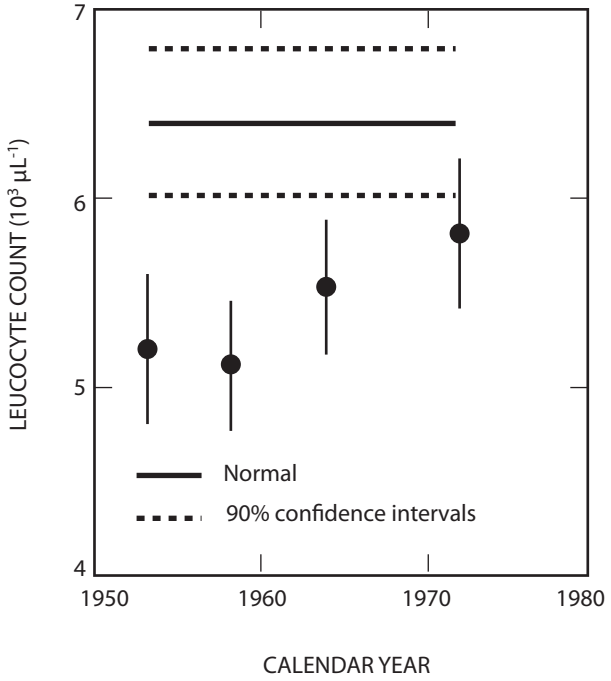
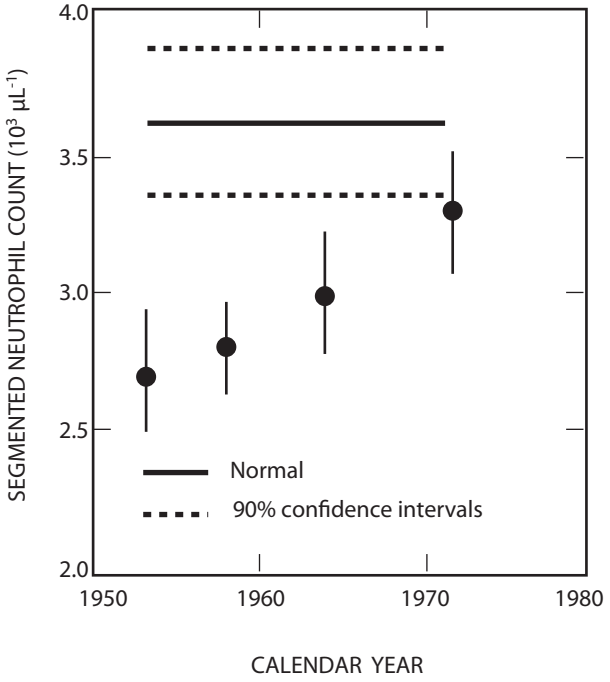


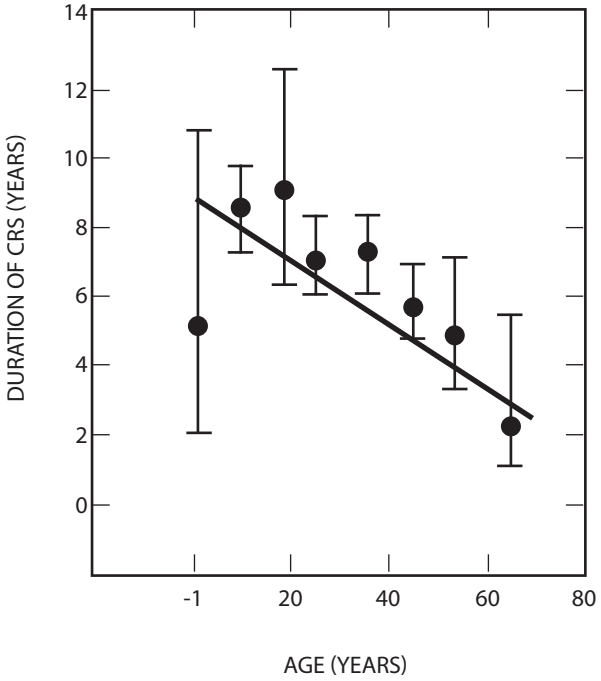
Figure X. Mean neutrophil counts at different times after the beginning of exposure [K19].



417. Recovery from these symptoms depended on exposure rates, with greater exposure leading to longer recovery times. Even after the cessation of external exposure and intakes of radionuclides, leucopenia and neutropenia persisted for a long period; this may have been due to incorporation of long-lived strontium, which contributed to irradiation and maintained a certain level of dose rate. The average duration of the disease was 7.35 years, but at doses in excess of 700 mSv to the red bone marrow, the repair process lasted for more than 9 years [K19]. Complete recovery from the haematological and neurological effects occurred within 13–16 and 14–20 years, respectively, following the beginning of exposure. The duration of the disease was presumably dependent on the patient's age at exposure. Recovery processes developed more slowly in children and teenagers who received the highest doses, since a close dependence of exposure dose on age was observed. The age dependence of the duration of CRS is shown in figure XI [K19]. Immunity disorders persisted for 30 years and longer after the exposures began. Long-term immunity changes involved cellular immunity (decreased expression of differentiating antigens of T-lymphocytes, T-lymphocyte blast transformation), natural toxicity (reduced counts of NK cells) and signs of immunological imbalance. At long times (43–48 years) after the beginning of exposure, the status of haematopoiesis and immunity was normal among most of the exposed subjects. However, the proportions of the exposed persons still showed an increased frequency of chromosomal aberrations

and CD3–CD4+ mutant T-lymphocytes in the peripheral blood [A18]. An increased frequency of TCR mutant lymphocytes was also noted [A17].

Figure XI. Dependence of CRS duration on age at time of exposure [K19].



418. CRS diagnosed in a number of residents of Techa riverside villages was found to be associated with higher death rates from cancer and leukaemia. The Extended Techa River Cohort (ETRC) includes 29,873 people born before 1950 who lived near the river sometime between 1950 and 1960. Between the years 1950 and 1999, 1,842 solid cancer deaths and 61 leukaemia deaths occurred. The maximum incidence of leukaemia was observed 15–19 years after the exposure began. The excess relative risk per unit dose for solid cancer was 0.92 (95% CI: 0.2, 1.7) Gy^{-1}, while the values for leukaemia, including and excluding chronic lymphocytic leukaemia, were 4.2 (95% CI: 1.2, 13) Gy^{-1} and 6.5 (95% CI: 1.8, 24) Gy^{-1}, respectively. It is estimated that about 2.5% of the solid cancer deaths and 63% of the leukaemia deaths were associated with the radiation exposure [K52]. Clinical manifestations of immune insufficiency with respect to certain infectious diseases (such as chronic pneumonia, pulmonary tuberculosis and non-traumatic osteomyelitis) occurred with higher frequency among exposed patients with tumours than in exposed individuals without tumours [A8].

419. The Techa River Offspring Cohort (TROC) comprises 10,459 children at least one of whose parents lived along the Techa River during the period 1950–1992. Of these children, 3,897 were born during the period of highest release, i.e. between 1950 and 1956, and might thus have been exposed in utero or during childhood. A total of 1,103 individuals have since died, mainly owing to infectious and respiratory diseases and trauma [K49]. A total of 75 cases of cancer were detected. The most frequent cancer types were found to be respiratory tract, malignant lymphoma and leukaemia. The overall cancer incidence rate was 24.3 per 100,000, this comparatively low morbidity rate being attributed to the fact that the highest age attained was 45 years, with only 26% of the cohort being over 40 years of age [K50].

3. Remarks on the Techa River study

420. Some remarks may be made concerning the information reviewed above. Residents of Techa riverside villages were exposed to external gamma radiation and internal contamination with both short-lived and long-lived fission products. Early effects on the immune system included leucopenia, neutropenia, inhibition of innate immunity and autoimmune disorders. Long-term effects include impaired cellular immunity and a decrease of NK cells. A long-lasting delayed maturation and differentiation of granulocytes was observed. Leucopenia and neutropenia persisted for around three decades after the beginning of the exposure. Continuous exposure from long-lived incorporated radionuclides may account for this long-lasting effect. Greater exposures were correlated with longer recovery times. Subjects exposed in utero or during the first two years of post-natal life presented more severe effects. Five decades after the beginning of the exposure, immunological parameters had normalized, but an increase in the frequency of chromosomal aberrations and mutations was found in the peripheral lymphocytes of residents of Techa riverside villages. Manifestations of immunodeficiency occurred more frequently among residents who developed tumours.

D. Hanford nuclear site

1. General considerations

421. As previously described, there is no statistically significant dose response in the prevalence of autoimmune hypothyroidism or thyroid autoantibodies in atomic bombing survivors. On the other hand, it was suggested that the immune system might be involved in the pathogenesis of thyroid diseases in Chernobyl accident victims. It is thus interesting to present here another epidemiological study developed for evaluating thyroid diseases in people exposed to ionizing radiation.

422. Approximately 2.73×10^{16} Bq of ^{131}I were released to the atmosphere from the Hanford nuclear site between the years 1944 and 1957. This facility manufactured plutonium for early nuclear weapons. This production process caused the release of a variety of radioisotopes, which drifted on the wind and the river, settled on vegetation and were consumed by grazing animals. The vast majority of radioactive releases came in a single year. A study was recommended to estimate radiation doses to area residents and another to examine the

feasibility of potential health effects of ^{131}I, the radioisotope that accounted for most of the exposures [R12].

2. Hanford Thyroid Disease Study

423. The Hanford Thyroid Disease Study was conducted as a retrospective cohort study (1992–1997) to determine if thyroid disease had increased among persons exposed as children to these atmospheric releases of ^{131}I. The cohort included a sample of all births between the years 1940 and 1946 to mothers whose usual residence was in one of seven counties near the Hanford site. The thyroid doses ranged from 0.0029 to 2,823 mGy (mean 174 mGy, median 97 mGy) [K47]. Assessments of thyroid disease, including a thyroid ultrasound, a physical examination, and a fine needle biopsy if required to evaluate thyroid nodularity, were carried out in 3,440 individuals.

424. There was no evidence of a relationship between radiation dose and the cumulative incidence of any of the following outcomes: total neoplasia, thyroid cancer, benign thyroid nodules, autoimmune thyroiditis and hypothyroidism. Although ultrasound abnormalities were observed in 55.5% of women and 37.4% of men, they were not significantly associated with the dose. The Hanford Thyroid Disease Study has sufficient statistical power to test for dose–response relationships between thyroid outcomes and radiation exposure [D16, K48, R12].

3. Remarks concerning the Hanford nuclear site

425. As seen in the preceding paragraphs, ^{131}I was the radioisotope that accounted for most of the dose received by residents near the Hanford nuclear facility. Fifty years after exposure, the Hanford Thyroid Disease Study did not find evidence of a relationship between radiation dose and thyroid pathologies, including autoimmune thyroiditis. The results of this study support the hypothesis that exposure during infancy and childhood to ^{131}I at these dose levels and in these exposure circumstances does not increase the risk of these forms of thyroid disease.

E. Patients undergoing radiotherapy

1. General considerations

426. Ionizing radiation is an important and often indispensable strategy for cancer treatment. More than 50% of people with cancer undergo radiotherapy at some time during their illness [F9]. Although more alternatives for fractionation are now available, conventional fractionation schedules for local external radiotherapy (9–10 Gy/week delivered at dose rates of approximately 50 Gy/h over 5–6 weeks) continue to be the main modality. The potential for increased tumour control with protocols combining low-dose-rate with high-dose-rate irradiation is now under discussion [G11]. The rapid expansion of the number of elderly individuals in the world population will lead to a substantial increase in the prevalence of cancer and hence in the number of individuals undergoing radiotherapy. Second primary malignancies among cancer patients account for 16% of all cancer incidences [T13]. An extensive body of literature concerning second cancers in patients undergoing radiotherapy has recently been published [A20, D25, G33, R15, R16, R17]. However, papers concerning changes of immunological parameters after radiotherapy are scarce, and the actual impact of these changes on health has not been well established.

2. Review of published data

427. Nakayama et al. investigated changes in peripheral blood lymphocyte subsets of 15 lung cancer patients who had undergone thoracic irradiation [N17]. After radiation therapy, the percentage and the absolute number of CD4+CD45RA+ cells (naive T-cells) and CD56+ and/or CD16+ cells (NK cells) decreased. The percentage of HLA-DR+ CD4+ cells (activated CD4+ T-cells) and HLA-DR+ CD8+ cells (activated CD8+ T-cells) increased, although the absolute number did not change significantly. Changes in local inflammatory cells in bronchoalveolar lavage fluid were analysed by the same authors in a similar group of patients [N18]. The percentage of lymphocytes and eosinophils, the percentage of HLA-DR+ CD4+ and CD8+ cells (activated CD4+ and CD8+ T-cells, respectively) and the incidence of ICAM-1+ T-cells was higher in lung cancer patients who had undergone thoracic irradiation than in controls (lung cancer non-irradiated patients). Naive T-cells seem to be more selectively damaged than memory T-cells by thoracic irradiation. The reduction of NK cells may be disadvantageous for antitumour immunity. On the other hand, thoracic irradiation enhanced both peripheral and local T-cell activation, which may promote antitumour effects. Nakayama's results are in good agreement with the findings of Ishida et al. in patients who underwent thymectomy and post-operative radiation therapy, which also indicated that irradiation was associated with a higher percentage of activated T-cell subsets [I10].

428. Van Mook et al. studied 24 B-cell chronic lymphocytic leukaemia patients after splenic irradiation. Radiation treatment consisted of a weekly dose of 1 Gy up to a total dose of 10 Gy to the spleen. Six weeks after splenic irradiation, total leucocytes decreased significantly, with a decrease in the fraction of lymphocytes and an increase in the neutrophil and platelet counts. The number of CD4+ and CD8+ cells decreased significantly without significantly changing the CD4+/CD8+ ratio. No significant changes in immunoglobulin levels were observed [V11].

429. A long-term deficit in total CD4+ T-cell counts after radiation treatment for Hodgkin's disease (HD) was reported many years ago [P14]. In patients who received mediastinal irradiation for HD, Watanabe et al. found a marked depletion in both CD4+ and CD8+ naive T-cell counts that persisted for up to 30 years after completion of treatment.

In contrast, CD4+ and CD8+ memory T-cell subsets and total CD8+ T-cells recovered to normal or above normal levels by five years post-treatment, with different kinetics (early expansion of CD8+ memory T-cells versus the gradual recovery of the others). Thus the long-term deficit in total CD4 T-cell counts in irradiated HD patients was due to specific depletion of the naive T-cell subset. Similarly, total CD8+ T-cell counts returned to normal values by 5 years post-treatment, particularly because CD8+ memory T-cells expanded to higher than normal levels. As the thymus is the main source of naive T-cells, these findings suggest that mediastinal irradiation results in a long-term depletion of the CD4+ naive cell pool, probably owing to thymus impairment. This dysregulation of T-cell subset homeostasis may explain the altered T-cell function observed in treated HD patients, including the poor response to immunization after treatment. An extrathymic (peripheral) expansion of mature T-cells may partially compensate for the loss of thymus-derived T-cells, but this expansion is primarily restricted to the memory population, thus resulting in a selective expansion of memory T-cells, while naive T-cell numbers remain low [W10].

430. Safwat et al. [S35] studied immunological parameters in 35 non-Hodgkin's lymphoma patients undergoing WBI consisting of two cycles of four daily fractions of 0.2 Gy separated by 2 weeks of rest (total dose of 1.6 Gy over four weeks). WBI was associated with a significant decrease in the percentage of lymphocytes and a significant increase in the percentage of CD4+ T-cells, with a consequent significant increase in the CD4+/CD8+ ratio. In terms of absolute values, WBI leads to a significant reduction in the absolute number of all the lymphocyte subsets. The significant increase in the percentage of CD4+ cells in the peripheral blood was interpreted as indicating a higher radiosensitivity of the CD8+ T-cell subset. This contrasts with previous data published by Clave et al. [C11] for patients given WBI (total dose 12 Gy) before bone marrow transplantation, which revealed that all major T-lymphocyte subsets appeared equally radiosensitive, while the NK cells were relatively radioresistant. In that study, however, blood samples were collected 6 h after a single dose of 2 Gy, while in Safwat's study the samples were collected 24 h after a total dose of 1.6 Gy delivered over 4 weeks.

3. Remarks concerning data on patients undergoing radiotherapy

431. As presented in the preceding paragraphs, the effects on the immune system observed in cancer patients undergoing local radiotherapy include decreases in the absolute number of total leucocytes, total lymphocytes, CD8+ and/or CD4+ T-cells, and NK cells. Naive T-cells are more selectively damaged than memory T-cells. While CD4+ and CD8+ memory T-cell subsets and total CD8+ T-cells return to normal levels within 5 years after irradiation, CD4+ naive T-cell depletion may be long-lasting (recovery several decades after radiotherapy). This is particularly evident following mediastinal irradiation, probably owing to thymus impairment. Extrathymic (peripheral) expansion of memory T-cells results in a lower naive/memory cell ratio.

432. Although reduction in the absolute number of all lymphocyte subsets is observed after therapeutic WBI, the effects differ according to the fractionation schedule. While an early increase of the CD4+/CD8+ ratio is observed after a total whole-body dose of 1.6 Gy protracted over four weeks (low-dose fractions of 0.2 Gy), indicating a higher radiosensitivity of CD8+ T-cells, this effect was not observed after a total whole-body dose of 12 Gy given over three days (high-dose fractions of 2 Gy).

F. Summary

433. This section reviewed the effects observed on the immune system of human populations exposed to ionizing radiation in very different conditions. Diverse immunological parameters were evaluated in these populations, at different times after exposure. There are similarities and differences among the results reported by different authors. The analysis of similarities might aid identification of the predominant effects of ionizing radiation on the human immune system. However, findings should be interpreted taking account of the specific characteristics of each population.

434. The detonation of the atomic bombs in Hiroshima and Nagasaki in 1945 resulted in a short burst of external neutron and gamma irradiation. Radiation-induced cell death accounts for the short-term effects observed in the immune system, mainly associated with the development of acute radiation syndrome. An almost complete recovery of the haematopoietic system of atomic bombing survivors took place within the first year after the exposure. Little evidence of dose-dependent effects on the immune system of the survivors was found soon after the bombings. Studies of the long-term effects of ionizing radiation on the immune system began about 20 years after the atomic bombings.

435. The Chernobyl accident released large amounts of radionuclides into the environment over a period of around 10 days. External irradiation was predominant among emergency and recovery operations workers. Doses to residents of contaminated areas resulted from external irradiation from radionuclides deposited on the ground and from internal irradiation mainly due to ingestion of short-lived (e.g. ^{131}I) and long-lived (e.g. ^{137}Cs) radionuclides present in foodstuffs. Large quantities of radioiodine were internalized during the early period. Ingested and inhaled radioiodine was preferentially incorporated into the thyroid, resulting in higher levels of exposure to this gland compared with the rest of the body. As seen in the atomic bombing survivors, short-term effects on the immune system were mainly associated with the symptoms of acute radiation syndrome.

436. The residents of Techa riverside villages were exposed predominantly during the early 1950s to external

gamma irradiation from fission products and internal incorporation of short-lived (mainly ^{89}Sr) and long-lived (mainly ^{90}Sr and ^{137}Cs) radionuclides via drinking water and foodstuffs. In the early period, chronic radiation sickness (CRS) was diagnosed, including leucopenia, neutropenia, thrombocytopenia, impaired innate response and autoimmunity. The effects were more severe in children exposed in utero or at 1–2 years old. Normalization of haematological parameters in CRS patients was observed 30 years after the beginning of the exposure. Long-lived radionuclides contributing to chronic irradiation may explain why immunity disorders persisted for such a long period, even after cessation of external exposure and intakes. Long-term effects included decreased cellular immunity and reduced counts of NK cells.

437. Major radioactive releases into the air, water and soil occurred at the Hanford nuclear site between 1944 and 1957. Thyroid diseases were selected for the study of health effects because the release of ^{131}I caused the highest exposures in the population. There is no evidence of a relationship between radiation dose and the incidence of total neoplasia, thyroid cancer, non-cancer thyroid diseases, hypothyroidism and autoimmune thyroiditis.

438. Most cancer patients undergoing radiotherapy receive a very localized high-dose irradiation protracted over several weeks. Most of the published data concern mainly short-term effects on the immune system: total leucocyte decrease, total lymphocyte decrease, selective damage to naive T-cells compared with memory T-cells, enhancement of local and peripheral T-cell activation and decrease of NK cells. Long-term effects described in Hodgkin's disease patients show long-lasting (30 years) depletion of naive T-cells with a more rapid (5 years) recovery of memory T-cells. Studies concerning short-term effects of ionizing radiation on the immune system of patients receiving WBI indicate that the fractionation regime influences the resulting effects. Although a decrease in total lymphocytes is a common feature, the behaviour of lymphocyte subsets differs according to the WBI schedule.

439. The following findings may be considered as similar effects on the immune system observed in human populations exposed to ionizing radiation:

- Impairment of T-cell immunity: decreased total T-cells and CD4+ T-cells, lower T-cell responsiveness to mitogens and alloantigens;
- Increased humoral immunity: higher levels of total Ig, IgA and IgM;
- Shift towards an inflammatory profile: inflammatory cytokines, activated complement.

440. Atomic bombing survivors presented with a reduction of naive CD8+ and CD4+ T-cell pool, and clonal expansion of memory CD8+ T-cells associated with TCR repertoire deviation. This kind of perturbation of T-cell homeostasis was not evident in Chernobyl workers and residents. While total CD4+ T-cells were diminished in both populations, total CD8+ T-cells were decreased only in the Chernobyl population.

441. Findings concerning NK cells also differ: while significant changes were found in neither their number nor their cytotoxic activity among atomic bombing survivors, a decrease in both parameters was found in the Chernobyl population. Many workers who took part in recovery operations after the Chernobyl accident were contaminated with heavy metals, which may account, at least in part, for the observed changes in their immune status. An increase in the NKT lymphocyte subset was observed in Belarussian recovery operations workers, an observation that was not reported in atomic bombing survivors.

442. The development of autoimmunity is a relevant finding among Chernobyl workers and residents. There were regions with iodine deficiency in most affected territories of Belarus, the Russian Federation and Ukraine. Ionizing radiation might have induced thyroid gland changes affecting the expression of endemic goitre. These factors should be considered in interpreting the increase in thyroid autoantibody levels and the development of autoimmune thyroid diseases, which were not observed in the atomic bombing survivors or in residents near the Hanford site.

443. Studies of the long-term effects of ionizing radiation on the immune system of atomic bombing survivors began about 20 years after the bombings and continue to be carried out. Because the Chernobyl accident occurred in 1986, data concerning long-term effects of ionizing radiation on the immune system are today limited to the first 20 years after the event. These should be considered for cross-comparison of the long-term effects observed in the two populations.

V. FINAL SUMMARY

444. The immune system is certainly one of the most complex systems of the body. It is composed of a large variety of cells spread widely throughout the body and of different organs where stem cells can differentiate into one of the major lineages. Immune cells communicate via cytokines, which are soluble molecules that stimulate immune cell proliferation and/or differentiation. Consequently the immune cells can differentiate towards specific cell types. Clusters of differentiation (CD) are cell surface glycoproteins associated with specific functions; their expression may change depending on cell environment, e.g. the effect of ionizing radiation.

445. One of the main functions of the immune system is the recognition of foreign antigens and the development of subsequent actions of protection, for example against infection and cancer. Autoimmune disease may result from the alteration of self tolerance mechanisms.

446. For protecting the body, the immune system can use two different but interrelated forms of immunity, i.e. innate and acquired. While innate immunity provides a rapid defence because it is always ready for use, acquired immunity develops only after a pathogen has entered the body. Acquired immunity is very antigen-specific and keeps the memory of a previous exposure, yielding a stronger response at the time of a subsequent exposure to the same antigen. Acquired immunity can respond to the diversity of foreign antigens, those antigens being processed by APCs. The major histocompatibility complex, in humans called human leucocyte antigen (HLA), plays a fundamental role in the processing of antigens by APCs and their presentation for recognition by T-cells via specific receptors. HLA-G is a family of particular molecules involved in immunotolerance; cells expressing HLA-G can escape from immune surveillance.

447. The effects of ionizing radiation on each component of the immune system (e.g. organs of the immune system, cell populations, expression of CDs) have been documented. Although significant changes occur, the results of the publications on these changes are difficult to compare, because the circumstances and the protocols of exposure to ionizing radiation (dose, dose rate, quality of radiation, cell type) differ considerably.

448. The data reviewed in this annex indicate that exposure to ionizing radiation often leads to immunosuppression, particularly following high-dose irradiation. Immunosuppression is most often ascribed to lymphocytes being highly radiosensitive, owing to their proclivity to undergo radiation-induced apoptosis. In addition to these cytotoxic effects, ionizing radiation may induce “danger signals”, which may in turn influence cell responses in the immune system. Such evidence has led to the emerging notion that ionizing radiation has much more to offer than its qualities as a powerful cytotoxic agent, and because of this, ionizing radiation is probably better considered an immunomodulatory agent rather than an immunosuppressive one.

449. Although many questions remain open, the role of the immune system with regard to cancer development is better understood. In the classical immune surveillance theory, tumours may develop when cancer cells escape from immune surveillance either by reducing the expression of tumour antigens or by modifying the immune response to them. Although a strong antitumour activity can be efficiently developed as a result of the activation of both innate and acquired immune responses, some immunological promotion of tumours may result from low-grade persistent inflammation, chronic activation of innate immune cells or the blocking of cell-mediated cytotoxicity by antibodies.

450. The effects of ionizing radiation on the immune system at low doses (<200 mGy) and low dose rates (<100 mGy/h) remain controversial. In animals, although depletion of different categories of immune cells is observed, as well as changes in lymphocyte subsets, there is some evidence that low-dose WBI can be immunostimulatory. Data concerning suppressive effects of low doses on tumour growth have been reported. Such data have been obtained after low-LET radiation exposure, they are dependent on a number of factors and they are very variable. In humans, although a decrease of CD4+ T-lymphocytes, a decrease of HLA-DR+ lymphocytes and decrease of the CD4+/CD8+ ratio due to an increase in CD8+ are frequently reported, these numerical findings could not be directly related to a decrease of immune function. Some studies regarding people living in areas with high levels of natural radiation suggest the existence of an adaptive response induced by chronic radiation exposure.

451. In atomic bombing survivors and the residents of Techa riverside villages, the haematolymphoid system was damaged in a dose-dependent manner, although the groups underwent different types of radiation exposure. Several months or even years later, their systems regenerated, and haematolymphoid function recovered almost completely. However, even after several decades, with different effects in the two populations, significant effects have been observed in the haematolymphoid systems.

452. In atomic bombing survivors, long-lasting effects are still observed more than a half-century after their radiation exposure. These effects include an increase in the frequency of somatic mutations and chromosome aberrations in lymphocytes, as well as significant changes in lymphoid cell composition and function. TCR-defective cells could result in the impairment of the immune function. Although low frequencies of somatic mutations or chromosome aberrations would not influence the regeneration or homeostasis of the immune system, the extensive proliferation of a single cell bearing a radiation-induced mutation may result in clonal expansion, particularly in haematopoietic stem cells, committed lymphoid precursor cells and memory T-lymphocytes. With regard to changes of lymphoid cell composition and function, the main kinds of damage observed among atomic bombing survivors include: impairment of T-cell immunity, especially owing to a decreased proportion of CD4+ helper, CD4+ naive and CD8+ cytotoxic T-cells; a dose-dependent increase in the proportion of B-cells and of immunoglobulin production; and impairment of viral immunity and other T-cell functions, such as PHA-dependent proliferation, ability to produce IL-2 and alloantigen responses.

453. In Techa riverside village populations chronically exposed to radiation, long-term immunity changes involved decreased expression of differentiating antigens of T-lymphocytes, reduced counts of cells involved in natural cytotoxicity and signs of immunological imbalance. As in the atomic bombing survivors, a preferential CD4+ cell deficiency was observed many years later in persons affected by the Chernobyl accident. Proliferative response to mitogens was also altered. The dynamics of post-irradiation recovery of CD4+ and CD8+ were different, suggesting that radiation may induce damage to the thymus, accelerating the natural ageing of the immune system by a progressive decline in thymic function. Both short-term and long-term effects were detectable in the B- and T-cell function profiles, as well as in the synthesis of immunoglobulins, depending on the population studied. Some of these changes were dose-dependent and characterized by phases. The immune system is involved in the pathogenesis of the thyroid diseases observed in victims of the Chernobyl accident, probably owing to antigenic mechanisms leading to autoimmune damage.

454. Animal data involving low-dose irradiation reinforced some of these results, for example the gradual reconstitution of peripheral blood and bone marrow patterns with partial deficiency of haematopoietic and lymphopoietic precursors, suggesting that ineffective haematopoiesis could cause restriction of myeloid and lymphoid cell reserves and consequent disturbances of cellular and humoral immunity. Enhancement of immunity may be observed under certain circumstances, in particular following low-dose irradiation, and modulation of oxidative status seems to be involved in this effect.

455. Recent developments in immunology have contributed to our understanding of how human diseases may be related to abnormalities in the immune system. The study of these disorders from an immunological viewpoint could therefore provide further insight into the mechanisms involved in certain radiation-associated diseases. Radiation-induced perturbation of T-cell homeostasis may have important implications for human health, not only by reducing the ability of the immune system to fight against new pathogens but also by compromising the control of recurrent and latent infections. Increasing evidence indicates that persistent exposure to infections leads to more rapid senescence of the immune system. An association between non-cancer diseases and radiation dose has recently emerged among atomic bombing survivors. This association has led to the hypothesis that radiation-induced effects on the immune system may account at least in part for this phenomenon, although the mechanisms involved remain incompletely understood.

456. Epidemiological findings suggest that the radiation-induced impairment of immunocompetence may increase the risk of diseases that normally occur in elderly people. The data reviewed in this annex reinforce the hypothesis that ionizing radiation may accelerate immunosenescence by perturbing T-cell homeostasis in the same direction that ageing does. Immunological homeostasis has critical implications for human health, for example with respect to the relationship between the immune system and disease susceptibility, and the possible interaction between hereditary and environmental factors such as ionizing radiation.

457. A statistically significant dose–response relationship has been found for several inflammatory biomarkers in irradiated subjects. The persistent inflammatory status induced by ionizing radiation may increase the risks of both cancer and non-cancer diseases. The negative correlation between plasma levels of inflammatory biomarkers and the percentage of CD4+ helper T-cells in peripheral blood indicates an association between radiation-induced impairment of cell-mediated immunity and a preclinical inflammatory status that could further promote the development of various diseases.

458. The proportion of CD4+ cells was significantly decreased among atomic bombing survivors with increased dose and history of myocardial infarction. It has been suggested that a diminished immune response against certain infections implicated in the development of cardiovascular diseases could account for this finding. Moreover, inflammatory biomarkers were significantly elevated in these patients, indicating that inflammatory responses may be playing a role in the development of cardiovascular diseases, such as myocardial infarction, in irradiated people.

459. In order to explain the possible links between radiation-induced perturbation of immunological homeostasis and human diseases, the “Th1/Th2 paradigm” has been invoked. Experimental data, further reinforced by human data, seem to sustain the hypothesis that ionizing radiation reduces cellular responses controlled by Th1 cells and increases humoral responses controlled by Th2 cells by triggering a shift from Th1 towards Th2 (Th1/Th2 imbalance). Recent findings demonstrated that radiation-dose-dependent

increases observed in irradiated individuals involved not only Th2-related cytokines but also Th1-related cytokines. This evidence leads to the postulate that ionizing radiation might better be considered an agent that modulates the production of cytokines towards a pro-inflammatory response rather than towards a Th2 response.

460. Programmed cell death (apoptosis) is essential for the development and maintenance of cellular homeostasis of the immune system. The functional balance of proapoptotic versus anti-apoptotic influences determines whether a lymphocyte will live or die. Multiple molecules, often working in concert, control serial stages of lymphocyte development and homeostasis. Radiation-induced apoptosis is one of the mechanisms by which ionizing radiation alters the homeostasis of the immune system.

461. Evaluations of the human health risks associated with radiation exposure have been based primarily on the assumption that the effects of radiation occur in irradiated cells. Non-targeted cellular responses to ionizing radiation, such as bystander effects and genomic instability as well as adaptive responses, have also been demonstrated in the immune system, although their implications for human health are still poorly understood. These effects, which predominate at the low doses of relevance to radiation protection, need to be fully characterized; they pose new challenges to evaluating the risks associated with radiation exposure. In contrast, local radiotherapy may facilitate the expansion of dendritic cells and the generation of antitumour immune responses outside the radiation field (abscopal effect).

462. The idea that the immune system functions by discriminating "self" from "non-self" has a long history in immunology. The "self/non-self theory" proposes that lymphocytes with reactivity against host constituents are destroyed during development, and only those tolerant lymphocytes that are not self reactive are left to engage foreign antigens. Although the notion that the immune system has evolved to recognize (dangerous) pathogens is not new, recent discussions have emerged concerning the notion that antigenicity in the immune system may be seen as a question of degree, where "self" evokes one kind of response (tolerance) and "foreign" evokes another kind of response (destruction) based not on intrinsic foreignness but rather on the immune system's recognition of foreign antigens in the context of "danger signals". Whichever theory is correct, ionizing radiation has emerged as an agent capable of disturbing the ability of the immune system to deal with this sort of recognition. As a result of radiation-induced damage, the immune system may tolerate what should be destroyed and, conversely, it may destroy what should be tolerated. Examples of these two opposing consequences are the diminished ability to mount an immune response against some infections and the development of autoimmune reactions as a consequence of radiation exposure, both of which have been demonstrated by experimental and human data.

463. Individual genetic susceptibility to ionizing radiation has been clearly demonstrated in patients. Some of the molecular and cellular mechanisms that determine sensitivity to ionizing radiation have been elucidated, most of them representing a defect in the response to DNA damage. Research on this subject will be relevant, since most of the human genetic disorders involving such defects are also associated with alterations of immune system functioning.

464. Finally, the question of how radiation-induced effects on the immune system may impact on human health remains unanswered. There are many issues that need to be more thoroughly investigated before firm conclusions can be reached. Possible future directions for research concerning the effects of ionizing radiation on the immune system that should provide new insights about the underlying mechanisms may include:

- Effects of low-dose and low-dose-rate irradiation versus intermediate- and high-dose irradiation;
- Combined effects of ionizing radiation and other agents;
- Differential effects of external and/or internal irradiation;
- Immunomodulation and cancer;
- Perturbation of T-cell homeostasis;
- Immune function and disease development;
- Immunogenetic background and disease susceptibility;
- Immunological ageing and inflammatory response;
- Effects on the skin immune system.

VII. CONCLUDING REMARKS

465. This annex reviews data related to radiation-induced alterations of immune response, considers the possible mechanisms involved and reviews epidemiological studies of the effects of ionizing radiation on the human immune system.

466. The effects of ionizing radiation on the immune system can be assessed by estimating changes in cell numbers or by using a variety of functional assays. The impact of such alterations in immune response depends on factors such as the dose of radiation, its temporal relationship with immune system challenge and individual genetic constitution.

– High doses of radiation produce immunosuppression mainly through the destruction of cells. Lymphocytes are very radiosensitive, and their reduction is currently used as an early indicator of the level of an accidental acute exposure. Radiation-induced changes in immune parameters seem to be more dependent on total dose than on dose rate. Persisting effects on the immune system have been observed after exposure to ionizing radiation.

– At low doses and dose rates, the effects of ionizing radiation on the immune system may be suppressive or stimulatory. The long-term impact of low radiation doses on the immune function in relation to human health needs to be further evaluated.

Abbreviations

AIDS	acquired immunodeficiency syndrome
AIRE	autoimmune regulator
AP	alkaline phosphatase
APC	antigen-presenting cell
ARS	acute radiation syndrome
AT	ataxia telangiectasia
ATLD	ataxia-telangiectasia-like disorder
ATM	ataxia-telangiectasia-mutated gene
ATP	adenosine triphosphate
BCR	B-cell receptor
C	constant gene segment
cAMP	cyclic adenosine monophosphate
CD	cluster of differentiation
CFU	colony-forming unit
CFU-F	fibroblastoid colony-forming units
CFU-GM	granulocyte–macrophage colony-forming units
CFU-S	stem cell colony-forming units
cGMP	cyclic guanosine monophosphate
CH	heavy chain constant domain
CL	light chain constant domain
Con A	concanavalin A
COX-2	cyclooxygenase-2
CRP	C-reactive protein
CRS	chronic radiation sickness
CS	Cockayne syndrome
CTLA-4	cytotoxic T-lymphocyte-associated antigen-4
D	diversity
DC	dyskeratosis congenita
DNA	deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
DNF	dinitrofluorobenzene
DSB	double-strand break
DTH	delayed type hypersensitivity
ESR	erythrocyte sedimentation rate
ETRC	Extended Techa River Cohort
FA	Fanconi anaemia
Fas/CD95	Fas death receptor
FasL/CD95L	ligand for Fas death receptor
GM-CSF	granulocyte–macrophage colony-stimulating factor
GSH	glutathione
Gy	gray
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus

HD	Hodgkin's disease
HER2	human epidermal growth factor receptor 2
HETE	hydroxyeicosatetraenoic acid
HGPS	Hutchinson–Gilford progeria syndrome
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HLNRA	high-level natural radiation area
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HR	homologous recombination
HVC	hepatitis C virus
ICE	interleukin-converting enzyme
IFN	interferon
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
ILT	immunoglobulin-like transcript
J	joining
JNK	Jun N-terminal kinase
KIR	killer cell immunoglobulin-like receptor
KLH	keyhole limpet haemocyanin
LC	Langerhans cell
LD	low dose
LDR	low dose rate
LET	linear energy transfer
LPB	lipopolysaccharide-binding protein
LPS	lipopolysaccharide
MALT	mucosa-associated lymphoid tissue
MAP kinase	mitogen-activated protein kinase
MBP	mannan-binding protein
MCP-1	monocyte chemoattractant protein 1
MHC	major histocompatibility complex
MICA	class I MHC chain-related A molecule
MNCA	modified neutral comet assay
MP	myeloperoxidase
mRNA	messenger ribonucleic acid
NBS	Nijmegen breakage syndrome
NCR	natural cytotoxicity receptor
NER	nucleotide excision repair
NHEJ	non-homologous end-joining
NK	natural killer (cell)
NKG2	lectin-like receptor
NKT	lymphocyte with certain characteristics of both T- and NK cells
NPP	nuclear power plant
p38 MAP	p38 mitogen-activated protein
PAMP	pathogen-associated molecular pattern

PCNA	proliferating cell nuclear antigen
PGE2	prostaglandin E2
PHA	phytohaemagglutinin
PKA	protein kinase A
PKC	protein kinase C
PLA2	phospholipase 2
PLC	phospholipase C
PRRs	pattern recognition receptors
RBE	relative biological effectiveness
ROS	reactive oxygen species
RRD	recurrent respiratory disease
RT-PCR	reverse transcription polymerase chain reaction
SCID	severe combined immune deficiency
SIS	skin immune system
SLE	systemic lupus erythematosus
SOCS	supressor of cytokine signalling gene
STAT proteins	signal transducer and activator of transcription proteins
TCR	T-cell receptor
TCR	transcription-coupled repair
TCR/CD3	T-cell receptor/CD3 complex
TGF-β	transforming growth factor β
Th	lymphocyte T-helper
Th1	lymphocyte T-helper subset or subclass 1
Th2	lymphocyte T-helper subset or subclass 2
TLR	Toll-like receptor
TLS	translesion synthesis
TNF	tumour necrosis factor
TNFR	tumour necrosis factor receptor
TRAIL	tumour-necrosis-factor-related apoptosis-inducing ligand
TRDS	Techa River Dosimetry System
Treg	T-regulatory cells
TROC	Techa River Offspring Cohort
TSH	thyroid-stimulating hormone
TTD	trichothiodystrophy
UDS	unscheduled DNA synthesis
UV	ultraviolet
UV-DDB	UV-damaged-DNA-binding protein
V	variable
VH	heavy chain variable domain
VL	light chain variable domain
WBI	whole-body irradiation
WLM	working level month
WS	Werner’s syndrome
XP	xeroderma pigmentosum
XP-V	xeroderma pigmentosum variant

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Effects of Ionizing Radiation: United Nations Scientific Committee on the Effects of Atomic Radiation 2006 Report to the General Assembly, with Scientific Annexes—Volume II

Annex E ("Sources-to-effects assessment for radon in homes and workplaces"), paragraph 460

The ninth sentence *should read*

For residential exposure to 150 Bq/m^3, the authors estimated a combined OR of 1.1 (95% CI: 1.0, 1.3).

V.11-82963 (E)

ANNEX E

Sources-to-effects assessment for radon in homes and workplaces

Contents

INTRODUCTION

1. For many years, the Committee presented its evaluations of information and data on the "Sources" and "Effects" of ionizing radiation separately. These were divided into two volumes in the UNSCEAR 2000 Report [U2]. During its 50th session, in April 2001, the Committee discussed the feasibility of preparing documents in an integrated "Sources-to-Effects" approach. This document provides a "test case" for future UNSCEAR "Sources-to-Effects" assessments.

2. Radon is an inert noble gas. Its most common isotope, and the one that is commonly known as radon, is ^{222}Rn, which arises in the radioactive decay chain of uranium-238. Uranium occurs naturally in varying levels in all rocks and soils. Some fraction of the radon produced in rocks and soils escapes to the air; therefore radon is present in the atmosphere. Thus, simply by breathing, people everywhere are exposed to radiation from radon itself and also from short-lived radon decay products (RDPs).[1] Moreover, radon is soluble in water, and groundwater that passes through uranium-bearing soils and rocks contains radon. When radon-rich groundwater is used as drinking water, people are exposed both through water consumption and by radon being released from the water to the air and being inhaled.

3. Thoron (^{220}Rn) is an isotope of radon, and therefore also an inert noble gas, which arises from the decay chain of thorium-232 (^{232}Th). Thorium is a common element in the earth's crust and therefore, like radon, thoron is found in air at varying concentrations. Since radon and thoron are members of different decay chains, the ratio between radon and thoron (or between the decay products of radon and thoron) will depend in part on the ratio of uranium to thorium in local soils, rocks or building materials. As discussed in the UNSCEAR 2000 Report [U2], the radioactive half-lives of radon and thoron and their respective decay products are also very important in determining the exposures of people in workplaces and homes. Since thoron has a much shorter half-life ($t_{½}$ = 55 s) than radon ($t_{½}$ = 3.82 days), the distance it can travel before undergoing radioactive decay is very much shorter than the distance radon can travel in the same medium, and therefore its expression in the environment is quite different from that of radon.

4. Sources of radon and thoron and of potential exposures to workers and the public are briefly discussed in section I. A more comprehensive discussion of sources of exposures to radon and thoron in the workplace is provided in the UNSCEAR 2000 Report [U2]. The Committee is presently updating its assessments of these sources.

5. Historically, a wide variety of quantities and units were used to assess radon exposure. Appendix A provides a short summary of the historical units used in this report and the relevant conversion factors. To maintain the integrity of the historical data, the original units used in the papers cited in this annex are preserved. Most historical, and indeed current, measurements of radon in mines are in units of working level (WL) or working level month (WLM). The former is a measure of the potential alpha energy concentration in the air and the latter is a measure of exposure for an assumed working month of 170 hours. The unit of WLM is the traditional unit used to report exposure to RDPs in studies of miners. The modern quantity for expressing concentration of RDPs is the equilibrium-equivalent concentration (EEC), which represents the concentration of ^{222}Rn in equilibrium with its decay products that would have the same potential alpha energy. To convert from an exposure in WLM, it is necessary to multiply by 6.4×10^5 (see appendix A) to obtain Bq h m^{-3} (EEC). Where appropriate, the RDP measurement expressed as EEC is provided in brackets or is otherwise discussed.

6. For many years, radon was recognized as constituting a hazard to underground miners. However, while it was also recognized that domestic exposure to radon might carry a risk, there was no direct evidence of this until recently. The risk from residential exposure to radon and its decay products is of great interest in many countries; thus methods to evaluate exposure to radon and thoron and their decay products as well as the subsequent risks from exposure are of great interest.

7. In the past, exposures to thoron and its decay products were often ignored. As will become evident from the discussion in section I, it has become increasingly clear that the exposure to thoron and its decay products cannot be ignored in some environments (both workplace and residential) as it contributes to the risks otherwise assigned solely to exposure to radon and its decay products (e.g. [S41]). In some epidemiological studies, no distinction is made between exposure to radon and its decay products and exposure to thoron and its decay products. Measurement techniques for discriminating between them do, however, exist (e.g. [C21, T12, T14, T15, T16, T17, Z3, Z4]).

8. The use of recent measurements to estimate exposures received many years ago, for example in the uranium miner studies and in the studies of residential radon exposures,

[1] The term "radon" is used generically in this report to indicate both radon and its decay products, the latter in fact contributing most of the dose to lung tissue.

carries particular difficulties. In the case of mining, ore grade, mining methods, ventilation practices and other factors have changed over time. Residential radon studies experience similar problems in estimating past exposures, because of, for example, changes in heating and ventilation practices over time. It is thus important to recognize factors that have a substantial impact on exposure estimates and to assess the potential magnitude of these impacts. For example, the entry of radon into structures is an important consideration, is well studied and is described in many reports, including references [U2, U5, U6].

9. To understand how radon exposure estimates can be transferred from one miner population to another, or from conditions in mines to conditions in homes, it is important to understand the differences in dosimetry of exposures in mines compared with homes. The 1991 National Research Council companion report [N10] to the report of the BEIR IV Committee [C19] provides a comprehensive discussion of these differences, as do BEIR VI [C20] and the UNSCEAR 2000 Report [U2]. Section II of this annex provides a concise overview of current issues in radon and thoron dosimetry.

10. An understanding of the mechanisms for the carcinogenicity of radon and its decay products and of how radon interacts with other agents is important. Much information is available on these topics (e.g. [C20, N11, U2]). Information from animal experiments and experiments at the cellular and subcellular levels relevant to understanding the mechanisms of radon carcinogenicity is discussed briefly in section III.

11. Until recently, the main basis for estimating risks from residential exposure to radon and its decay products was provided by epidemiological studies of underground miners that extrapolated the results down to the levels of exposure seen in homes. Studies of historical miners require retrospective estimation of exposure conditions many years in the past. Often there are few or even no actual measured exposure data from the early years of mining; the results from such studies are less certain because of this and other factors. Extrapolation from risks estimated in miner studies to residential exposure conditions involves additional assumptions. For example, such extrapolations must consider the impact of the relatively short exposures at high exposure rates seen in mines compared with the longer exposures at lower exposure rates in homes. The different dosimetry of exposures in mines and in homes, as well as other factors, must be considered in selecting the most appropriate exposure– (or dose–) response model for extrapolation down to residential levels.

12. Consequently, direct estimates of residential risk from the exposure of the general population are of great interest, and numerous studies of risk from residential exposure to radon were made using case–control studies. The results of these studies demonstrate an excess risk at the levels of radon seen in homes and suggest a pattern of increasing risk with increasing exposure that is generally consistent with the experience of epidemiological studies of miners. Section IV discusses the epidemiological studies of miners exposed to radon and section V discusses the epidemiological studies of residential exposure to radon.

13. While an increased risk of lung cancer associated with exposure to radon and its decay products is well established from epidemiological studies of underground miners and more recently from residential radon studies, the potential risks to tissues and organs other than the lung are also of interest and are the subject of section VI.

14. There is great interest in predicting future risks to people who are exposed to radon and its decay products either in the workplace or in the home. It is necessary to understand the limitations of risk projections and how such projections may be affected by consideration of exposures to other agents (cigarette smoke being the most important) in the workplace or in the home. The characteristics and limitations of existing models, including biologically based models, and a recommended approach to risk projection are the subjects of section VII.

15. The reliability of estimates of radiation exposures is one important factor in assessing the risk of cancer following radiation exposure. The sources of uncertainty in epidemiological studies and how these uncertainties affect the dose–response analysis [N5] was discussed in the UNSCEAR 2000 Report [U2]. Uncertainties arise in estimating exposures of miners at work, historically reported in WLM, and of people at home, reported in Bq h m^{-3}.

16. The sources and characteristics of uncertainty in the exposure of miners are of considerable interest and constitute a major focus of this report. The report of the BEIR VI Committee [C20] discusses this subject at length, as does the most recent radon report of the National Council on Radiation Protection and Units (NCRP) [N11], which provides the most comprehensive examination to date of the underground miner data. There are large difficulties in developing reliable estimates of underground radon exposure for epidemiological studies of miners, especially for the pre-1960 miners [C20, L10]. Studies of miners employed more recently have relatively reliable exposure information (e.g. [H35, S12, S14]). Uncertainties in assessment of exposure are similarly important considerations in residential radon studies (e.g. [D15, D17, L8]).

17. Finally, section VIII provides an overall summary of the main observations from this annex.

I. SOURCES AND LEVELS OF RADON EXPOSURES

18. The majority of the dose to the lung arises from exposure to the short-lived decay products of radon and thoron. Concentrations of the potential alpha energy of these short-lived decay products are estimated by considering the state of equilibrium between the parent nuclides and their respective decay products. In practice, an equilibrium factor F_{eq} is used to characterize the state of equilibrium. The equilibrium factor F_{eq} is defined as the ratio of the actual potential alpha energy concentration (PAEC) to the PAEC that would prevail if all the decay products in each series were in equilibrium with the parent radon or thoron, as the case may be. However, as discussed in reference [U2], it is simpler to evaluate this factor in terms of an equilibrium-equivalent radon or thoron concentration.

19. The Committee customarily reports concentrations of radon or thoron (and also the lung dose) in terms of equilibrium-equivalent concentration (EEC). This is defined as the equivalent concentration of the decay products in equilibrium with the parent gas that yields the same potential alpha energy per unit volume as the existing mixture.

$$EEC(^{222}Rn) = 0.105\ (^{218}Po) + 0.516\ (^{214}Pb) + 0.379\ (^{214}Bi)$$
$$EEC(^{220}Rn) = 0.91\ (^{212}Pb) + 0.087\ (^{212}Bi)$$

where ^{218}Po, ^{214}Pb, etc., and EECs are in Bq/m^3. Older publications often report activity in curies (Ci). The Appendix presents relevant conversion factors.

20. Radon and thoron are ubiquitous in the air at ground level and are significant contributors to the average dose from natural background sources of radiation [U2]. In homes, in underground mines and in other situations where radon (and thoron) may be present and where ventilation may be limited, the levels of these radionuclides and their decay products can accumulate to high levels. Soils and rocks are often the main sources of radon. In unsaturated soils or rocks, radon moves with air through pores and fractures. In saturated zones, radon moves with groundwater to underground openings, such as mines and caves, and to buildings [N9].

A. Outdoors

21. Concentrations of radon in the outdoor environment are affected not only by the magnitude of the release rate from the ground to the atmosphere but also by atmospheric mixing phenomena. Solar heating during the daytime induces turbulence, so radon is more readily transported upwards and away from the ground. Doi and Kobayashi [D18] provide information on the vertical distribution of outdoor radon and thoron in Japan. At night and in the early morning hours, atmospheric (temperature) inversion conditions are often found; these tend to trap the radon closer to the ground. This means that outdoor radon concentrations can vary diurnally by a factor of as much as 10. Seasonal variations, related to the effects of precipitation or to changes in prevailing winds, also exist [U2]. An evaluation of exposure to outdoor concentrations of radon in Iowa and Minnesota [S40] in the United States concluded that outdoor exposure to radon in some areas can be a substantial fraction of an individual's exposure to radiation and moreover is highly variable across the population. Outdoor levels of radon provide a baseline for indoor levels of radon. In tropical climates, indoor and outdoor concentrations are essentially the same because of rapid exchange between indoor and outdoor air [C43].

22. The UNSCEAR 2000 Report [U2] suggests that typical outdoor levels of radon and thoron gas are each of the order of 10 Bq/m^3. There is, however, a wide range of long-term average concentrations of radon, from approximately 1 Bq/m^3 to more than 100 Bq/m^3, with lower levels typical of isolated small islands or coastal regions and higher levels typical of sites with high radon exhalation over large surrounding areas. Although data are relatively sparse for thoron, considerable variability from place to place would be expected because of thoron's short half-life, which amplifies the effect of local variations in exhalation rate. Thoron decay products were measured continuously outdoors in suburban New Jersey, United States, for a 2-year period [H20]. The average outdoor concentration of ^{212}Pb was 0.09 Bq/m^3 and the variability over seasons was a factor of 2. Bismuth-212 was not detectable. The average outdoor concentration of thoron gas was 15 Bq/m^3, yielding a value of 0.005 for the equilibrium factor outdoors [C21].

B. Indoors

23. In buildings with high radon levels, the main mechanism for entry of radon is pressure-driven flow of soil gas through cracks in the floor. This arises because the air inside buildings is normally at a slightly lower pressure than the air outdoors. This underpressure is the consequence of the air inside the building being warmer than that outside. In temperate zones especially, this causes a convective flow ("chimney effect"), which, together with the effect of the wind blowing over chimneys and other openings ("Venturi effect"), draws soil gas and hence radon into the building. However, in addition to pressure differences, other factors, including relative humidity and soil moisture, can also influence radon levels in buildings [S66].

24. While most building materials produce some radon, certain materials can act as significant sources of indoor radon. Such materials have a combination of elevated levels of ^{226}Ra (the radioactive parent of radon) and a porosity that allows the radon gas to escape. Examples are lightweight concrete with alum shale, phosphogypsum and Italian tuff.

25. Groundwater, particularly in granitic areas, can have high levels of radon. Workplaces such as laundries and restaurant kitchens can have high radon levels from the use of such water. Because municipal water supplies are often from rain catchment surface reservoirs, radon levels in public water supplies are normally not high, and any problems are normally limited to wells in geological formations containing naturally elevated levels of uranium. In the United States, groundwater supplies were reported to contain radon at levels of as high as 10^6 Bq/m^3 or more [N9]. In Germany, treatment and distribution workplaces for groundwater supplies also were found to contain elevated radon concentrations in air, with up to several hundred thousand becquerels per cubic metre [U2].

26. There is a considerable amount of data available on radon concentrations in indoor air, and new information is becoming increasingly available on thoron concentrations indoors. Substantial compilations of radon measurements appeared in the UNSCEAR 2000, 1993 and 1988 Reports [U2, U5, U6]. On the basis of current data, the UNSCEAR 2000 Report [U2] deduced values of 40 and 30 Bq/m^3 for the arithmetic and geometric means of indoor radon gas concentrations worldwide, with a geometric standard deviation of 2.3. On the basis of the information collected for the present report, these values are still appropriate.

27. Published literature provides additional insight into the sources and levels of radon and thoron. Examples include publications on the assessment of radon in drinking water [N9, T22] and numerous papers on residential radon levels in Iowa, United States [F3, S40], Italy [B15, F4, P6], Poland [Z9], the former Yugoslavia [Z2], China [W13, W16], the Isle of Man [G6] and the Republic of Korea [C33], among others. The influence of groundwater on radon concentrations in drinking water and subsequently on residential radon levels in air from the use of potable water indoors was also investigated in radon-prone areas of Japan [K27, I6, N17].

28. A great deal of information is available from radon measurements in homes in the United Kingdom. A national survey carried out in the early 1980s provided results of gamma ray dose rate and radon concentration measurements in more than 2,000 dwellings selected systematically according to postal codes [W14]. The mean radon level from the survey, after adjustment for dwelling types, was 20.5 Bq/m^3. The same study also reported data from studies carried out in regions of the United Kingdom with elevated uranium mineralization. The regional surveys showed average radon levels of up to 300 Bq/m^3 for areas of south-west England, which are about 15 times the national average. In addition, simultaneous measurements of thoron decay product levels in high-radon regions showed a mean thoron decay product level of about 0.6 Bq/m^3, though the national average was estimated to be 0.3 Bq/m^3 [W14]. Subsequently, more than 400,000 measurements were made throughout the United Kingdom aimed at identifying dwellings with elevated radon levels; this information is available for England and Wales [G10], Scotland [G11] and Northern Ireland [G12].

29. A nationwide indoor radon survey carried out since 1982 in France has updated estimates of the population exposure [B38]. Indoor radon measurements were performed using passive dosimeters left in place for 2 months in the main room. A questionnaire was completed regarding housing characteristics. In total, the survey included 12,261 radon measurements distributed over the whole country. Corrections for seasonal variations [B36] and housing characteristics were applied. The crude average of indoor radon concentrations was 89 Bq/m^3, and the average corrected for season and housing characteristics was 83 Bq/m^3 (the range over French districts was 19 to 297 Bq/m^3). Weighting by district population density yielded a national average of about 63 Bq/m^3.

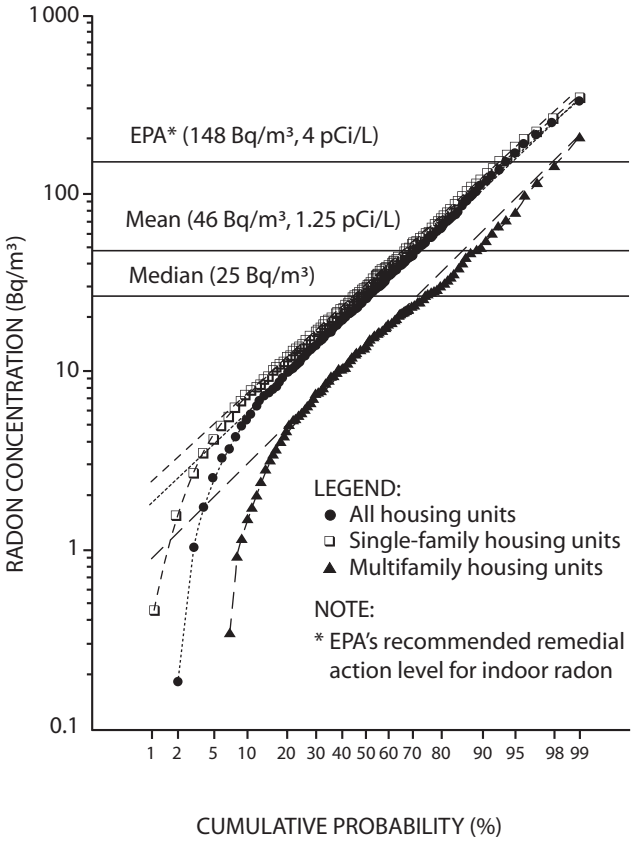
30. Bochicchio et al. [B40] report a national radon survey of some 5,631 dwellings distributed in 232 towns across Italy. The authors report a national population-weighted average of 70 Bq/m^3 and a geometric mean and geometric standard deviation of 52 Bq/m^3 and 2.1, respectively. The authors also report seasonal differences. On a national scale, over the 21 regions studied, the authors report a winter radon to summer radon ratio with a geometric mean and standard deviation of 1.23 and 1.71, respectively.

31. European Union (EU) efforts to develop a radon map of European countries are under way as part of an EU project to develop a European atlas of natural radioactivity [D20].

32. A major survey of radon concentrations in United States homes measured radon levels in homes in 125 counties in 50 states with the results shown in figure I (after reference [M1]). Approximately 6.1% of the homes surveyed exceeded the United States Environmental Protection Agency (EPA) action level of 148 Bq/m^3 (4 pCi/L). This study indicated that the distribution of radon concentrations in homes could be reasonably described by a log-normal distribution. The overall geometric mean (median) of the radon concentration data was 25 Bq/m^3 with a geometric standard deviation of about 3.1.

33. Information on radon levels in Latin American countries is also becoming available (e.g. [C41, M41, S65]). According to reference [C41], most indoor radon levels in Latin America are below 100 Bq/m^3. However, levels can be quite variable in different regions within a country, as evidenced by data for 17 states in Mexico, which report mean radon levels across the 17 states surveyed ranging from less than 40 Bq/m^3 to near 200 Bq/m^3. The overall mean value reported across the 17 states surveyed was 111.6 Bq/m^3.

Figure I. Distribution of indoor radon levels in homes in the United States (after reference [M1]).



34. A study of indoor and outdoor radon levels in Brazil reported that a variability in radon levels of about 50% in a single day could be measured on an hourly basis, with the highest values in the morning and the lowest values in the afternoon. The authors also reported a seasonal variability of two orders of magnitude in outdoor levels, with the highest levels in the dry winter season and the lowest levels in the wet summer months [M41]. The mean indoor radon concentration within the urban area of Rio de Janeiro is 40 Bq/m^3, while the indoor radon concentrations within urban and rural areas in Poços de Caldas are 61 and 204 Bq/m^3, respectively [M41].

35. Several Japanese studies, including two nationwide indoor radon surveys, showed significant differences [F14, F15, S64]. The first survey of more than 7,000 dwellings, using passive radon detection, reported a mean radon concentration of 20.8 Bq/m^3 [F14]. To investigate potential confounding by thoron, a survey of 900 dwellings used a detector that could discriminate between radon and thoron. The mean radon concentration from the second survey was 15.5 Bq/m^3 [T12]. The authors noted a gradient in thoron concentration in the homes, the concentration decreasing by nearly a factor of 2 with increasing distance, up to about 1.5 m, from walls made of soil. Radon concentrations are reported as generally homogeneous in rooms, except for places near the inlet and outlet of indoor airflow [Z8]. This result should be considered when choosing locations for detection.

36. In the 1980s and 1990s, two nationwide surveys [R12, W21] of indoor radon decay products were performed in China using various grab sampling measurements (e.g. scintillation cell method and two filter methods) or short-term measurements (2–4 days of activated charcoal adsorption). The arithmetic mean (AM) of the indoor radon concentration was 24 Bq/m^3 and the geometric mean (GM) was 21 Bq/m^3 [U2]. Since 1996, China's housing situation has greatly changed. A new survey of indoor radon in 26 cities and regions during 2001 and 2005 used alpha track detection (exposure period of 3–6 months) [S67]. The AM of indoor radon concentration was 43.8 ± 37.7 Bq/m^3 and the GM was 34.4 ± 1.95 Bq/m^3, with the median of the concentration at 32.9 Bq/m^3. These results are significantly higher than those of previous surveys. The radon concentration in some high-rise buildings exceeds the national action level of 400 Bq/m^3.

37. Traditional soil-brick and mud-wall structure houses are still popular in the countryside of China. At least 100 million people are currently living in such houses. Surveys of thoron and its decay products in Chinese traditional soil structure houses have been conducted. Fan et al. observed a thoron EEC of 4.0 Bq/m^3 (n = 56) in 1991 in Shaanxi soil houses [F20]. More recently a survey using improved alpha track monitors was completed in Guangdong, Gansu, Yunnan, Xinjiang, and Guizhou provinces: thoron and its decay product concentrations (AM) were 318 Bq/m^3 and 3.8 Bq/m^3 (n = 148, maximum value 15.8 Bq/m^3), respectively [S68]. Applying a dose conversion factor of 40 nSv/(Bq h m^{-3}), the average annual effective dose from inhalation of ^{220}Rn was estimated to be in the range 1.1–4.4 mSv. The dose contribution from thoron is obviously significant in this situation.

38. Surveys of radon and thoron decay products were carried out in Fujian province in China, where the natural levels of ^{238}U and ^{232}Th in soil are elevated (about 53.4 Bq kg and 116.8 Bq/kg, respectively) [Z5]. Radon and thoron decay products were measured in homes and various other locations, including outdoors, in mineral processing plants, underground and in railway tunnels. The average radon and thoron decay product concentrations (expressed as EEC) measured in 204 dwellings were 12.9 Bq/m^3 and 0.87 Bq/m^3, respectively. The mean outdoor level of radon decay product concentrations (expressed as EEC) derived from measurements made at 180 locations was 9.69 Bq/m^3. The authors also assessed potential exposure of people living in Fujian province, and concluded that thoron (and decay products) contributed about 20% of the estimated effective dose of 1.28 mSv/a from radon and thoron combined.

39. Measurements of outdoor (1,100 samples) and indoor (1,050 samples) levels of radon and thoron decay products were made at a university campus in northeastern Japan over a period of 4 years [K20]. The authors analysed the data using a variety of statistical methods. A seasonal variation was observed, with higher radon concentrations for autumn and winter, both outdoors and indoors. A seasonal variation of thoron levels was not as clear.

40. Shang et al. [S67] report the results of a countrywide survey of 3,098 homes in China carried out with alpha track detectors between 2001 and 2005 by the Chinese National Institute for Radiological Protection. Measurement sites were statistically distributed in the municipalities (Beijing, Shanghai and Tinjing) and 15 provinces, using a sampling rate of 0.09 in 10,000 houses across the country. The authors report an arithmetic annual average radon concentration of 43.8 ± 37.7 Bq/m^3 and a geometric mean of 34.4 ± 2.0 Bq/m^3, and note that about 6.4% of the measured houses had levels of above 100 Bq/m^3, although only 0.7% were above 200 Bq/m^3. The authors also report that in dwellings made of soil or mud, exposure from thoron may be significant, and that a study of thoron has been designed.

41. Additional data on indoor levels of thoron in Europe and Asia are also reported in reference [S41], which notes that the doses from thoron and its short-lived decay products can be comparable to, or even larger than, the dose from radon and its short-lived decay products. Others also reported data on thoron and thoron decay products for some areas of East Asia (e.g. [T21, Z5]).

42. It was not practical to calculate lung dose directly from thoron gas measurements because the equilibrium factor (F_{eq}) between the gas and decay products was not well established. Past dose estimates for thoron were made mainly from filtered air measurements of the thoron decay product ^{212}Pb. However, much work on methods of measuring radon and thoron and their decay products was carried out in Japan (e.g. [T12, T13, T14, T15, T16, T17, T18, T19, T20, T34, T41, Z3]). A Japanese study [I15] observed that it was important to consider the influence of thoron on the measurement of RDPs. A great deal of work was also carried out in Japan concerning the measurement of radon and thoron and their decay products, including passive and continuous systems, and the measurement of various factors (e.g. F_{eq} and the fraction f_p of decay products attached to particulates) which are important in assessing lung dose [K28, T42, T43, T44, T45, T46, T47, T48). Shang et al. also investigated the effect of thoron on the measurement of RDPs in Chinese cave dwellings [S69]. Since thoron will be present in many homes, if a detector is used that responds to thoron and radon without distinguishing between them, the contribution from thoron will be attributed to radon. For example, in testing the sensitivity of one thoron monitor, the average thoron level in 20 homes in southern India was 168 Bq/m^3 [Z3]. More detailed information on the average thoron levels in these homes is provided in reference [T33].

43. Evaluation of exposure to radon and thoron and their decay products thus must take account of the actual activity concentrations of the various alpha-emitting radionuclides from the two series in the air that is inhaled. As noted previously, the total alpha particle energy yet to be released by the decay of inhaled radon or thoron is reported in terms of potential alpha energy concentration (PAEC), with units of either J/m^3 or WL (working level). This quantity can be calculated once the activities of the individual radionuclides are determined. In most cases, the individual activities are not directly measured. Thus the exposure rate must be indirectly determined using assumptions on concentration ratios, i.e. equilibrium factors, which lead to the determination of the EEC. The environmental factors that influence concentration ratios in each of the radioactive series are of great significance for assessments of both exposure and dose [M33, U2].

44. Many measurements of RDPs have been reported. These suggest that a rounded value for the equilibrium factor of 0.6 may be appropriate for the outdoor environment [U2]. Ramachandran and Subba Ramu [R6] reported variations in indoor equilibrium factors. The UNSCEAR 2000 Report also noted that there is a wide range of values from individual measurements. This is understandable given the many environmental factors, including exhalation rates and atmospheric stability conditions, that influence the various activity ratios. The range of the equilibrium factor for outdoor radon is from 0.2 to 1.0, indicating a high degree of uncertainty in the application of a typical value to derive EECs [U2].

45. Measurements of both thoron and radon gas using passive alpha track detectors have been reported (e.g. [B16, D11, G13, I8, I9, L37]). Measurements were obtained over a 2-year period for both thoron gas and its ^{212}Pb decay product in four locations — three indoors and one suburban outdoor location [C22, H20, H21]. Tokonami et al. [T48] provide information on the contribution from thoron for several radon detectors. The authors also provide a comparison of small indoor surveys in the Gunna prefecture in Japan and in Kovagoszlos, Hungary. The average radon/thoron ratio from the Japanese survey was 1.3, compared with a mean of 4.5 in the Hungarian survey. The authors concluded that measurements without discrimination of radon isotopes have the potential to affect risk estimates. Sugino, Tokonami and Zhuo [S70] report radon and thoron concentrations in offices and dwellings of the Gunna prefecture. The average radon concentrations in offices were about 29 Bq/m^3, higher than the 17 Bq/m^3 reported for dwellings. As for reference [T48], the mean ratio of thoron and radon was estimated as approximately 1.3.

46. The UNSCEAR 2000 Report [U2] assumed average equilibrium factors obtained from ^{212}Pb/^{220}Rn ratios of 0.003 outdoors and 0.02 indoors to derive estimates of a dose conversion factor for thoron EEC and recommended a value of 40 nSv/(Bq h m^{-3}) for dose estimation.

47. Determinations of the equilibrium factor for radon indoors generally confirm the typical value of 0.4 previously assessed by the Committee [U5, U6]. While indoor measurements show a range from 0.1 to 0.9, most are within 30% of the typical value of 0.4 [H22, R6]. A study [H22] in seven North American houses showed that the equilibrium factor varies significantly with time, typically by a few tens of per cent. Measurements were carried out over a 4-year period to gain an understanding of the characteristics of radon and its decay products in air-conditioned office buildings in Tokyo, Japan. The equilibrium factor was evaluated during working

hours and over the whole day in this survey; there was little difference between the two conditions. These values were within 30% of the assumed typical value of 0.4 [T34]. Although the measurement of radon gas concentration may be a surrogate for direct measurement of the decay product concentration in the determination of exposure, EECs or PAECs estimated using this assumed typical value may be in error, frequently by several tens of per cent, though rarely by as much as a factor of 2.

48. More caution should be exercised in assuming the average values of the equilibrium factor for dose assessment from inhalation of thoron decay products. An objection to the use of thoron gas measurements for dosimetric purposes is that thoron may not be well mixed in the indoor air because of its short half-life. As indicated previously, some data indicate that indoor thoron concentrations vary with the distance from walls and floors [Z8]. In many samples, the thoron concentrations in the centre of the room or more than 1 m from the surface of building material containing ^{224}Ra were as low as in outdoor air, while the thoron concentration near the surface of the building material was more than 10 times that in the centre of the room. Only where a room fan is used would thoron be well mixed and a large variation of the thoron concentration in the room not be found [M40].

49. Equilibrium factors for estimating ^{220}Rn EEC are given above as single values. Because of the large spatial variations in thoron concentrations in a room, these single values should be regarded as being subject to large uncertainties. Thus the use of an equilibrium factor for thoron should be limited to situations where large spatial variation is not found. On the basis of the results of simultaneous measurements of thoron concentration and thoron EEC by long-term passive methods, Yonehara et al. were unable to find a good correlation between thoron concentration and the EEC [Y9]. However, Yamada et al. were able to find good agreement in a study of 265 cave dwellings [Y10].

50. Although only a limited number of measurements of thoron in indoor air are available, several investigations reported both radon and thoron EECs. While acknowledging the uncertainty noted earlier, this allows some generalizations to be made from the derived ratios of the radon and thoron EECs. On the basis of the physical characteristics of radon and thoron, model entry rates to buildings and a ventilation rate of 0.7 h^{-1}, the International Commission on Radiological Protection (ICRP) estimated expected concentrations in buildings [I5], which in terms of EEC are 2–50 Bq/m^3 for radon and 0.04–2 Bq/m^3 (mean = 0.5 Bq/m^3) for thoron. This corresponds to a thoron/radon EEC ratio of 0.03 [U2].

51. Table 1 provides a summary of concentrations of radon in indoor air determined from surveys. Many of the data in table 1 are from annex B of the UNSCEAR 2000 Report [U2]. Data carried forward from reference [U2] are indicated as such. Original sources of data in [U2] are from UNSCEAR surveys of Natural Radiation Exposure and literature as cited in [U2]. Since the publication of [U2], the Committee has carried out three surveys of the natural radiation environment, in 2001, 2004 and 2006. Summary results from these surveys are provided in table 1. Not surprisingly, many of the indoor radon data were collected for areas or regions where indoor radon levels were thought to be elevated. Thus, where the survey results provided to the Committee make such a judgement possible, a note is given in the last column of table 1 indicating whether the data provided in the table may be considered as some form of national average or whether they are more indicative of a regional or local area of elevated levels. In some instances the national authorities provided this judgement, while in others the judgements were made by the Committee. In all instances, precedence was given to data provided to the Committee, as opposed to literature values. The data in table 1 show considerable variability both within countries and from country to country, with, for example, reported nominal geometric mean indoor levels ranging from <10 Bq/m^3 in Egypt and Cuba, to more than 100 Bq/m^3 in a number of European countries, and to above 600 Bq/m^3 in parts of the Islamic Republic of Iran.

52. The UNSCEAR 2000 Report [U2] gives worldwide arithmetic mean values of 46 Bq/m^3 (unweighted) and 39 Bq/m^3 (population-weighted). Worldwide geometric mean values of 37 Bq/m^3 (unweighted) and 30 Bq/m^3 (population-weighted) with corresponding geometric standard duration of 2.2 (unweighted) and 2.3 (population weighted) are also presented in [U2]. Given the wide variety and disparity of data currently available, no attempt was made to update the nominal values from [U2], and the values provided in [U2] are retained for the purposes of this report.

53. The UNSCEAR 2000 Report [U2] gives an annual per caput dose estimated at 1.15 mSv from exposure to natural sources of radon. This value is still appropriate. The UNSCEAR 2000 Report also gives an annual dose of 0.1 mSv from natural sources of thoron [U2]. While this value is still reasonable, data collected for the present study indicate that the levels of thoron (and hence doses from exposure to thoron and its decay products) are highly variable and that thoron may provide a larger contribution to natural background dose than previously thought. Doses from radon and thoron represent approximately half of the estimated dose from exposure to all natural sources of ionizing radiation.

Table 1 Concentrations of radon in indoor air

Region/country	*Population (10^6)*	*Indoor radon (^{222}Rn) (Bq/m^3)*				*Notes*
		Arithmetic mean	*Geometric mean*	*Maximum value*	*Geometric standard deviation*	
Africa						
Algeria [U2]	28.78	30		140		–
Egypt [U2]	63.27	9		24		–
Ghana [U2]	17.83			340		–
North America						
Canada [U2]	29.68	34	14	1 720	3.6	
Canada [L47]	32.27	28.35	11.2	1 720	3.9	
Mexico	107.03	140	90	1 193		[M39]
United States [U2]	269.4	46	25		3.1	National survey [M1, U18]
South America						
Argentina	38.75	35	25	211	2	Countrywide average[e]
Brazil	186.40	81.95		310.0		[C41]
Chile [U2]	14.42	25		86		–
Cuba	11.20	7.7	5.2	15.3	3.3	Countrywide average[f]
Ecuador		200				[Z11]
Paraguay [U2]	4.96	28		51		–
Peru	27.97	32.29		50.20		[C41]
Venezuela	26.75	52.50		346		[C41]
East Asia						
China	1315.84	43.8	34.4	596		National average based on sampling 3098 dwellings [S67]
China [U2]	1 232	24	20	380	2.2	–
Hong Kong SAR [U2]	6.19	41		140		–
Taiwan	22.89	10.0	8.5	63.5	0.6	Countrywide average[f]
India [U2]	944.6	57	42	210	2.2	–
Indonesia	213.67	35.1	35.1	165	1.2	Countrywide average[e]
Japan [U2]	125.4	16	13	310	1.8	–
Kazakhstan	14.83			5 000		Countrywide average[e]
Republic of Korea	48.85	53.4	43.3	1 350	1.8	Countrywide average[e]
Malaysia [U2]	20.58	14		20		–
Pakistan [U2]	140.0	30		83		–
Philippines	75.90	23	22	62	1.13	Countrywide average[e]
Philippines	76.57	23	23	62	±6	Countrywide average[g]
Russian Federation		50–60				[Z11]
Thailand [U2]	58.7	23	16	480	1.2	–

Region/country	*Population (10^6)*	*Indoor radon (^{222}Rn) (Bq/m^3)*				*Notes*
		Arithmetic mean	*Geometric mean*	*Maximum value*	*Geometric standard deviation*	
West Asia						
Armenia [U2]	3.64	104		216	1.3	
Islamic Republic of Iran	63.76	82		3 070		Countrywide average
Islamic Republic of Iran	795	2 745		31 000		High-background areas
Islamic Republic of Iran		600		1 000		High-background areas
Kuwait	1.69 [U2]	14 [U2]	10.6	119.2	0.74	Countrywide average
Palestine (Gaza) [Y7]	0.95	34		105		–
Saudi Arabia [A25]		16		36		–
Syrian Arab Republic [U2]	14.57	44		520		
North Europe						
Denmark	5.2	59[a]	39[a]	1 200	2.2	Countrywide average
Estonia [U2]	1.47	120	92	1 390		
Finland	5.2	120	84	20 000[d]	2.1	Countrywide average
Iceland	0.3	10		26		Countrywide average[g]
Lithuania	3.73	49	38	1 900		Countrywide average
Lithuania	3.49	55	36.5	636		Countrywide average
Norway [U2]	4.35	73	40	50 000		–
Sweden	8.88	108	56	84 000		Countrywide average
West Europe						
Austria [U2]	8.11		15	190		–
Belgium	10.22	48	38	12 000	2	Countrywide average
France [U2]	58.33	62	41	4 690	2.7	–
		89.3	53.5	4 964		Population-weighted mean of 63 Bq/m^3 based on 12,261 measurements in dwellings
Germany [U2]	81.92	50	40	>10 000	1.9	Countrywide average
Ireland	3.84	89	57	7 000	2.4	Countrywide average
Liechtenstein	0.03	80		1 098		*e*
Luxembourg	0.22	110	70	2 500	2.0	Countrywide average
Netherlands [U2]	15.58	23	18	380	1.6	–
Switzerland	6.71	75	41	10 000		Countrywide average
Switzerland		142[b] 73[c]	81[b] 59[c]	15 000[b] 15 000[c]	2.6[b] 1.8[c]	Countrywide average
United Kingdom [U2]	58.14	20	14	17 000	3.2	[C26]
England [G10]		90	50			Average (20 Bq/m^3) population-weighted average
Wales [G10]		84	48			

Region/country	*Population (10^6)*	*Indoor radon (^{222}Rn) (Bq/m^3)*				*Notes*
		Arithmetic mean	*Geometric mean*	*Maximum value*	*Geometric standard deviation*	
East Europe						
Belarus		31.8		221		Countrywide average
Bulgaria	8.10		22	250	2.1	Countrywide average
Czech Republic		118	94.4	70 000	1.84	Countrywide average
		442		20 000		High-background area
		214		20 000		High-background area
		124		70 000		High-background area
		112		20 000		High-background area
		136		6 000		High-background area
		214		6 500		High-background area
Hungary [U2]	10.05	107	82	1 990	2.7	–
Poland	38.12	49.1		1 300		Countrywide average[e]
Poland	38.17	49	31	3 260	2.3	Countrywide average[g]
Romania	22.55	25.0		564		Countrywide average[e]
Slovakia [U2]	5.35	87		3 750		–
South Europe						
Albania [U2]	3.40	120	105	270	2.0	–
Croatia [U2]	4.50	35	32	92		–
Cyprus [U2]	0.76	7	7	78	2.6	–
Greece [U2]	10.49	73	52	490		–
Greece		55	44	1 700	2.4	Countrywide average[g]
Italy [U2]	57.23	75	57	1 040	2.0	–
Italy	57.3	70	52	1 036	2.1	Countrywide average[g]
Montenegro (Yugoslavia)	0.60	184	110	1 128	2.74	Countrywide average[e]
Portugal [U2]	9.81	62	45	2 700	2.2	–
Slovenia [U2]	1.92	87	60	1 330	2.2	–
Spain	36.72	90.38	45.69	15 400		Countrywide average[e]
Spain	0.001	748.5	242.64	15 400		High-background areas[e]
Spain	40.84	90.4	45.7	15 400	2.9	Countrywide average[g]
Spain	0.02	610.0		1 400.0		High-background areas[g]
Oceania						
Australia [U2]	18.06	11	8	420	2.1	–
New Zealand	3.81	21.5	19.5	80		Countrywide average[e]

[a] Population-weighted average.
[b] Upper value, unweighted data.
[c] Lower value, data weighted for floor dependence, population distribution, error of exposition and mobility.
[d] Annual mean.
[e] Data from *UNSCEAR Global Survey on Exposures to Natural Radiation Sources (2001-2006)*, submitted in 2001.
[f] Ibid., submitted in 2004.
[g] Ibid., submitted in 2006.

C. Workplaces

54. The spectrum of workplaces other than mines where radon can present a hazard is large. While it includes below-ground workplaces such as subways, tunnels, underground parking, stores, caves, spas and closed-out mines open to visitors, the majority of such workplaces, such as factories, shops, schools and offices, will be above ground.

55. Other workplaces where large quantities of materials with elevated radium concentrations are stored or processed, for example phosphate fertilizer production [R11] or monazite sand mining, can similarly exhibit elevated radon and thoron levels [A30, C42]. One study [S71] reporting on radon and thoron concentrations in the monazite production area of a rare earth facility in Kerala, India, found radon levels to be below the detection level of about 1.7 Bq/m^3 (see reference [S72] for discussion of monitoring techniques), but the corresponding thoron levels were 5.9 kBq/m^3.

56. Underground workplaces, including mines other than uranium mines, especially coal mines (e.g. [B17, J8, S43, U16, V5]), can accumulate high radon levels in the same way as natural caves or abandoned mines. High radon levels in underground workplaces will not be limited to only those areas where elevated levels were found in above-ground workplaces. The experience of the Newfoundland fluorspar miners illustrates this. The levels of ^{226}Ra in the host rock were low, but the miners were exposed to elevated levels of radon and short-lived decay products arising from radon in the groundwater, which entered the mine and subsequently the mine atmosphere (e.g. [A14, M16, M17]).

57. Exposure to environmental sources of radon from mining and mineral processing is common, and the inhalation of RDPs can be a significant exposure pathway. For workers involved in the nuclear fuel cycle, radon exposure from mining and milling is a relatively important contributor to the per caput dose.

58. The total number of workers exposed to human-made sources and enhanced natural sources was given in the UNSCEAR 2000 Report [U2] as 11.1 million. Approximately 4.6 million workers were exposed to human-made sources at an annual average (effective) dose of about 0.6 mSv. Some 6.5 million workers were exposed to enhanced natural sources at an annual average dose of about 1.8 mSv, of which approximately half was from radon. The estimate for radon in above-ground workplaces (for example in the phosphate industry) is still considered to be crude.

59. In the case of occupational exposure, there are several situations where workers who have the highest radiation doses receive a significant contribution from radon. These include the situations listed in table 2 (from reference [U2]). For workers involved in nuclear power production, those involved in the mining of uranium typically receive the highest collective doses; a significant part of that exposure is from radon inhalation. The group of workers in the category of above-ground workplaces (see table 2) are the second largest group identified in annex B of the UNSCEAR 2000 Report [U2]. These workers were estimated to receive an average annual effective dose of 4.8 mSv. This is the largest average annual dose received by any type of worker and was due entirely to radon. According to the UNSCEAR 2000 Report [U2], radon inhalation is also a significant contributor to the doses to the other categories of workers in table 2. While information to fully update table 2 is not available, a paper by Liu et al. [L46] indicates that there are about 6 million miners in Chinese coal mines alone, which nearly doubles the number of coal miners reported in reference [U2].

Table 2 Situations where doses from radon are significant [U2]

Source/practice	*Number of monitored workers*	*Average annual effective dose (mSv)*
Nuclear fuel cycle (including uranium mining)	800 000	1.8
Mining (other than coal and excluding uranium mining)	760 000	2.7
Coal mining[a]	3 910 000	0.7
Mineral processing	300 000	1.0
Above-ground workplaces (radon)	1 250 000	4.8

[a] See paragraph 59.

D. Measurements of radon and radon decay products

60. It is well established that the inhalation of the short-lived decay products of radon (^{222}Rn) and their subsequent deposition along the walls of the various airways of the bronchial tree are the main pathways of radiation exposure of the lungs [U2]. As discussed elsewhere in this report, the lung exposure arising from the decay products of thoron (^{220}Rn) is also of increasing interest. Traditionally, the potential alpha decay energy per litre of air (referred to in units of working level month (WLM)) was the measure of exposure to RDPs used in evaluations of exposures in mines. Miner epidemiological studies use data based on measurements of this type. Later, time-integrated radon measurement techniques were

developed and are the method of choice for modern studies of residential radon. The discussions of each miner cohort (section IV) or residential case–control (section V) epidemiological study include a brief description of the methods used for measuring radon and its decay products; in addition, a few general comments are provided below. Further information is available in published reports (e.g. NCRP Report No. 97 [N15]).

61. Various techniques were used in the past to assess residential radon exposure, including: instantaneous grab samples and subsequent analysis in a scintillation cell; accumulation on a charcoal absorber with subsequent gamma spectroscopy; various solid state detectors; and a variety of other techniques, including continuous measurements [I12, N15]. Overall, long-term track etch radon measurements are widespread and are almost universally used for residential epidemiological studies. In recent years, increasing attention was given to measurements that assist in the reconstruction of past exposures, including the measurement of polonium (^{210}Po) activity on glass surfaces or in volume traps (e.g. [F1, F9, L31, M33, N11, N15, P15]). Bochicchio et al. [B41] describe a comprehensive quality assurance programme for radon measurements carried out as part of a residential radon case–control study.

62. A paper on the use of (outdoor) radon levels in meteorology provides additional information on environmental radon monitoring and experimentally available lower limits of detection [Z7]. Tokonami et al. [T42] conducted an intercomparison of measurement methods for radon, RDPs and particle size using a radon/aerosol chamber. The authors report good agreement (typically within $\pm$5%) across institutions and methods for continuous radon monitoring. However, the ratios of the radon concentrations measured by various institutions to those measured by alpha track detectors ranged from about 0.71 to 1.34. The authors noted that these types of detector (alpha track detectors) are often used in surveys and that quality control is always needed.

63. Much of the miner epidemiology is based on exposures characterized by relatively short-term (grab) measurements of WL in one or more areas of a mine (combined with an evaluation of the hours worked in the same location). According to reference [L13], the first radon gas measurements in United States mines were in 1949, and the first RDP measurements were in 1951. In 1973, the American National Standards Institute (ANSI) published a consensus standard for radiation protection in uranium mines in the United States [A22]. According to this standard, a monitoring system for RDPs must be capable of measuring the annual accumulated exposure in WLM within an uncertainty interval of 50% at the 95% confidence level. To satisfy the ANSI criterion, uranium mining companies in the United States adopted a procedure based on periodic measurements of the air concentration of RDPs. All of the measurement methods in routine use at the time involved drawing a known volume of air through a filter. After a specified time delay, the activity on the filter was analysed. The concentration of short-lived RDPs, in units of working levels (WL), was calculated from the measured activity on the filter. The most common method was that of Kusnetz [K23], who devised a procedure for estimating WL from a single alpha count. (The Kusnetz method generally involves a 5-minute sample of air drawn through a glass fibre filter paper at a rate of 1–2 L/min; the filter paper is counted for total alpha activity after a delay of 40–90 min and the concentration in WL is determined from the total alpha count using a correction factor based on the decay time [K23].) For control purposes, a single measurement was used to establish whether or not a work area was safe for occupancy by miners. For routine dosimetry, the concentration estimated from the grab sample was assigned to the appropriate work area. As discussed later in section IV, similar practices were followed in many uranium mines outside the United States.

64. Twelve grab sampling methods for measuring airborne radon decay product concentrations were analysed by Schiager et al. [S56] to determine whether they would satisfy the ANSI standard [A22]. The evaluations considered six independent sources of uncertainty that together, according to reference [S56], comprised the overall uncertainty of the method. The results of their evaluation are summarized in table 3.

Table 3 Uncertainties in grab sampling (adapted from reference [S56])

Nature or source of uncertainty	*Total uncertainty (Percentage of measured value)*
Variations in airborne RDP concentrations	36
Inherent errors of the method	4–20
Precision based on counting statistics (dependent on concentration)	1.4–9.3[a]
Human error	1–4
Estimation of occupancy time	4
Record-keeping and data transcription	1.5
Overall uncertainty	37–41

[a] Range for 10 out of 12 methods analysed at 0.3 WL.

65. For comparing the uncertainties involved in different grab sampling methods, Schiager et al. [S56] assumed that all of the sources of uncertainty were independent, multiplicative and normally distributed. The total relative uncertainty in an individual measurement was estimated, by conventional propagation of error techniques, as the square root of the sum of the squares of the individual relative uncertainties. The result was an approximation, since the shape of the distribution of errors was not determined. In spite of the limitations of the analysis, Schiager et al. [S56] determined that the estimated uncertainties are entirely adequate to indicate the nature and magnitude of the various sources of potential errors and the adequacy of the sampling methods used for meeting the ANSI criterion.

66. Makepeace and Stocker [M35] presented a statistical analysis of WL measurements in Canadian uranium mines. No attempt was made to establish the accuracy or precision of the individual measurements. A total of 2,427 observations were obtained at 33 mine locations. The number of observations per location varied from 22 to 188, and the sampling periods ranged from 4 to 12 days. The combined data at each location had a coefficient of variation ranging from 5 to 95%, with an arithmetic mean value of 30%, consistent with the ANSI standard. Francis et al. [F18] evaluated the effects of autocorrelation on workers' daily exposures and concluded that sampling programmes that rely on measurement on consecutive days (a possibility in the uranium mine environment) can result in biased estimates of exposure if autocorrelation is present. They suggested a random sampling strategy be employed where day-to-day correlation is high.

67. The possible role of exposure to thoron and its decay products is of increasing interest, and a number of authors report that the contribution of thoron and its decay products can be a significant component of the total exposure (radon plus thoron); thoron can thus be a source of error in residential radon studies that do not distinguish the two contributions to exposure (e.g. [C21, T11, T17]). Future measurement studies should therefore consider the contribution of both radon and thoron.

II. DOSIMETRY

68. The health risk associated with radon arises from the inhalation of the short-lived decay products and the consequent dose to critical cells of the respiratory tract. Estimates of the absorbed dose to the critical cells of the respiratory tract per unit radon exposure can be derived from an analysis of information on aerosol size distribution, unattached fraction, breathing rate, fractional deposition in the airways, mucous clearance rate and location of the target cells in the airways. Such estimates are model-dependent and necessarily subject to all the uncertainties associated with the input data as well as with the assumptions built into the particular model. The dose calculation procedure and assumptions are described in the UNSCEAR 2000 Report [U2]. The magnitude of the risk from exposure to RDPs was quantified in epidemiological studies of the increased rate of lung cancer among uranium mine workers and more recently in residential case–control studies.

69. ICRP Publication 65 [I2] recommended the use of the risk factors determined from epidemiological studies of uranium miners as the preferred method for converting RDP exposure to effective dose. Since that time, as discussed in section VI, numerous case–control studies of residential exposure to radon suggest risk factors that are generally consistent with those from miner epidemiology. These case–control studies provide direct evidence of risk from residential radon, removing the dependence on dosimetric adjustments from conditions in mines to conditions in homes. Thus, as discussed in section V, the assessment of risks from exposure to radon in the home can now be based on the evidence from residential case–control studies.

70. The ICRP has provided guidance on a dose conversion convention for radiation protection purposes [I2]. For radon, the ICRP recommends the RDP conversion convention that all exposures are combined on a dose- and risk-equivalent basis. The use of a single value for the dose conversion factor implicitly assumes: *(a)* that the distribution of RDP particle size for occupational exposure, in particular the fraction and particle size of the ultrafine mode, is not too different from the particle size distribution in uranium mines; and *(b)* for exposures of the general public, that the differences in aerosol conditions are offset by lower breathing rates for members of the public, particularly children. The current ICRP conversion convention is not applicable to thoron decay products.

A. Dosimetric models

71. Dosimetric models can be used to estimate the radiation doses arising from the inhalation of airborne radioactive material. Such models incorporate data on respiratory tract deposition, including data on deposition of ultrafine particles in nasal passages (e.g. [H22, I3, I13, J1, J2, N8, N10]). These models, widely used for the assessment of dose for most inhaled radionuclides, show that the dose per unit intake of RDPs is dependent upon the site of deposition within the respiratory tract. The site of deposition in turn is strongly dependent on the particle size of the airborne RDP, particularly for those particles of below 10 nm diameter (typically the “unattached fraction” of the RDP, i.e. the fraction not attached to ambient particulates). A weighted dose conversion factor for a particular exposure location can be derived from measurements of RDP size distribution combined with the particle-size-dependent dose conversion factors, calculated using one of these dosimetric models.

72. Various techniques are used to measure size distributions of RDPs associated with fine (5 nm) and ultrafine (0.5 nm) particles. These are extensively reviewed elsewhere (e.g. [N8]). Many of these methods rely on fractionation of the sub-micrometre radioactive particles by diffusion processes, using sets of wire screens, or by inertial and impaction processes, using cascade impactors. The latter systems are also capable of resolving particle sizes exceeding 1 μm. These systems have been used to measure RDP size distributions in homes, workplaces and uranium mines [G1, G2, R7, S44, S57, T23, T25]. A simpler technique involving the measurement of the unattached fraction is widely used [H23]; this method relies on the separation of the ultrafine and accumulation modes (particles in the size range 50–500 nm, which grow on inhalation, affecting the pattern of deposition in the lungs) using a diffusion sampler, usually a single wire screen. When used with the optimized configuration, a system with a set of wire screens can measure size distributions in both modes concurrently [F16, F17]. Tokonami et al. [T49] describe two methods of measuring the particle size distribution of RDPs and report data measured in a mine in Japan, as well as in a well-controlled radon chamber.

B. Dosimetry of radon and thoron

73. A number of researchers have used the ICRP dosimetry model to assess the doses from radon and thoron and their decay products (e.g. [B35, I7]). The ICRP advises against using the human respiratory tract model for risk estimation [I3].

74. Tokonami et al. [T36] report measurements of physical parameters related to dose assessment in an actual room where environmental conditions such as ventilation rate, air-conditioning and the operation of an air cleaner were varied. Using the data and the ICRP Publication 66 respiratory tract model, dose conversion factors were calculated under different situations and compared with each other. The paper investigated the wide variation of dose conversion factors with environmental conditions and concluded that the most sensitive parameter is the unattached fraction of decay products (f_p).

75. Other dosimetric models have also been described [H3, N10], and current models are relatively consistent in their dose estimates, particularly in showing a strong dependence of the radiation dose per unit intake on the size of the inhaled radioactive particles (aerosol in the range 0.8 nm to >1 μm). The publication of James [J5] contains an illustration of the dose conversion factors according to various models.

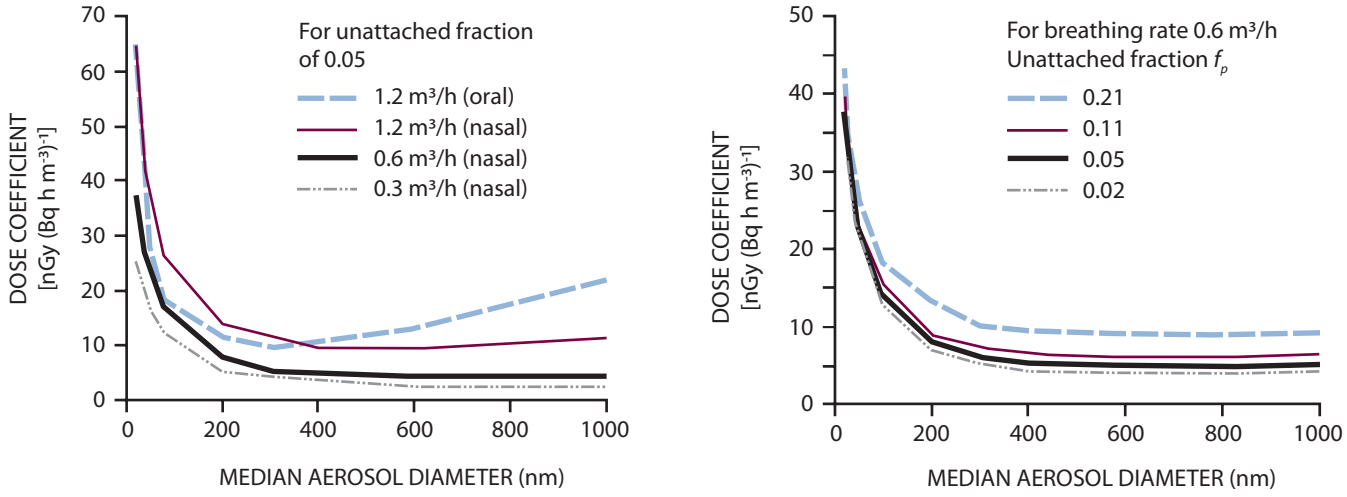
76. The UNSCEAR 2000 Report [U2] listed, in table 26 of annex B, the principal dosimetric assessments for the lung dose from deposited RDPs. For convenience, this table is reproduced here as table 4. Graphs of the dose coefficient as a function of median inhaled aerosol diameter, breathing rate and unattached fraction are reproduced from reference [U2] in figure II. The current RDP dosimetric models use the standard values for tissue and radiation weighting factors to convert tissue dose to effective dose. The effective dose estimates vary, but are within a factor of 3 higher than the estimates derived from the epidemiological approach used by the ICRP conversion convention. Considering the uncertainties in both the epidemiological and dosimetric approaches, this agreement is remarkable.

Table 4 Principal dosimetric assessments of lung dose from deposited RDPs [U2]

Year	*Investigator*	*Parameter values*		*Target region*	*Model type*	*Dose factor*[a] *[nGy(Bq h m^{-3})$^{-1}$]*
		Unattached fraction	*Breathing rate (m^3/h)*			
1956	Chamberlain and Dyson [C1]	0.09	1.2	Average in 45 μm epithelium	Cast of trachea and bronchi	11
1959	ICRP [I4]	0.1	1.2	Mean tracheobronchial region	Deposition retention assumptions	6.7
1964	Jacobi [J1]	0.25		Basal cells (30 μm)	Findeisen–Landahl 6-region anatomical model	24
1964	Altshuler et al. [A3]	0.085	0.9 basal	Cells (22 μm)	Findeisen–Landahl 6-region anatomical model	32
1967	Haque and Collinson [H3]	0.35	Basal	Cells (30 μm)	Weibel dichotomous airway model	71
1972	Harley and Pasternack [H5]	0.04	0.9 basal	Cells (22 μm)	Weibel dichotomous airway model	5.7
1980	Jacobi and Eisfeld [J2]	0.1	1.2	Mean epithelium	Weibel dichotomous airway model, correction for upper airway turbulent diffusion [M2]	8.9
1980	James et al. [J6]	0.1	1.2	Mean epithelium	Yeh–Schum anatomical model [Y2]	14
1982	Harley and Pasternack [H6]	0.07	1.1 basal	Cells (22 μm)	Same as Jacobi and Eisfeld [J2]	6.4
1982	Hofmann [H10]	0.2	0.9	Mean epithelium	Same as Jacobi and Eisfeld [J2]	11
1991	National Research Council [N10]	0.16	1.2 basal	Cells (35–50 μm)	Yeh–Schum anatomical model [Y2], correction for upper airway turbulent diffusion	21
1996	Harley et al. [H4]	0.1	1.2 basal	Cells (27 μm)	Nikiforov and Schlesinger [N12] anatomical model, airway deposition from empirical data from human airway casts	9
1998	Marsh and Birchall [M2]	0.08	0.8	Bronchial cells: basal (35–50 μm), secretory (10–40 μm) Bronchiolar cells: secretory (4–12 μm)	ICRP lung model [I1]	8.5 19 14

[a] Per unit ^{222}Rn concentration (EEC). WLM converted to Bq h m^{-3} using 0.27 × 10^{-3} WL (Bq/m^3)$^{-1}$ and 170 h per working month.

Figure II. Absorbed dose in bronchial epithelial cells per unit exposure to radon decay products as a function of aerosol size [U2].



77. It is not possible to assess the radiation dose from inhalation of thoron decay products by epidemiological means, and therefore it must be estimated using dosimetric modelling. In annex A of the UNSCEAR 2000 Report [U2], a conversion factor for thoron decay products of 40 nSv (Bq h m^{-3}) was used. According to reference [U2], this value was intended to include the dose to organs other than the lungs due to the transfer of ^{212}Pb from the lungs. Table 5 provides a summary of the principal dosimetric assessments of lung dose from deposited thoron decay products and supports the continued use of a conversion factor of 40 nSv (Bq h m^{-3})$^{-1}$. Marsh and Birchall [M42], in a comment on the review of thoron dosimetry issues [N16], report thoron dose conversion factors based on the latest ICRP biokinetic models and provide a range of dose conversion factors from 1.1 mSv/WLM to 3.8 mSv/WLM, which encompasses the full range of previously estimated thoron dose conversion factors.

Table 5 Principal dosimetric assessments of lung dose from deposited thoron decay products

Year	*Investigator*	*Parameter values*		*Target region*	*Model type*	*Effective dose[a] [nSv(Bq h m⁻³)⁻¹]*
		Unattached fraction ^{212}Pb	*Breathing rate (m³/h)*			
1956	Chamberlain and Dyson [C1]	0.02	0.3	Average in 45 μm epithelium	Cast of trachea and bronchi	30
1959	ICRP [I4]	0.02	1.2	Mean tracheobronchial region	Deposition retention assumptions	43
1973	Harley and Pasternack [H31]	0.02	0.9	Basal cell generations 2–15	Weibel	43
1980	Jacobi and Eisfeld [J2]	n.a.	1.2	Basal cell generations 2–15	Weibel	35
				Bronchial from whole lung × 0.06	Weibel	64
1981	ICRP 32 [I10]	n.a.	1.2	Bronchial from whole lung × 0.06	Based on Jacobi and Eisfeld [J2]	73
1982 (1993)	James et al. [J16] (James [J5])	n.a.	1.2	Bronchial basal cell	Birchall–James model using either Weibel or Yeh–Schum [Y2] model	34–103
1983	NEA 1983 [N8]	0.02	1.2	Mean bronchial	Jacobi and Eisfeld [J2]	36
2000	UNSCEAR [U2]	n.a.	1.2	Whole body	ICRP 50 [I5]	40
2001	Porstendorfer [P12]	0.005–0.02	0.75	Bronchial	Modified from ICRP 66 [I3] weighting by basal and secretory cell density	53

[a] Per unit ^{220}Rn concentration (EEC). WLM converted to Bq h m^{-3} using 3.6 × 10^{-3} WL (Bq/m^3)$^{-1}$ and 170 h per working month.

C. Dose conversion factors

78. The health risks from exposure to radon and thoron are principally due to the inhalation of the short-lived decay products and alpha particle irradiation of the bronchial airways. Radon and thoron decay product exposure rates are specified by the measure of potential alpha energy concentration (PAEC), given in units of J/m^3 or working levels (WL), and the equilibrium-equivalent concentration (EEC), given in Bq/m^3. The potential alpha energy concentration is derived from a linear combination of the activities of the short-lived decay products in each radon decay series (see paragraph 122, annex B, UNSCEAR 2000 Report [U2]). The constants in the linear combination are the fractional contributions of each decay product to the total potential alpha energy. The EEC (in Bq/m^3) can be converted to the PAEC by the relationships:

$$1\ \text{Bq/m}^3 = 5.56 \times 10^{-9}\ \text{J m}^{-3} = 2.7 \times 10^{-4}\ \text{WL}\ (^{222}\text{Rn});$$

and

$$1\ \text{Bq/m}^3 = 7.6 \times 10^{-8}\ \text{J m}^{-3} = 3.64 \times 10^{-3}\ \text{WL}\ (^{220}\text{Rn}).$$

79. For occupational exposure to inhaled ^{222}Rn decay products, the ICRP 65 [I2] recommended the use of a single factor (conversion convention) to relate the ^{222}Rn decay product exposure to the effective dose to an individual. This conversion convention is based on a comparison of the risk to a uranium miner, based on epidemiological studies, with the risk to a radiation worker from an effective dose of 1 Sv, in other words, comparison of the radiation detriment coefficient (risk per unit dose) with the miner detriment (risk per PAEC exposure). For worker exposure, this factor is 1,430 mSv (J h m^{-3})$^{-1}$ (rounded to 1,400), 5.06 mSv/WLM (rounded to 5 mSv/WLM), or 7.95 nSv (Bq h m^{-3})$^{-1}$ (rounded to 8 nSv (Bq h m^{-3})$^{-1}$) EEC (tables 7 and 8, ICRP Publication 65 [I2]).

80. In recommending that a similar approach be used for members of the public, ICRP Publication 65 [I2] assumed that the lung cancer risk per unit exposure in a home was the same as that in an underground uranium mine, in order to derive a conversion factor for members of the public. Since the detriment coefficient for the public is greater than for workers (7.3% Sv^{-1} against 5.6% Sv^{-1}), the derived conversion convention for members of the public was calculated to be 1,100 mSv (J h m^{-3})$^{-1}$, or 3.88 mSv/WLM (rounded to 4 mSv/WLM), or 6.1 nSv (Bq h m^{-3})$^{-1}$ EEC.

81. In developing ICRP Publication 65 [I2], data from epidemiological studies of seven miner cohorts were used to derive a central estimate of the excess relative risk (ERR) of exposure to RDPs. The ICRP (table A.2 of reference [I2]) estimated a mean ERR coefficient of 3.79 (J h m^{-3})$^{-1}$ (1.34% WLM^{-1}), by averaging the results from the seven selected cohorts, weighted according to person-years of risk for each cohort. Lowe and Chambers [L7] considered three additional epidemiological studies (the Port Radium uranium miners, the Chinese tin miners and the Newfoundland fluorspar miners, as discussed in section IV) that were not included in ICRP's calculations, though cited in them. Their inclusion yielded a mean ERR coefficient of 3.08 (J h m^{-3})$^{-1}$ (1.09% WLM^{-1}), about 80% of the 3.79 (J h m^{-3})$^{-1}$ (1.34% WLM^{-1}) value estimated by the ICRP.

82. Alternative methods to ICRP's weighting by person-years can also be used to arrive at a mean risk estimate. One alternative considered by Lowe and Chambers [L7] was based on the confidence interval (CI) associated with each estimate: in this approach, the estimates with the least assigned uncertainty are given the most weight in estimating an overall mean. The method of combining values with different CIs was to weight each value by the inverse of its variance. It was assumed that the 95% CI about each estimate approximated to a range of ±2 standard deviations about the estimate, and that the variance was the square of the standard deviation. This weighting method gave a mean ERR of 0.73 (J h m^{-3})$^{-1}$, or 0.26% WLM^{-1}.

83. Stather [S62], in a discussion of the dosimetric and epidemiological approaches, arrives at a similar conclusion and suggests that the difference of about a factor of 3 is "surprisingly good". The Committee agrees with this view and simply notes that the calculated doses are in reasonable agreement with risk factors derived from epidemiology, uncertainties in both approaches considered.

D. Uncertainties in dose conversion factors

84. Uncertainties are present in both epidemiological (see sections IV and V) and dosimetric approaches, and the dosimetry of inhaled RDPs is quite complex and depends on many factors (biological, physical and behavioural). Many authors have reported estimates of doses, including references [A3, C1, H4, H5, H6, H10, J1, J2, J5, J6, N1, Y2, Y3, Y4, Y5], and both sensitivity (e.g. [M2]) and uncertainty analyses (e.g. [M32]) have been conducted. It may be that a single dose conversion factor cannot adequately cover the variety of natural and occupational exposure situations.

85. Marsh et al. [M32] described a detailed parameter uncertainty analysis for the weighted equivalent lung dose (absorbed dose averaged over the lung that is weighted for the relative biological effectiveness of alpha radiation) per unit exposure to RDPs in the home. The authors commented that the ICRP (para. 356 of reference [I3]) recommended that the risks from residential radon be based on epidemiological studies of miners, from which a conversion factor (effective dose per unit exposure to radon) of 5 mSv/WLM for workers was estimated. In contrast, Birchall and James [B18] calculated a conversion factor of 13.4 mSv/WLM for miners based on the ICRP's human respiratory tract model [I3], a factor of 2–3 times larger than that estimated from miner epidemiological studies. In carrying out the parameter uncertainty analysis, Marsh et al. [M32] assumed a dosimetric tissue weighting factor of 0.12 for the lung and an alpha radiation weighting factor of 20. Their analysis considered various aerosol parameters, target cell parameters,

and parameters such as breathing rate and fraction of breathing through the nose, related to the characteristics of individuals at home. Using the ICRP's weighting factors for exposure at home, the mean ratio of the distribution of millisieverts per working level month was found to be about 15. It was further concluded that a conversion factor of as low as 4 mSv/WLM was extremely unlikely from a dosimetric perspective [M32].

86. In a separate analysis of the physical parameters and (dose) conversion factors for RDPs, Porstendorfer [P12] calculated a range of (dose) conversion factors of 4.2–11.5 mSv/WLM, depending on aerosol concentration and other factors. Homes with higher aerosol concentrations had lower dose conversion factors. Porstendorfer [P12] noted that, while dose conversion factors calculated for homes with "high" aerosol levels were comparable to those derived from miner epidemiological studies, the discrepancy between the dose conversion factors derived from miner epidemiology and dosimetry remained for homes with "normal" aerosol concentrations. Dose conversion factors for exposure to thoron decay products of 2–3 mSv/WLM were also calculated.

87. Nikezic et al. [N13] discussed the importance of the absorbed fraction (of alpha particles in sensitive cells) when estimating dose conversion factors for RDPs. The authors noted that the most important parameter in estimating the absorbed fraction was the depth of the sensitive cell layers. In another paper [N14], the ICRP's human respiratory tract model [I3] and microdosimetric considerations were used to investigate the dose conversion factors for RDPs. The authors concluded that having the alpha particles deposit their energy only in the nuclei of sensitive cells reduced the dose conversion factor from 15 mSv/WLM to about 11 mSv/WLM.

88. All of the above calculations assumed the standard alpha radiation weighting factor of 20. Brenner et al. [B19] suggested that a quality factor of 20 may be too large, on the basis that RDP alpha particles deposit most of their energy in a region of relatively low biological effectiveness, and recommended a value of about 10 for residential radon exposure. The suggestion of Brenner et al. [B19] seems quite reasonable on the basis of their review of the extensive data available at present on the in vitro transformation of cultured mouse cells. However, Brenner's suggestion would also be likely to raise questions at the tissue level, where the cell loss by killing may stimulate cell renewal that eventually promotes the development of cancer (i.e. increasing the weighting factor). In ICRP Publication 92 [I11], the ICRP noted that the current radiation weighting factor (w_R) of 20 for internally deposited alpha emitters can serve as a guideline, and suggested that for specific situations such as exposure to radon and its progeny "more meaningful weighting factors can be derived", whether based on specific assumptions about target cells and dosimetric models or on epidemiology.

89. Little [L21] compared lung cancer risk in the survivors of the atomic bombings in Japan (using data from reference [P13]) and the Colorado Plateau uranium miners (using data from references [R8] and [H9]). Models of ERR were used, and time since exposure, smoking and sex were considered. Little found that, although there are statistically significant differences between the two data sets in how ERR varied with time since exposure, these differences were no longer statistically significant when only male atomic bombing survivors were used as the basis for comparison with the Colorado Plateau miners. Little concluded that the conversion factor based on the atomic bombing survivors was 18 (95% CI: 6.1, 110) mSv/WLM, using a model with exponential adjustments for the effects of radiation with time since exposure and age at exposure, and 19 (95% CI: 6.2, 160) mSv/WLM, using a model with adjustments for the effects of radiation proportional to a power function of time since exposure and attained age. The absence of smoking data for the Japanese atomic bombing survivors was acknowledged to be a potentially important confounding factor. Little's estimates compare with the range developed by Birchall and James [B18] of 17.2–22.5 mSv/WLM (with 95% CI extending from at least two times smaller to at least two times larger) using the ICRP lung model [I3] and with the ICRP's estimate of 5 mSv/WLM [I5] based on the Japanese atomic bombing survivor data and the uranium miner data. Overall, Little concluded that the various estimates of the dose conversion factor are "very close" to that predicted by dosimetry and "statistically compatible" with the epidemiological derivation of the conversion factors [L21].

E. Exposures in homes and in workplaces other than uranium mines

90. Both the BEIR IV and the BEIR VI Committees [C19, C20] used dosimetric models to examine the comparative doses for exposures of miners and people exposed at home. As a follow-up to the BEIR IV report, the United States National Academy of Science compared the dosimetry of radon in mines with that in the home [N10]. This study expressed differences in exposure–dose relationships in terms of a "K-factor", defined in reference [C19] as the ratio of dose per unit exposure at home to the dose per unit exposure to a male miner. Thus, for $K < 1$, the dose per unit exposure at home is less than in a mine, and for $K > 1$, the dose per unit exposure at home is larger than for exposure in a mine. As reported in reference [N10], the K-factors for children and infants, calculated for a wide range of exposure scenarios, were somewhat larger than those calculated for adults, but nevertheless did not exceed 1. BEIR VI [C20] used the concept of the K-factor and included various environmental and physiological factors. BEIR VI reviewed measurements of RDP activity size distributions for data collected in Germany, the United Kingdom and the United States (annex B of reference [C20]). These size distributions were used with the RDP dosimetric model to estimate the K-factor for exposure in homes. The values obtained were close to 1 for male and female adults and for children. James et al. [J15] described the technical basis for the comparative radon dosimetry applied to BEIR VI [C20], including a thorough review of the basis for the BEIR VI

[C20] Committee's choice of unity for the K-factor. James et al. concluded that a K-factor of unity is appropriate, but noted that it is not yet established that the conditions in the houses considered in the BEIR VI analysis are representative of conditions in houses in other regions across the United States (including the effects of seasonal and climatic conditions) [C20].

91. No similar review of RDP activity size distributions has been undertaken for occupational exposure. While it might be expected that RDP exposure in uranium mines would be consistent with the ICRP conversion convention, namely 8 nSv (Bq h m^{-3})$^{-1}$, there are a significant number of workers exposed to elevated levels of environmental radon in workplaces other than uranium mines (buildings, other kinds of mine, caves, etc.). A series of RDP aerosol measurements carried out in Australia in a range of workplaces [S45] showed that some workplaces have aerosol conditions that differ markedly from those found in operating uranium mines, particularly those operating during the 1950s and 1960s. Adjustment factors for these workplaces, also referred to as K-factors, were derived from the Australian measurements for occupational exposures and are in the range 1–2.6. Use of the ICRP conversion convention in these exposure situations would lead to a significant underestimation of radiation doses. Thus it is important to understand the factors that affect the estimation of dose in workplaces other than uranium mines.

92. As discussed in the UNSCEAR 2000 Report [U2], the Committee adopted the dose conversion factors set out in ICRP Publication 65 [I2], which had been based on epidemiological evidence. Dosimetric evaluation of the absorbed dose to the basal cells of the bronchial epithelium per unit exposure gave values in the range 5–25 nGy (Bq h m^{-3})$^{-1}$, and a value of 9 nGy (Bq h m^{-3})$^{-1}$ was estimated for average indoor conditions. Using a tissue weighting factor of 0.08 for the bronchial and bronchiolar regions and a radiation weighting factor of 20 for alpha particles, the effective dose per unit equilibrium-equivalent concentration (EEC) became 15 nSv (Bq h m^{-3})$^{-1}$. The epidemiological approach provides a value of 6 nSv (Bq h m^{-3})$^{-1}$, a factor of 2½ times lower, and the dosimetric evaluations provide dose coefficients in the range 6–15 nSv (Bq h m^{-3})$^{-1}$. The UNSCEAR 2000 Report concluded that the value used by the Committee in earlier evaluations [U5, U6], 9 nSv (Bq h m^{-3})$^{-1}$, is within this range, and recommended that this value continue to be used in dose evaluations [U2].

III. EXPERIMENTAL STUDIES

93. Information from animal experiments and from cellular and molecular biology is helpful in understanding the underlying mechanisms of cancer induction, potential interactions among agents and the uncertainties associated with extrapolating risks from exposure in mines to residential exposure. The UNSCEAR 2000 Report [U2] discussed the biological effects of low doses of ionizing radiation (annex G) and the combined effects of exposure to radiation and other agents (annex H), and provided much information from animal experiments, studies of DNA, and cellular and molecular responses to various forms of radiation. This section provides a concise summary of experimental results, including more recent data than reported in reference [U2].

A. Animal experiments

94. Animal studies have been conducted for several decades to identify the nature and levels of the uranium mine air contaminants responsible for producing the lung cancers observed among uranium miners (e.g. [C40, M29]). Many of the initial studies were concerned with early effects or short-term pathological changes (e.g. [R4]). Exposures were based primarily on radon gas concentrations, and provided little or no information on the radon decay product concentrations that contribute the greatest radiation dose to the lung. The early studies (e.g. [K10]), in which lung tumours were produced, were methodologically or statistically inadequate to show an unequivocal association of lung tumours with exposure to radon and/or RDPs.

95. In the 1950s, there was growing concern that the increased incidence of respiratory cancer observed in European uranium miners would also be found in United States miners. This led to the initiation of systematic studies in the United States to identify the agents responsible for increased incidence of lung cancer in miners and to develop exposure–response relationships in animals. Investigators at the University of Rochester began to focus attention on the biological and physical behaviour of RDPs as well as on their contribution to the radiation dose to the respiratory tract [B1, M12, M13]. Shapiro [S33] exposed rats and dogs to several levels of radon alone and in the presence of RDPs attached to room dust aerosols. He also showed that the degree of attachment of RDPs to carrier dust particles was a primary factor influencing the alpha radiation dose to the airway epithelium. This dose was further demonstrated to be due primarily (>95%) to the short-lived RDPs rather than to the parent radon. Cohn et al. [C8] reported the relative levels of radioactive material found in nasal passages, trachea and major bronchi, and in other portions of rat lungs after exposure to radon and/or RDPs. The respiratory tracts of animals that inhaled radon plus its decay products contained 125 times more radioactive material than those of animals that inhaled radon alone [C44].

96. Beginning in the mid-1950s, at the University of Rochester in the United States, Morken and Scott initiated a pioneering series of experiments (e.g. [M13]) to evaluate the biological effects of inhaled radon and radon decay products in mice; later experiments used rats and beagle dogs. The essentially negative biological results of these studies suggested that alpha irradiation alone was relatively inefficient in producing tumours in the respiratory system. NCRP Report No. 78 [N7] provides a comprehensive summary of many of the early animal data.

97. In the late 1960s and early 1970s, other studies in France (Compagnie Générale des Matières Nucléaires (COGEMA)) and the United States (Pacific Northwest Laboratory (PNL, later PNNL)) were initiated and later proved successful in producing lung tumours from RDPs. The French investigators exposed rats to RDPs, either alone or in combination with stable cerium, uranium ore dust or cigarette smoke, to produce tumours in the lung (e.g. [C4, P4]). Later, the potential co-carcinogenic effects of various environmental and industrial airborne pollutants, e.g. minerals from metallic ore mines and diesel exhausts, combined with radon and/or radon progeny exposure were also investigated [M31]. The later United States studies were designed to systematically determine the pathogenic role of RDPs, either alone or in various combinations with uranium ore dust, diesel engine exhaust and cigarette smoke. These studies involved lifespan exposures of beagle dogs and Syrian golden hamsters, and chronic exposures of rats (e.g. [C13, C14]). A joint review of PNNL (United States) and the Commissariat à l'énergie atomique (CEA)–COGEMA (France) animal experimental data was published in 1999 [C40]. Bronchial dose models were published for the Syrian golden hamster, rats (Long–Evans, Wistar, Sprague–Dawley, Fischer) and beagle dogs [D23, H39, H40].

98. A review of the animal studies through 1970 appeared in the final report of subgroup IB, Interagency Uranium Mining Radiation Review Group [R4]. That report, which addressed the early acute radon toxicity studies, concluded (as had an earlier Federal Radiation Council report [F2]) that experimental work prior to the 1970s had not demonstrated that pulmonary carcinomas could be produced in animals in a systematic way from controlled exposures to radon and its

decay products. Since that review, discussions of the biological effects of inhaled radon and RDPs in animals have appeared in ICRP Publication 31 [I1] and NCRP Report No. 78 [N7]. A more detailed review of animal studies was provided in the most recent NCRP SC65 Report [N11].

99. Gilbert et al. [G3] analysed data on the risk of lung tumours in rats exposed to radon. Male, specific-pathogen-free rats were exposed starting at about 90 days of age to RDPs (and uranium ore dust) at levels of from 20 WLM to more than 10^5 WLM, and at exposure rates of 10 WL to 10^3 WL. (The experiments are reported by the authors to have resulted in an estimated dose of about 5 mGy/WLM at the cellular level.) The authors used a time-dependent hazard model to accumulate risk and examined several exposure–response functions, the simplest being a simple linear model. They concluded that the rat data were in "reasonable agreement with a linear exposure–response model" and estimated an overall linear (risk) coefficient of 237 per 10^6 WLM. In addition, their analysis showed "no observed evidence of exposure-rate effect below 1,000 WLM".

100. Bijwaard et al. [B20] used equations based on a two-mutation biological model for two large data sets of radon-exposed rats (10,000 in total). The improvement in fitting these data sets separately compared with fitting them jointly was statistically insignificant, indicating that a joint fit represented the data well. The joint solution exhibited a first mutation rate two orders of magnitude larger than the second mutation rate, which was strongly suppressed by a mutation killing term acting at high exposure rates. Maximum cancer incidence occurred for exposure rates of 1–10 WLM/d, which is in good agreement with reference [M10]. An inverse exposure-rate effect was evident at higher exposure rates, with incidence orders of magnitude larger than for lower exposure rates. A very pronounced effect of age at exposure was also observed. In all cases, ERR ranged between 0.007WLM^{-1} and 0.025 WLM^{-1}. The proposed model compared reasonably well with a similar model derived for Colorado uranium miners [L23], when the data for rats were scaled by the ratio of human to rat lifetimes.

101. Heidenreich et al. [H8, H26] used a two-step clonal model, based on Luebeck et al. [L9] and Moolgavkar et al. [M11], to investigate the risk of lung cancer induction in rats exposed to radon. The authors concluded that only fatal lung tumours among the rats could be used for generalizations to models for lung cancer induction in humans. This model for fatal tumours showed an inverse dose-rate effect at average exposure rates of above 20 WL, but below 10 WL the lung cancer risk per unit exposure decreased with increasing duration of exposure. Finally, on the basis of their analysis, the estimated ERR for rats at low exposure rates was in the range from 0.003 WLM^{-1} to 0.012 WLM^{-1}, depending on the exposure periods; this is of the same order of magnitude as the ERR seen in humans. Because a statistical test had given a strong indication that the results from different rat strains should not be pooled together, Kaiser et al. [K25] carried out separate risk analyses for two rat cohorts: the PNNL cohort of Wistar rats, and the combined cohort of Sprague–Dawley rats at the CEA and at AEA Technology Plc (AEAT), United Kingdom. The study was restricted to fatal tumours. Using a refined technique of age adjustment [H34], the lifetime absolute risk was standardized with the survival function for competing risks in the control population. The age-adjusted excess risks for both strains of rats were of similar magnitude, despite the higher lifetime excess absolute risks per unit exposure (LEAR at 1 WLM) in the European cohort because of the very low mortality in the control group.

102. Rats were exposed to tobacco smoke and radon (1,000 WLM) at the CEA. When animals were exposed to cigarette smoke prior to radon exposure, a slight decrease in lung carcinoma incidence was observed compared with rats exposed to radon only, but when the cigarette smoke exposure occurred after the radon exposure, there was a highly significant factor of 4 increase in lung carcinoma incidence. This resulted mainly from an increase in squamous-type tumours. These data were used to estimate smoke-dependent parameters in the biologically based two-stage clonal expansion model [H36]. Although smoke had no effect on tumour initiation, an effect was seen on tumour promotion. Promotion by cigarette smoke was also seen in levels of adenomatosis in the PNNL studies. It appears that preneoplastic lesions induced by radon are promoted by cigarette smoke.

103. Monchaux and Morlier [M28] reported the results from a study of the effect of exposure rate on lung cancer induction in radon-exposed Sprague–Dawley male rats. The study was conducted at relatively low cumulative exposures of about 100 WLM, which is comparable to lifetime exposures in high-radon houses or to current underground mining exposures. The risk of lung cancer in rats decreased with potential alpha energy concentration (PAEC), i.e. exposure rate, confirming the results obtained at lower exposures [M30]. These results and those from former experiments [M31] indicated that the risk of lung tumour induction in rats was maximal for cumulative exposures ranging from 25 to 200 WLM and PAEC ranging from 50 to 150 WL, i.e. exposure rates ranging from 5 to 25 WLM per week. These data suggest that there is a "watershed" at cumulative exposures of about 50 WLM. Below this exposure, decreasing the exposure concentration (WL) or protracting the time over which the dose is delivered results in a reduction in the lung tumour risk. Above this level, the reverse is true: decreasing exposure concentrations or protracting the exposure time results in an elevated lung cancer risk [M29]. These results were confirmed by Collier et al. [C38] in a series of lifespan experiments in which the effects of radon and its decay products were investigated at different total doses, dose rates and unattached fraction. Collier et al. [C16, C34] also reported on studies of the factors that affect the risk of inducing lung tumours in rats exposed to radon and RDPs.

104. The results of rat experiments conducted in parallel at the CEA and AEAT (as described in Work Package 4 of reference [T30]) have been reviewed by various individuals. The studies were carried out specifically to investigate the

effect of exposure rates on induction of lung cancer at cumulative exposures of about (100 WLM) (0.36 J h m^{-3}). Except for the fact that rat exposures in the CEA experiments were carried out for a working day without a carrier aerosol and the AEA Technology experiments were for continuous exposure with carnauba wax as a carrier aerosol, the experimental designs were similar. The joint analysis comprised more than 4,000 exposed rats and 1,500 non-exposed control rats. The authors calculated both relative and absolute risks and found, in general terms, increased risks of lung cancer both with increasing cumulative exposure and with increasing exposure rate. These results indicated that, at low exposures comparable to those in modern mines or high-radon homes and for cumulative exposures of up to about 100 WLM, the risk of lung cancer in rats decreased with decreasing exposure rates, confirming earlier results [L43, M30] at lower cumulative exposures. A parallel analysis of these experimental data with data from European uranium miner cohort studies was performed [L44, T30]. It confirmed that epidemiological and animal data are consistent in showing an increase of risk with cumulative exposure protracted at low exposure rates.

105. Mitchel et al. [M9] reported an experimental study using a nose-only breathing system with male Sprague–Dawley rats exposed to one of two concentrations of natural uranium ore dust (44% U, at 50 mg/m^3 or 19 mg/m^3) without significant radon exposure. They concluded that chronic inhalation of ore dust alone posed a risk of lung cancer in rats that was directly proportional to dose rate.

106. Although it was established that the rat constitutes a valuable model to study radon-induced cancers, Petitot et al. proposed a system which is designed to allow direct exposure of isolated cell populations cultured in vitro to radon and its decay products [P18]. One advantage of this is that cells cultured in vitro are irradiated directly by a natural radon emanation and a mixture of RDPs in exposure conditions similar to those used for inhalation exposures of rats (in vitro studies). This new method could help to identify biological markers of irradiated cells in radon-induced cancers by an in vitro approach.

107. Overall, animal data support the conclusion from epidemiology that exposure to radon and its decay products is carcinogenic. Moreover, animal data also confirm the observations from epidemiology that the risk from exposure to radon and its decay products increases with increasing cumulative exposure, even for protracted exposures at low exposure rates.

B. Biomarkers

108. Traditionally, biomarkers have been used to determine exposures or doses. Jostes [J14] provides an overview of the use of biomarkers as measures of effects of exposure to radon. It is becoming increasingly apparent that biomarkers of effect are potentially of great value in evaluating potential health impacts. The following discussion provides an overview of selected studies of biomarkers involving exposure to RDPs. Readers interested in a more extensive discussion of this subject are referred to annex C, "Non-targeted and delayed effects of exposure to ionizing radiation".

109. In biodosimetry, the emphasis has traditionally been on assessing doses per se; however, an important perspective nowadays is to think of these biomarkers as possible surrogates for measuring the body's integrated response to radiation damage, rather than as simply indicators of absorbed dose. The focus of this section is on the potential use of biomarkers of effects of radiation exposure rather than on biological dosimetry per se.

110. In addition to interactions with DNA, ionizing radiation also damages other cellular components. Cellular responses to various forms of radiation include structural and functional changes to cells and cell organelles. In addition to the morphological signs related to cell death, several reversible alterations are seen in the structure of different cell organelles. Radiation-induced changes in the supramolecular organization of the membranes, including the plasma membrane as well as different cell organelle membranes, can play a significant role in the development of radiation effects. Various morphological alterations of nuclear chromatin (e.g. changes of fine structure, development of chromosome aberrations, etc.) are thought to originate from radiation-induced damage to the supramolecular organization of DNA and/or nuclear proteins [N3, S35]. Changes in chromosomes are considered to be useful as biological indicators or even biological dosimeters of radiation injury [A26, A27, A28, A29, B2, C3], and can be evaluated qualitatively and/or quantitatively by various techniques, such as morphological analysis of metaphase chromosomes, fluorescence in situ hybridization (FISH) and the scoring of micronuclei. Brenner et al. [B32] argued that there was a need for a biomarker to distinguish between the effects of exposure to high-linear-energy-transfer (LET) radiation and other carcinogens. They suggested that exposure to high-LET radiation produced a distinctly low ratio of stable interchromosome to intrachromosome aberrations, and recommended this ratio as a candidate for such a biomarker.

111. Miller et al. [M7] exposed cultures of C3H 10T½ cells either to microbeam irradiation using the Columbia University microbeam or to broadbeam irradiation in order to investigate oncogenic transformation rates, and demonstrated that cells exposed to exactly one alpha particle each had a significantly lower response than cells exposed to a Poisson mean of one alpha particle each. Miller's group concluded that cells intersected by multiple alpha particles (as may occur in high-exposure miner studies) contributed most of the response. This suggested that extrapolating from the high-exposure conditions of miners could result in an overestimation of the risk of cancer induction at domestic levels of radon exposure, in which situation essentially no target cell is ever traversed by more than a single alpha particle.

112. Brenner and Hall [B10] commented on a phenomenon known as the inverse dose-rate effect. They noted that it is

widely observed for radiation with medium to high LET, such as neutrons and alpha particles, and that a given dose delivered over a longer time (i.e. protracted) had a greater effect than an acute exposure at the same total dose. They suggested that the dose-rate effect was significant only when a certain combination of dose, dose protraction and radiation quality was present. When uranium miners are exposed to low WLs for a long period of time, the possibility of the (inverse) dose-rate effect occurring exists, but such dose-rate effects in cases of typical domestic exposures to radon are unlikely, because the average cell is traversed by one or zero alpha particles in a lifetime. Hence when risk estimates for domestic exposure are extrapolated using data from miners, the radon risk may be overestimated.

113. Similar to the conclusion reached by Brenner and Hall [B10], Jostes [J14] noted that epidemiological studies have shown that radon is a risk factor for both smoking and non-smoking miners, and that it is reasonable to suppose, on the basis of molecular and cellular considerations, that exposure to RDPs in the home also poses a risk of cancer induction. He also suggested that, while an inverse dose-rate effect has been seen in miners, such an effect may not apply to residential exposures, since the majority of lung cell nuclei would experience no alpha hits and only a few cells would receive even a single alpha hit.

114. Mutations induced in mammalian cells following irradiation with alpha particles have been studied using microbeam methods to irradiate the cytoplasm of individual human–hamster hybrid cells (A_L cells) [W11]. The aiming point of the microbeam was 8 μm from the ends of the major axis of each cell nucleus. The probability of a scattered alpha particle hitting the nucleus was 0.4%. Irradiation of the cytoplasm produced gene mutations in the nucleus in a process mediated by free radicals. While the microbeam irradiation induced minimal toxicity to the irradiated cells, it was effective in inducing mutations in the nucleus. An analysis of the mutational spectra induced by nuclear versus cytoplasmic irradiation suggested that different mechanisms of cancer induction were operative. Two approaches were used to investigate whether reactive oxygen species (ROS) mediated the process of mutagenesis through cytoplasmic radiation. The first involved the use of an antioxidant (dimethyl sulphoxide) to scavenge ROS, and the second involved the addition of a drug (buthionine-S-R-sulphoximine) to deplete endogenous scavenger sulphydryl groups. In the first approach, the induction of mutations was suppressed; in the second, the induction of mutations was enhanced. Indicators of cellular ability to scavenge ROS may have potential as a biomarker of effect. Observations from in vitro studies further demonstrate that cytoplasmic irradiation is clearly a risk factor.

115. An earlier study had also demonstrated that radiation damage from alpha irradiation reflected in the patterns of sister chromatid exchange (SCE) in human diploid lung fibroblast cells was independent of the number of alpha particles traversing a nucleus [D3]. The much larger cross-sectional area of the cell relative to that of the nuclear target was suggested to be an important consideration. It was hypothesized that the effect was mediated by the free radicals formed in the cytoplasm as the result of traversal by alpha particles. In support of this hypothesis, a paper subsequently described the production of superoxide anions and hydrogen peroxide by alpha particles traversing the cytoplasm [N1].

116. In recent years, evidence has accumulated that suggests that both directly hit and bystander cells may show effects of exposure to alpha radiation (e.g. [M36]). Goodwin and Lehnert [G8] found from in vitro studies that a low dose of alpha radiation increased levels of SCE, an indicator of genetic damage in the lungs of mice and humans. In addition, the amount of ROS increased. SCE and ROS levels were also increased in non-irradiated cells. The authors concluded that harmful effects of radiation are induced in bystander cells to the same extent as in irradiated cells. There was evidence that the ROS response triggered the up-regulation of cytokines that mediate the bystander effect [G8]. The authors anticipated that an important next step would be to show whether this work applied in vivo and whether carcinogenesis and other disease processes were a result of the bystander effect from ionizing radiation.

117. Azzam et al. [A15] postulated that the reaction of non-irradiated cells is due to gap junction-mediated intercellular communication (GJIC) of a damage signal between irradiated cells and their neighbours. The levels of a protein ($p21^{Waf1}$) induced by stress were compared in cells competent in GJIC and not competent in GJIC. In the competent cells, more cells showed elevated levels of the protein than could have been directly intersected by an alpha particle. The expression of $p21^{Waf1}$ correlated with micronucleus formation (indicating DNA damage) and with increased phosphorylation of the *p53* gene. While GJIC is certainly one mechanism by which a signal is communicated from an irradiated cell to a non-irradiated cell, there is also evidence for soluble factors being secreted into the culture medium to communicate the bystander signal [M43, S73].

118. A bystander effect and gene induction in non-irradiated cells occurred for situations where only a small fraction of the cell nuclei were traversed by an alpha particle [L5]. Oncogenic transformation can arise from the passage of single alpha particles through cell nuclei [M7]. The role of ROS in mediating DNA damage and cell-related effects is of great interest in understanding bystander effects. The bystander effect and other non-targeted effects are discussed at greater length in annex C, "Non-targeted and delayed effects of exposure to ionizing radiation".

119. Little and Wakeford [L35] discussed radon-induced lung cancer and the bystander effect in C3H 10T½ cells exposed to alpha radiation. These authors fitted a model of the bystander effect [B21] to experimental data [M7, S47] and to epidemiological data for various residential radon studies, for Colorado Plateau miners [L10] and for the combined analysis of BEIR VI [C20]. The best estimate of the number

of neighbouring cells that contributed to the bystander effect was between 0 and 1.0 (with an upper 95% confidence limit of between 1 and 6.5), and therefore the bystander effect seen in the experimental C3H 10T½ cell system probably did not play a large role in the radon-induced lung cancer seen in the epidemiological studies of humans. The authors also found that the ERRs in the Colorado Plateau miner data were statistically indistinguishable from those derived from residential radon studies.

120. One potential biomarker of radon exposure is interleukin-8 [N2]. When alpha particles hit a cell, the production of ROS increases; this causes the production and release of interleukin-8 to increase. However, there are triggers for ROS production other than alpha radiation; asbestos, ozone and cigarette smoke can all cause an increase in ROS levels and therefore in interleukin-8 levels.

121. In an early study of chromosome aberrations in uranium miners, Brandom et al. [B30] studied cultures of peripheral blood leucocytes in 15 uranium miners and 15 normal age-matched non-miner male controls. The miners' work experience ranged from 1 to 20 years, and cumulative exposures ranged from 10 to 5,400 WLM. Chromosome abnormalities were observed in 3 (0.28%) of the control cells and 37 (2.3%) of the miner cells. The differences were statistically significant and were considered by the authors to be biologically important.

122. Smerhovsky et al. [S58] described a study of chromosome aberrations in radon-exposed miners in the Czech Republic. The study included 1,323 cytogenetic assays of peripheral blood lymphocytes from 225 mine workers exposed to RDPs at levels ranging from about 1.7 to 662 WLM. Seventy-five of the workers were reported as non-smokers. In total, some 36 lung cancers were observed in this group. Kaplan–Meier survival analysis was used to investigate cancer incidences and Cox regression was used to model associations between chromosome aberration frequency and cancer incidence. The Kaplan–Meier survival analysis indicated a significant decrease in survival times dependent on frequency of aberrant cells and on frequency of chromatid breaks. The Cox regression showed that the frequency of aberrant cells was significantly related to the risk of cancer.

123. A pilot study on German miners determined the presence of biological markers related to radon exposure in uranium mines [P3]. The researchers looked at two categories of markers: markers in the blood and markers in bronchoalveolar lavage fluid. They found that former miners' leucocytes had a decreased ability to repair DNA. In addition, there were chromosome aberrations in their lymphocytes and an increased frequency of micronuclei in lung macrophages. There was an increase in the levels of tumour necrosis factor alpha in the miners compared with the control group, and this was weakly correlated to their radon exposure. Further study is needed to establish whether these measurements are reliable biomarkers and whether individual cancer risk can be predicted on the basis of the markers. A study of the relationship between residential radon and the occurrence of chromosome aberrations in peripheral blood lymphocytes was reported in reference [O3]. This study demonstrated an excess in the number of cells containing dicentric and/or centric rings for 61 people living in dwellings with radon concentrations of above 200 Bq/m^3 compared with a control group from the researchers' laboratory (53 people). However, no statistically significant difference was seen between the control group and people exposed to radon concentrations of between 230 and 13,000 Bq/m^3. Uncertainty in exposure was suggested as a possible confounding factor for this result [O3].

124. Another study done on German uranium miners tried to identify a specific genetic defect caused by alpha radiation that led to lung cancer [W4]. The authors were unable to find a mutation of the tumour-suppressor gene *p53* that was characteristic of a radon-induced cancer.

125. Hussain et al. [H28] described studies of the mutability of codons 249 and 250 of the *p53* gene patterns in normal human bronchial epithelial cells from a 15-year-old male who had never smoked. The cells were either unexposed or irradiated to a total dose of 4 Gy (equivalent, according to the authors, to 1,460 WLM of exposure to RDPs). In this study, exposure was from alpha particles from ^{238}Pu in six equal fractionated doses. The authors found that alpha radiation selectively increased mutation frequency in both codons 249 and 250 but noted that interindividual variability argues against extrapolation of their results based on a single donor.

126. Yngveson et al. investigated the association between residential radon exposure and *p53* mutation in lung tumours [Y6]. Their study included 83 lung cancer cases in non-smokers and 250 lung cancer cases in smokers. Lung cancer cases were selected with time-weighted average radon concentrations of below 50 Bq/m^3 or exceeding 140 Bq/m^3. Molecular analysis was carried out on samples obtained from the pathology departments where the cancer cases had been diagnosed. Statistical analysis was carried out to investigate associations between exposure to radon, tobacco consumption and the presence of *p53* mutations. A non-statistically-significant odds rates (OR) for increased mutation prevalence was indicated for those exposed to high levels of residential radon (OR = 1.4; 95% CI: 0.7, 2.6), especially among non-smokers (OR = 3.2; 95% CI: 0.5, 15.5). Mutations of *p53* were also found to be associated with smoking status and, in the case of non-smokers, with exposure to environmental tobacco smoke.

127. Although biomarkers such as mutations in the *TP53* gene (which encodes the *p53* protein) are recognized as important biomarkers of radiation damage, not all studies arrive at this conclusion. Besides the study in [W4], a more recent study of *p53* in serum samples from former uranium miners found no correlation between *p53* protein concentrations in serum and exposure to ionizing radiation (measured

in WLM), and the authors concluded that there was no benefit in screening for *p53* or *p53* antibodies at the present time [S7]. A study by Vahakangas et al. [V3] of lung tumours in miners found that their *TP53* gene mutations were different from those typically seen in lung cancers caused by tobacco smoke: there were no G:C to A:T transitions in the coding strand, and the mutations were mostly transversions and some small deletions, which are very uncommon in human lung cancers.

128. Albertini et al. [A2] studied the viability of using *HPRT* mutations in human T-cells as markers of the quality of radiation to which a person was exposed. They looked at the different mutations produced by high- and low-LET radiation. With high-LET radiation such as that from radon, the mutations were mostly small partial deletions, with fewer than 2% being total gene deletions. There were relatively more breaks in the DNA strand because of the energy transferred from the alpha particle to the strand. A high proportion of the breaks were lethal, so alpha radiation is efficient at killing cells. In the case of low-LET radiation, 10% of the mutations were total gene deletions and most of the damage to the DNA molecule occurred because of secondary ionization, not from the initial collision. These or related differences may eventually allow researchers to differentiate between high- and low-LET radiation.

129. Alpha particles, like other high-LET particles, induce the tumorigenic phenotype into BEP2D cells in the lungs [Z1]. This is achieved by deleting suppressor genes. Thus, by looking at the levels of the products of these genes, it may be possible to detect the early development of cancerous cells.

130. Jostes [J14] suggested that the cellular response to alpha radiation may depend on the repair status of the affected cell. This was based on studies by Schwartz et al. [S8] and Shadley et al. [S32], which compared the induction of chromosome aberrations in repair-proficient versus repair-deficient cells. In reference [S8], it was found that a cell line that was repair-deficient for single-strand DNA breaks was more prone to aberration induction than the parental repair-proficient cell line. In reference [S32], the cell line used was repair-deficient with respect to double-strand DNA breaks, and it was less prone to aberration production when alpha particles were used than when X-rays were the inducers. It also appears that there is an adaptive response to radiation. Jostes [J14] reported that when human peripheral blood lymphocytes were exposed to a small priming dose of low-LET radiation and later to a challenge dose from radon, the number of chromosome aberrations was smaller than expected [W8, W9]. However, when alpha particles and X-rays were used at the same time, the number of micronuclei was higher than the anticipated additive effect [B4]. Taylor et al. [T1] suggested a *p53* mutation hot spot in radon-associated lung cancer, noting that Jostes [J14] had also compiled results from various studies on mutations at the *HPRT* locus in Chinese hamster ovary cells exposed to alpha radiation. In general, half of the mutations were complete gene deletions, with the other mutations composed roughly equally of partial deletions and rearrangements (~25%) and undetectable changes (~25%). The proportions changed depending on cell type [J14].

131. In a study using comparative genomic hybridization, Dano et al. investigated gains and losses of genetic material in a series of radon-induced rat lung tumours [D13, D19]. Frequent losses occurred at various locations homologous to human chromosome bands. These regions are frequently (30–80%) deleted in human lung cancer and contain tumour suppressor genes or proto-oncogenes such as *MET*, *CDKN2A*, *CDKN2B*, *FHIT* and *RB1*, and genes yet to be identified. Frequently observed gains involved chromosomes homologous to those in human encoding *MYCN* and *MYC* oncogenes. The genetic similarities between rat and human lung cancer suggest common underlying mechanisms for tumour evolution in both species and provide an opportunity to study early events in carcinogenesis. Moreover, cytogenetic and molecular genetic analyses of radon-induced rat lung tumours could help to better understand the development and progression of radon lung cancer in humans.

132. Smoking remains the predominant cause of lung cancer. The effects of smoking per se and of exposure to environmental tobacco smoke (ETS) [A24] are of great interest for both miner and residential epidemiological studies of radon [A23]. On the basis of in vitro studies, Piao and Hei [P7] suggested that the combined effects of radiation and smoking are additive for both low- and high-LET radiation.

133. Bennett et al. [B31] reported a molecular epidemiological study of gene–environment interactions in promoting lung cancer in women who never smoked. The study was designed to assess the risk of lung cancer from exposure to ETS, radon and dust, as well as family history and occupational exposure in 106 white women with lung cancer who never smoked. The authors also carried out genetic analysis for cancer susceptibility genes. Odds ratios and 95% CIs were calculated by multiple logistic regression. A dose–response relationship was found between ETS exposure and increased lung cancer risk in women with a common genetic deficiency, loss of GSTMI enzymatic activity, with the trend test significant at the 2% level. This suggested that ETS exposure could possibly double the risk of lung cancer in nearly half of the white women in Western nations. According to Bennett, loss of GSTMI enzymatic activity occurs in about 50% of the white population in Europe and North America.

134. Alavanja [A23] reviewed biological damage from exposure to tobacco smoke and from radon, and concluded that strong similarities existed between the biological damage caused by the two agents. He also noted a protective effect arising from cruciferous vegetable consumption, at least in part attributable to their antioxidant properties, in both smokers and non-smokers [A23].

135. Traditionally, biological effects from irradiation of a population of cells are considered to arise as a result of

unrepaired or misrepaired damage to DNA in irradiated cells. It has been noted previously in this section that radiation effects can also occur in non-irradiated cells (e.g. [L5, L35, M7]), a phenomenon commonly referred to as the bystander effect (e.g. [M36, M37, M38]). As the bystander effect is thoroughly discussed in annex C, "Non-targeted and delayed effects of exposure to ionizing radiation", a detailed discussion here is unnecessary, other than to note that while the existence of the bystander effect raises many questions about traditional dosimetric modelling, risk factors derived from epidemiology will already implicitly take into account any contribution from the bystander effect, thereby supporting the use of epidemiological evidence for risk estimation.

136. At the present time, considerable uncertainty remains about the effect on risk estimation of the "new" biology outlined above and discussed in greater detail in annex C. Future dosimetry will need to consider the effect of mechanisms of carcinogenesis such as those discussed above. In the meantime, it should be noted that the effects of such mechanisms are implicitly included in the results of epidemiological studies. Although several potential biomarkers of radon exposure have been studied, chromosome aberrations appear to be the most promising at this time, owing particularly to the possible "signature" of high-LET exposures and the correlation with cancer risk.

IV. EPIDEMIOLOGICAL STUDIES OF MINERS

137. Studies of underground miners exposed to radon form the current basis for estimating risks from radon and its decay products. The UNSCEAR 2000 Report [U2], BEIR VI [C20] and others have reviewed the epidemiological studies of miners, but scant attention was given to the basis for the exposure estimates. Miner studies are reviewed in this section. Particular attention is given to the sources and effects of uncertainty in estimates of risk to miners.

A. United States: Colorado Plateau miners

1. Introduction

138. The discovery of radioactive ores in the Colorado Plateau dates to between 1881 and 1887. The ores contained vanadium, uranium and a small quantity of ^{226}Ra [H13]. Before there was a demand for uranium and radium, vanadium was mined on a small scale. Radium production became more important during the period 1916–1923. However, United States radium production eventually lost its competitiveness owing to the availability of radium from high-grade ores from the Belgian Congo, and during the period 1930–1945, the vanadium content of the Colorado Plateau ores was the principal objective of their mining. Subsequently, through the defence initiatives associated with the Second World War, the emphasis turned more towards the mining of uranium [L13].

139. The uranium mining industry had expanded somewhat by 1949, and by 1950, some 500 miners worked in the Colorado Plateau area mining uranium ores, mostly in small underground workings with an average production of about 1 ton (907 kg) per person-day [H12]. According to Holaday [H12], employment peaked in 1960, when about 5,800 underground miners were employed. By 1967, employment had declined to approximately 2,800 miners, who were then producing approximately 3 tons per person-day. Holaday noted that by 1967, the occupational health field station had some information on exposures in over 1,200 different mining operations; however, an unknown additional number of operations were never surveyed.

140. Cooper [C11], writing in a special supplement of the Journal of Occupational Medicine, noted that in 1953 or 1954, large deposits of primary uranium ores were discovered in the Moab, Utah, area and in the Grants area of New Mexico.

141. In 1949, as a result of concern about the possible health hazards of uranium mining, the United States Public Health Service (USPHS), in cooperation with the United States Atomic Energy Commission and the state health departments of Arizona, Colorado, New Mexico and Utah began studies of exposures in uranium mines. By 1950, medical studies had been initiated [A12], and uranium miners were subject to routine medical examinations. Beginning in 1954, medical examinations were performed every three years on all uranium miners who could be reached and who agreed to undergo examination [C11].

142. The initial study population, some 90% of these so-called "Colorado Plateau" miners, consisted of all miners examined in 1954. The USPHS began to collect data on radiation exposure, smoking history and mortality for these miners. Several analyses of these data, with different periods of follow-up, were published [A4, A10, A12, A16, A17, H17, L13, L14, L15, W1, W2, W3, W12].

143. Holaday [H11] recommended that all states adopt a tentative "working level" of 10^{-10} Ci/L of radon in equilibrium with its decay products [S31]. For practical purposes, the recommended standard was equivalent to 1 WL, or 12 WLM per year of exposure. Holaday indicated that this recommendation was adopted as a guide by official agencies in most of the uranium mining states. Holaday [H11] documented decreasing exposure to RDPs in the Colorado Plateau mines: in 1961, only 21% of the mines studied had WL measurements of below 1 WL, but by 1967, 70% of the mines fell in the <1 WL range.

144. Before 1964, the road systems serving the mines in the Colorado Plateau area were inadequate, and since most mines were located in remote areas, supplies of fresh water and electricity were often insufficient [H13]. Holaday and Doyle hypothesized that this insufficiency of electrical power contributed to high RDP levels during the early days of mining [H13]. In those days (early 1950s), the mines were typically shallow [H11], were usually entered by a horizontal adit or incline, and were almost always ventilated by natural draught only. In most mining operations, the miners would work throughout the mine in a variety of activities — drilling, mucking, handling the ore or setting timbers, etc. This pattern of working was established when the mining was for vanadium.

145. Uranium was identified in ores in the Shiprock area of New Mexico around 1918. At that time, it received little attention. By 1950, however, there was considerable interest in uranium, and various uranium outcroppings were discovered in limestone and sandstone areas in the Grants

mineral belt [S5]. At various times during the period 1950–1960, some 60 mines were in operation. Uranium market fluctuations starting in the early 1960s resulted in various expansions and shutdowns. In the period 1966–1978, the annual output of uranium in the Grants area of New Mexico represented approximately 45% of United States uranium production.

146. The New Mexico Health Department began monitoring RDP levels in New Mexico mines in the late 1950s, with the aid of the state mine inspector's office. According to Samet et al. [B47, S1, S3, S4, S5, S74], the maximum permissible concentration of RDPs decreased from 25 to 10 WL in 1960, to 5 WL in 1963, to 3 WL in 1967, to 1.75–2 WL in 1969, to 1.4 WL in 1973, and finally to 1 WL in 1976. The Grants clinic, which opened in 1957 to serve the miners, handled 80–90% of the pre-employment and follow-up medical examinations of miners, and kept records of the movements of most miners in this area. The majority of the investigations of New Mexico miners were examinations of morbidity and mortality among uranium miners and parallel investigations of the miners' exposures, with information on the exposures being published in the third annual report of the study by the University of New Mexico [S4]. A 1989 report provided the results of a case–control study to investigate lung cancer risk in a cohort of the New Mexico underground uranium miners [S1].

2. Radon and radon decay products

147. According to Lundin et al. [L13], radon gas measurements in United States mines were first made in 1949, and RDP measurements were first performed in 1951. In 1952, an effort was made to survey all operating uranium mines, and during that year, RDP measurements were made in 157 mines and radon gas measurements in 79 mines. In 1953–1954, the number of surveys was limited, but by 1955, many of the larger uranium mining companies initiated their own air sampling programmes. By 1956, the mining companies were performing most of the mine survey work, while agencies continued their own control programmes [L13]. These measurements were carried out across the four states of Arizona, Colorado, New Mexico and Utah.

148. Lundin et al. [L13] emphasized the large number of measurements made in the mines: "From the entire 1951–1968 period nearly 43,000 measurements of RDP concentrations were available to characterize the approximately 2,500 uranium mines from which ore was shipped" (p. 31). However, this represented typically less than one measurement per mine-year averaged over 18 years of study. The distribution of these WL measurements, moreover, was far from uniform (from no measurements to a fairly large number of measurements in a single mine). Lundin et al. tabulated the number of mines in which five or more RDP measurements were made in any one year. This amounted to 116 mine-years for the period 1951–1954, during which more than half of the cumulative exposure of miners was received. For the period 1955–1968, the total number of mine-years for which there were more than five measurements was 1,313 [L13]. An earlier study by the Advisory Council of the United States National Academy of Sciences [N4] observed, "Exposure values assigned to the period before 1956 are highly unreliable, being based almost entirely on estimates rather than measurements of concentrations" ([N4], p.7]).

149. The exposure estimates used by Lundin et al. [L13] were based on RDP concentrations derived in one of three ways:

- "Measured". Values were derived directly from one or more measurements of RDP concentrations in any given mine in a given calendar year. Before 1955, such measured data included only a few of the work areas in the mine.
- "Extrapolated". Extrapolated concentrations were obtained by extrapolating between measurements made in the same mine in other years, if not more than two years before or after the year to which the extrapolation applied. In some cases, regional extrapolations were also made for mines having no direct measurements; this was done by assigning a concentration value equal to the average for the other mines in the vicinity.
- "Guesstimates". These were provided by Holaday for mines in which RDP concentrations could not be obtained by any of the extrapolation techniques discussed in Lundin et al. [L13]. The "guesstimates" were based on general knowledge of the airborne concentrations that occurred in similar mines during the same time period and on expert knowledge of long-term trends.

150. The miner database of the United States Public Health Service/National Institute of Occupational Safety and Health (USPHS/NIOSH) contains a great deal of information on miners' exposures [S13]. There are significant temporal and spatial trends in RDP concentrations. Figure III illustrates the decrease in RDP concentrations over time. The figure is a plot of annual RDP concentration (WL) by years, estimated using the different methods described by Lundin et al. [L13]. The WL values estimated by the different methods are approximately comparable and show a similar temporal decrease. The high measurement average in 1951 was due to a high WL (895 WL) averaged over 8 samples at the Freedom Mine in Salt Lake, Utah. Figure IV illustrates the extent of variability in measured WL values for Colorado Plateau miners.

151. Given the uneven distribution of measurements on a per-mine basis and the large variability between mines, it may not be possible to estimate exposures for a specific year in those mines where no measurements were taken. SENES [S13] estimated variabilities at the four levels of "area estimate" by the pooled standard deviation of measurements, under the assumption that there was no spatial or temporal trend in the variation of exposure rate. At each level (i.e.

Figure III. Average radon decay product concentration for underground uranium mines in the Colorado Plateau area from 1950 to 1968, inferred using different methods [L13].

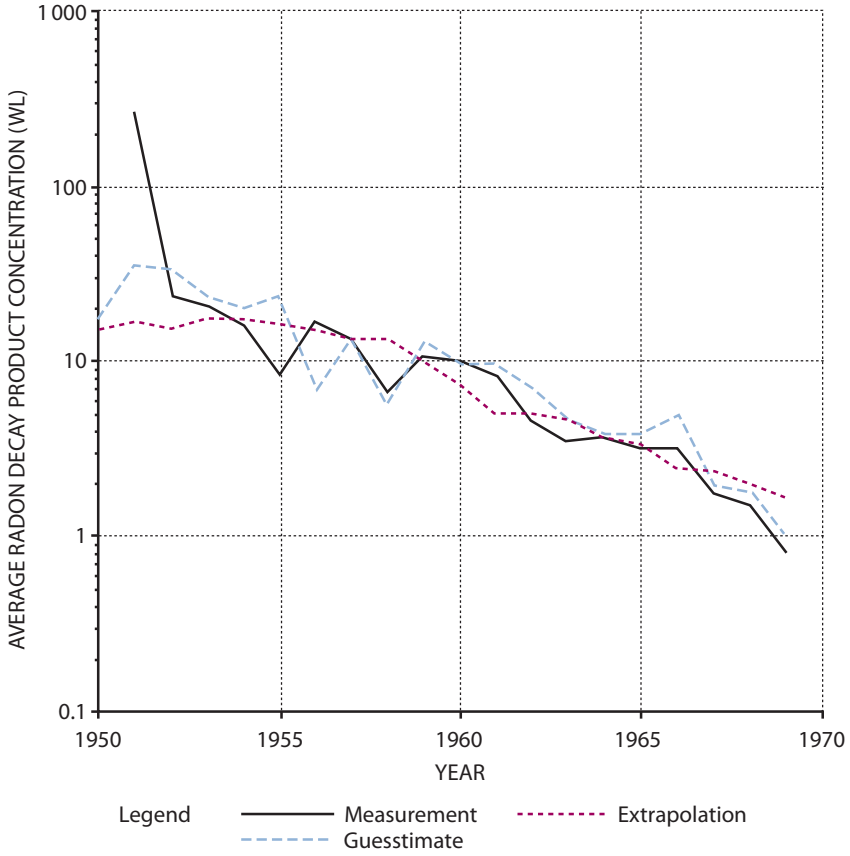
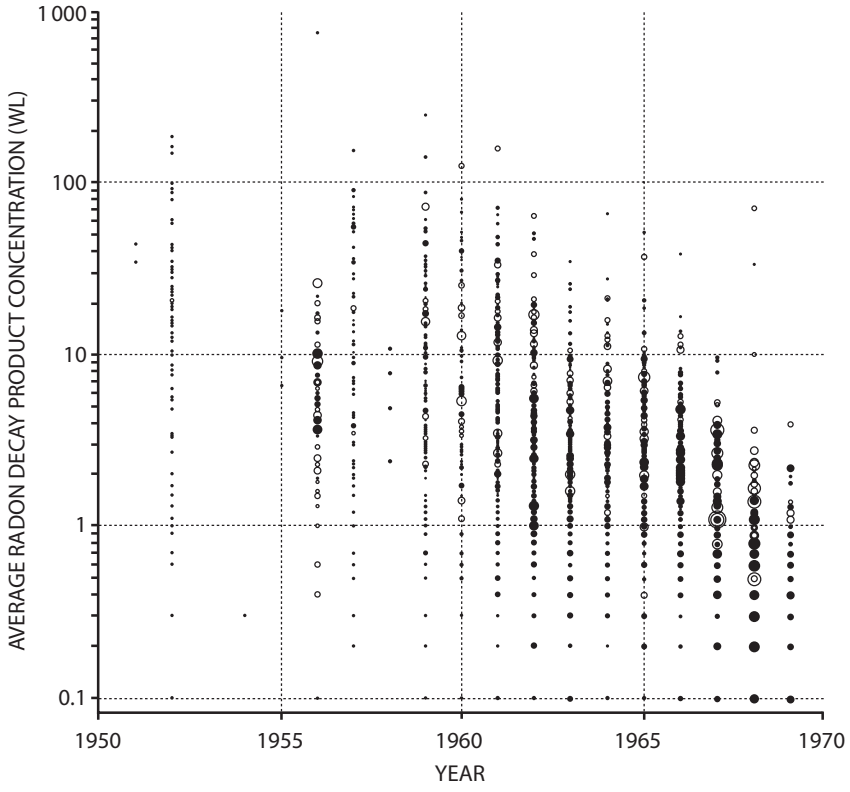


Figure IV. Range of average radon decay product concentrations measured in underground uranium mines in the Colorado Plateau area [S13].



local, district, etc.), only areas that met the criteria of having more than three mines and 10 samples were included in the calculation; the natural logarithm of measured concentration was used with sample size as a weighting factor. The results are listed in table 6 together with the equations used in the calculations to rate the large variability in WL values.

Table 6 Estimated variabilities at different levels of area estimate [S13]

Level of area estimate	*Symbol*	*Standard deviation of natural logarithm (WL)*
Locality[a]	S_L	3.16
District[b]	S_D	3.23
State[c]	S_S	3.39
Colorado[d]	S_C	3.66

$$S_X^2 = \frac{\sum_m \sum_\alpha \left(E_{m\alpha} - \bar{E}_\alpha\right)^2}{\sum_\alpha (N_y - 1)},$$

where
E is the natural logarithm of measured concentration (WL);
$\bar{E}$ is an average over E weighted by sample size;
N is the number of measurements;
$X = L, D, S$ and C, denoting locality, district, state and Colorado levels, respectively;
$\alpha = (ly), (dy), (sy)$; m = mine; y = year.

Summation over α denotes summation over both indices indicated in parentheses. In the case of the Colorado level, summation over α reduces to a single summation over variable y only.

[a] Locality: reflects variability among mines within the local mining area.
[b] District: reflects variability between mines within an entire mining district.
[c] State: reflects variability between states (Arizona, Colorado, New Mexico, Utah).
[d] Colorado: reflects variability among Colorado mines.

152. Schiager and Hersloff [S6], reporting on interviews with Holaday, noted that, while large uncertainties existed in the estimates of the RDP concentrations, these were the best that could be made and there was no conscious bias injected into the estimates of the early exposure conditions. This is in contradiction to other statements, which indicated that, where uncertainties existed, WL were deliberately overestimated [L13]. If, on average, exposures were overestimated, then on average the risk per unit exposure would be underestimated. As noted previously, mining companies began conducting the majority of exposure measurements after 1956. Lundin et al. [L13] felt that during this period, i.e. the 1950s and 1960s, there was a possibility of a bias towards underestimation of exposure, and therefore these authors tried to avoid this potential bias: "...efforts were made to exclude company measurements from data after 1960 from use in the epidemiological study of uranium miners" (p. 31). However, since the bulk of the exposures upon which risk estimates are based occurred prior to 1960, this selection had very little impact on the results overall.

153. In summary, most WL exposure estimates for Colorado Plateau miners for the period before 1950 seem unreliable, as essentially no data exist and all the estimates were based on extrapolation or "guesstimates". Exposures estimated for the period 1950–1956 were considered more reliable, although still highly uncertain. After 1956, the mining companies themselves started to make measurements on a systematic basis and, at least in the large mines, measurements became more reliable. However, there is still likely to be considerable uncertainty in exposure assessment up to what one might refer to as the "modern period", i.e. 1967 to the present.

3. Exposure estimation

154. Wagoner et al. [W2] concluded that the excess respiratory cancer rates among uranium miners were not attributable to age, smoking activity, heredity, urbanization, self-selection, diagnostic accuracy, prior hard rock mining or ore constituents. They attributed the excess risk to airborne radiation.

155. Miners who were examined in the Colorado Plateau area during the period 1951–1960 and for whom sufficient records existed made up the cohort studied by Wagoner et al. [W2]. The exposure calculation was performed individually for each miner; the miners were then categorized into groups according to exposure levels. The average RDP concentration (WL) and duration of work underground in working months (one working month is taken as 170 hours) were multiplied to arrive at average exposures (WLM) for each group of miners; additional details were provided in a subsequent paper by Wagoner et al. [W1]. At that time, approximately 12,000 RDP measurements were available for the approximately 1,200 mines under study. The RDP concentrations used in the calculations were derived as follows:

> "When there were multiple measurements in the mine during a calendar year, an average annual exposure was calculated. Measurements from non-work areas were excluded from all calculations. When no measurements were available for a mine during a calendar year, estimates were made from the average of measurements for the same mine during the preceding and following calendar year or other mines on the basis of geographic proximity, similar ore bodies, physical layout, ventilation and control efforts of regulatory agencies" ([W1], p. 184).

156. More detailed measurements began in 1967 with the intention of presenting these data for review by the Federal Radiation Council (FRC) [F2] so that the FRC guidelines for the control of radiation hazards in uranium mining could be updated. The results of this update were presented in a paper by Lundin et al. [L14], who examined a cohort of 3,414 white and 761 non-white underground uranium miners who had undergone medical examinations in 1950–1961. The results indicated that uranium miners who smoked had an excess lung cancer risk ten times greater than non-smoking miners. Prior hard rock mining experience had little effect on lung cancer mortality overall but was suspected of contributing more significantly to risks in the lower exposure categories.

157. In 1971, Lundin et al. [L13] reported on the cohort with exposures updated from the start of mining up to the end of September 1969. The cohort contained 3,366 white and 780 non-white uranium miners with at least one month of underground mining prior to 1 January 1964. Procedures were also developed by Lundin et al. [L14] for estimating RDP exposure from hard rock mining other than uranium mining.

158. As previously noted, radiation levels in uranium mines dropped sharply after 1967. This fact, combined with a drop-out rate of 10%–50% per year for the original cohort after 1960, justified the assumption that exposures received after September 1969 contributed only a relatively small additional exposure to this cohort.

159. According to Lundin et al. [L13], more than 50% of the collective exposure of some 2.8×10^6 person-WLM was received prior to 1955. For the calculated cumulative exposures (expressed in person-WLM) received up to 30 September 1960 by 3,325 men, more than 25% was received by 1,325 men and was based on actual measurements [L13]. Although the converse was not explicitly stated, it may be inferred from reference [L13] that, for the other 2,000 men in this cohort, less than 25% of the cumulative exposure was based fully on measured data. This emphasizes strikingly the uncertainties in the early exposure data; however, it does not necessarily imply any bias in exposure estimates and hence in risk estimates. The duration and periods of exposure of individual miners were determined in some cases from employment records, but in most instances they were determined from interviews with the miners themselves. Annual exposures for full-time miners were assigned on the basis of RDP concentrations averaged throughout the mine in which each miner worked. For calculating annual exposures, "it was assumed, unless we had information to the contrary, that a man worked in the mine at which he was found for six months before and six months after the census or questionnaire date (6 month rule). When a man was known to work in two different mines at less than a one year interval, the period of employment during the interval was equally divided between the two mines" [L13].

160. Full-time mining was assumed to consist of 12 full months underground with no adjustment for vacation or illness. This assumption was an important reason for believing that exposures were overestimated; however, no adjustments were made for overtime work or 'moonlighting' by any of the miners [S6]. People familiar with the uranium mining industry in the 1940s and 1950s recalled that many miners worked exceptionally long hours. A standard working week until 1960 was at least 48 hours. Many miners working entirely under production contracts spent 50–60 hours underground each week. It was not unusual for a miner to work a regular full-time shift for an established company and then spend his days off developing a mining claim of his own. Since estimates of cumulative exposures (i.e. WLM) were based on months rather than actual hours worked, the method tended to underestimate exposures. It is likely that any potential bias in exposure estimates resulting from not making allowances for vacation and sickness was more than compensated by not making allowances for underground time exceeding the normal (48 hour) working week.

161. The 1968 report of the National Academy of Sciences [N4] suggested that the problem of determining radiation exposure for individual uranium miners was also complicated because official mine records did not necessarily show the actual job assignment. Only the miner himself, and to a lesser extent his immediate supervisor, knew the areas in which he worked. The report also raised the possible problem of exposure from previous mining, and further noted, "there is some uncertainty in the average working values even in mines in which numbers of measurements have been made." Measurements consisted of spot sampling at a particular time and location, and therefore reflected only the conditions that existed at that time and location. (This is important since exposure in WLM is the product of time in working months, a working month nominally being taken as 170 hours, and RDP concentration in WL.) The report recognized that workplace conditions (dust levels, WL, etc.) varied with the nature of the operations being carried out, e.g. blasting, the ventilation provided and the amount of ore uncovered.

162. According to a review by the USPHS epidemiological study ([J10], p. 1,266), the basic information examined for individual uranium miners was the following:

- Mines worked;
- Dates of employment in mines;
- Fractional time in mines;

– Radon decay product concentration levels measured or estimated for each mine as a function of calendar year.

From these data, cumulative WLM values were calculated for each individual miner. Cumulative WLM values were calculated for both underground uranium mining alone and for uranium mining plus other hard rock mining. The average exposure of the group of miners in the USPHS study was of the order of 800 WLM [C18].

163. Some effort was made to estimate the exposure of miners to RDPs accumulated since 1967. As reported in reference [S13], exposure data were collected and examined at 23 separately managed large mining operations and also for more than 180 small mines representing in total some 25,000 miners. The major conclusions of this study (for 1967–1985) were: the average working time for the underground uranium miners studied was 3.3 years, and was 5.9 years for those who worked more than 2 years; the average lifetime exposure (i.e. 1967–1985) of underground uranium mine employees was 3.6 WLM; and the average yearly exposure per employee who worked in underground uranium mines in this period was 1.2 WLM.

164. Many New Mexico miners were included in the cohort study above. An independent epidemiological study of New Mexico uranium miners was initiated in 1977. This study was performed by the University of New Mexico under the direction of J.M. Samet. The focus of Samet's study group was retrospective analyses of a cohort of 3,055 underground uranium miners whose first underground experience occurred prior to 1971. An outline of the approach used to estimate exposure in the New Mexico study is provided in references [M34, S4, S75]. According to Samet et al. [S75], two large databases were developed to profile exposures to RDPs of Grants area miners:

> "The first comprises WLM measurements from 1957 to 1967. A total of 20,086 individual readings are available from the 186 visits made during the 11 years; after 1960, mine index values, which weight individual measurements by number of persons exposed, were generally reported. The second includes all individual WLM reports by companies for 1967 through 1982" [S75].

165. The mean WL values reported by Butler et al. [B47] were 42 in 1954, decreasing greatly to about 4.3 in 1955, and subsequently generally declining to about 1.7 in 1967. These authors also reported that the mean annual WLM exposure was below 10 from 1969 onward [B47]. It is perhaps worth noting that the (weighted) mean of the WL measurements was found to provide the best indicator of the total mine index values, which weight measurements by numbers of personnel exposed [B47, L35, S1, S4].

166. The employment history of United States miners was documented by various means. Miners were interviewed about mining experiences during medical examinations. In addition, supplemental information from subsequent annual uranium miner censuses, records of official agencies and mail questionnaires was available [L13]. Efforts were also made to account for other hard rock (OHR) mining experience, as many of the underground uranium miners in the Colorado Plateau had previous mining experience. For example, some had hard rock mining experience from other western mines, while others were coal miners from the eastern United States. However, the lack of radon or RDP measurements from these other mining activities may result in an underestimate of the miners' actual exposure.

4. Other hard rock mining exposures

167. Radon is a normal constituent of mine atmospheres, including those of non-uranium mines. For example, Holaday [H14] reported the results of several early measurements of radon in non-uranium mines in Colorado and New York. Radon levels surveyed in 35 metal and clay mines in Colorado ranged from 10 to 2,100 pCi/L. In the New York mines, radon concentrations ranged from 2 to 110 pCi/L. According to SENES [S9], about 40% (1,433 out of 3,359) of white male Colorado Plateau miners accumulated radiation exposures from OHR mining. To investigate this, exposures from OHR mining were calculated for miners whose exposures in uranium mines were less than 120 WLM. Following the procedure employed in the USPHS investigations as described by Lundin et al. [L14, L15], a miner was assumed to have completed his OHR mining activity by the year preceding the start of his underground uranium mining. Exposures were based on assumed RDP concentrations in OHR mines of 1.0 WL prior to 1935, 0.5 WL from 1935 to 1939 and 0.3 WL from 1940 onward.

168. When these OHR mining exposures were added to the exposures from uranium mining, the dates used in the previous analysis (i.e. the dates when each miner reached the limit of each exposure category) had to be recalculated. The assumed starting date of OHR mining was calculated on the basis of the total exposure and the appropriate rates. Next, from the OHR mining exposure rate and the known dates at which different uranium mining exposure levels were reached, the miner's exposure history was classified according to exposure rates and the number of months at each rate. The exposures were assumed to be accumulated at a constant rate during each period at a particular exposure level. The dates on which each miner reached a certain level of accumulated exposure were seen to be earlier when the OHR data were taken into account. The addition of OHR mining exposures caused a shift of the miners, and hence the lung cancers (and person-years), into the higher exposure categories. The average uranium and OHR mining exposures from the NIOSH cohort (with follow-up through 1985) are shown in table 7 [S13].

Table 7 Average uranium and OHR mining exposures by exposure category — white miners [S13]

Exposure category for uranium mining (WLM)	*Average time spent underground (years)*	*Average exposure for uranium mining (WLM)*	*Average exposure for uranium and OHR mining combined (WLM)*	*Average exposure rate while underground (WLM/year)*
<120	1.11	51.7	68	46.58
120–359	3.33	233.4	249	70.09
360–839	5.24	572.6	594	109.27
840–1 799	7.47	1 229.6	1 252	164.61
1 800–3 719	10.66	2 515.5	2 534	235.98
3720+	14.22	5 787.3	5 808	406.98

169. Although the impact of OHR mining on the overall cohort average WLM is small, the effect for individual miners can be appreciable. Table 8 summarizes the estimated exposures of miners from uranium mining only and from OHR mining, for those whose uranium-only exposure was estimated at less than 120 WLM [S9]. When OHR mining exposure was added, 5 of the 10 miners shifted from the 0–120 WLM category into the next higher exposure category. The average exposure of this group increased from 51 to 101 WLM. These data suggest that, particularly in the low exposure categories that are of greatest interest to present-day miners, a potential bias towards underestimation of exposure is introduced if OHR mining exposure is neglected. In the absence of an agreed procedure for estimating OHR mining exposures, studies could be done either on miners with no pre-1950 exposure (which minimizes this effect) or with the use of categorical variables denoting the presence or absence of pre-1950 exposure.

Table 8 Effect of OHR mining for lung cancer cases with less than 120 WLM uranium mining exposure [S9]

SENES ID number	*Uranium mining exposure (WLM)*	*OHR mining exposure (WLM)*	*Total exposure (WLM)*
1911	8	0	8
1011	13	136	149
1092	28	0	28
4017	30	0	30
3321	33	0	33
3633	42	0	42
3297	42	187	229
3588	44	62	106
2023	68	29	97
1563	73	68	141
50	82	0	82
534	83	122	205
560	119	44	163
Average	51	50	101

170. Limited data are available on the characteristics of mine atmospheres in the past. For example, the attached fraction of RDPs will depend on a number of characteristics of the mine atmosphere, among them WL, dust levels, dust particle size and humidity. Such data are important for both dosimetry and epidemiology. Useful data are summarized in various publications, including references [C10, G1, N7, N10, S23]. A 1957 report on the control of radon and its decay products [H27, U16] compared the operating conditions in uranium mines with those in conventional hard rock mines. While few data were presented, the report states that “dust counts in uranium mines indicate concentrations from 5 to 20 million particles per cubic foot of air” and “the silica content of uranium ore (carnotite) ranges from 50 to

75 percent" [H27]. It is important to remember that the majority of measurements in mines are from the mid-1960s onward, and that the major portion of exposure of miners dates from earlier times when few actual measurements were made.

5. Epidemiological analyses

171. In 1950, the United States Public Health Service (USPHS) began to collect various data on uranium miners in Arizona, Colorado, New Mexico and Utah, including radiation exposure, smoking history and mortality. Numerous analyses of the data have been published [G4, H9, H17, L13, L16, W12]. The miner data were incorporated into analyses carried out by BEIR IV [C19], Lubin et al. [L10] and BEIR VI [C20], among others.

172. Hornung and Meinhardt [H17] reported a proportional hazards analysis of the Colorado Plateau cohort, originally described by Lundin et al. [L13], with follow-up to 31 December 1982. The cohort consisted of 3,366 (white) miners with 256 lung cancer deaths, a median (cumulative) exposure of 10.3 WLM and a median duration of employment of 48 months underground. This study evaluated several risk models and chose a power function model since it provided the best fit to the data, and permitted analysis of the effects of several temporal factors and smoking. The study estimated excess relative risk (ERR) to be 0.9–1.4% WLM^{-1}, compared with a previous smoking-related estimate of 0.31% WLM^{-1} reported by Whittemore and McMillan [W12]. The relative risk increased with age at exposure and decreased with increasing time since exposure (a reduction of about 55% 10 years after cessation of mining compared with miners with the same exposure, smoking history and age) [H17]. Hornung et al. [H9] conducted further studies of modifiers of lung cancer risk in the Colorado Plateau miner cohort; follow-up to 31 December 1990 added an additional 121 lung cancer deaths, bringing the total number of lung cancer deaths to 377. This analysis confirmed the earlier finding of a strong interdependence of relative risk and age. It also suggested an exposure-rate effect resulting in a concave downward dose response; i.e. the relative risk was reduced at low exposure rates and low cumulative doses. For risk estimation, the authors recommended the use of ERR after stratification to lower exposure rates (<10 WL) and cumulative exposures (<800 WLM). The authors also briefly discussed the potential for errors in the exposure estimates to bias epidemiological analysis, and suggested that such errors might result in the underestimation of the ERR.

173. A 1992 report [S9] described exploratory analyses performed on white male underground uranium miners with follow-up to 31 December 1985. The purpose was to investigate the effect of other hard rock (OHR) mining experience and smoking on the risk of lung cancer. The report also included an analysis of a subgroup of miners with cumulative exposures of below 2,000 WLM, using Poisson regression to estimate model parameters using iteratively reweighted non-linear regression. Simple linear ERR and absolute risk models were among the dose responses evaluated. The results are shown in table 9. While the regressions in table 9 are all significant ($p < 0.01$), on the basis of the F-ratio (a statistical test to see whether the amount of variation explained by the regression is significant), the absolute risk model accounts for more of the variability in the data for all miners and smoking groups than the relative risk model. The effect of OHR mining and the joint effect of OHR mining and smoking were also investigated (table 10). The miner groups included all of the miners with no exposure from OHR mining, those with OHR mining experience but no uranium mining exposure prior to 1950, those with neither OHR mining experience nor pre-1950 exposure in uranium mines, and those with only OHR mining experience. All of the regressions in table 10 are significant ($p < 0.01$). On the basis of the F-ratio, the results in table 10 show that, in this case, the relative risk model accounted for more of the variability in the data than the absolute risk model. The absolute risk to miners who never smoked (2.8×10^{-6} a^{-1} WLM^{-1}) was about half that calculated for "all" smokers (6.6×10^{-6} a^{-1} WLM^{-1}). In this analysis, the risk was calculated relative to the baseline risk in a non-smoking reference population. Finally, the authors fitted the parameters of the BEIR IV model with the exposure–response model using the data for white Colorado Plateau uranium miners. The parameter estimates for time since exposure and attained age were comparable to factors presented by BEIR IV; however, the basic risk factors (b2 in BEIR IV terminology) estimated in the regressions of Colorado Plateau data alone (not correcting for exposure in OHR mining) were an order of magnitude lower than those estimated for the combined cohorts by BEIR IV [C19].

Table 9 Summary of regression analyses by smoking category [S9]

Category	*Number of miners*	*Number of lung cancers*	*Excess absolute risk*			*Excess relative risk*		
			Slope (PY/WLM)[a]	*Intercept (PY⁻¹)*[a]	*F-ratio*[b]	*Slope (WLM⁻¹)*	*Intercept*	*F-ratio*[b]
All miners	3 359	305	5.0 × 10–6	–0.18 × 10–3	3 960	2.3 × 10–3	0.30	619
Non-smokers	493	14	3.1 × 10–6	–0.61 × 10–3	139	21.1 × 10–3	–3.8	203
Ex-smokers	318	38	7.5 × 10–6	1.1 × 10–3	45	9.2 × 10–3	1.2	55

Category	*Number of miners*	*Number of lung cancers*	*Excess absolute risk*			*Excess relative risk*		
			Slope (PY/WLM)[a]	*Intercept (PY^{-1})*[a]	*F-ratio*[b]	*Slope (WLM^{-1})*	*Intercept*	*F-ratio*[b]
All smokers	2 754	282	5.7×10^{-6}	-0.30×10^{-3}	1 954	2.2×10^{-3}	0.30	607
Light smokers	501	29	5.2×10^{-6}	-0.35×10^{-3}	63	3.5×10^{-3}	1.3	11
Heavy smokers	1 910	215	5.5×10^{-6}	-0.0035×10^{-3}	7 882	1.8×10^{-3}	0.29	264

[a] PY = person-year.
[b] A statistical test to see whether the amount of variation explained by the regression is significant.

Table 10 Summary of regression analyses by smoking category for miners with no OHR mining exposures [S9]

Category	*Number of miners*	*Number of lung cancers*	*Excess absolute risk*			*Excess relative risk*		
			Slope (PY/WLM)[a]	*Intercept (PY^{-1})*[a]	*F-ratio*[b]	*Slope (WLM^{-1})*	*Intercept*	*F-ratio*[b]
All miners with no OHR mining	1 926	152	5.5×10^{-6}	-0.92×10^{-3}	599	2.6×10^{-3}	–0.031	1 266
Never smoked	338	9	2.8×10^{-6}	-0.64×10^{-3}	344	20.7×10^{-3}	–4.1	438
Ex-smokers	177	22	8.6×10^{-6}	-1.7×10^{-3}	56	9.7×10^{-3}	3.4	27
Light smokers	318	15	5.1×10^{-6}	-0.80×10^{-3}	108	3.3×10^{-3}	1.1	11
Heavy smokers	1 015	99	5.9×10^{-6}	-0.84×10^{-3}	977	2.4×10^{-3}	–0.15	498
All smokers	1 528	138	6.6×10^{-6}	-1.3×10^{-3}	310	2.6×10^{-3}	–0.033	1 034

[a] PY = person-year.
[b] A statistical test to see whether the amount of variation explained by the regression is significant.

174. Stram et al. [S60] reported an analysis which used a measurement error correction of lung cancer risk based on fitting a multilevel statistical model to the Colorado Plateau uranium miner cohort data within the same mine, locality and mining district. The authors used two subcohorts from the cohort of 3,347 white miners employed for at least one year in the period 1950–1960 as defined by Roscoe [R8]. The first cohort (referred to as the 1950 cohort) included 2,074 miners with 263 lung cancer deaths who had their initial mining experience commencing in 1950 or later. The second cohort (referred to as the 1952 cohort) included 2,388 miners with 209 lung cancer deaths. The authors noted that the reason for the selection of the 1952 cohort was that systematic measurement of radon in mines did not start until 1952. The approach to (exposure) error correction was based on a computation of the exposures (WLM) for each year and mine of interest. The authors investigated a number of models for lung cancer mortality, including a simple linear ERR model of the form $1 + \beta\chi(t)$, where β is the ERR per 100 WLM and $\chi(t)$ is the miner's cumulative workplace radon exposure up to 2 years prior to the attained age. In addition, the authors investigated several models, including a simplified BEIR VI model, of the effect of smoking, attained age and time since exposure. For the simple linear risk model, the authors report $\beta = 0.28$ (SE = 0.075), and for the exposure error adjusted model, $\beta = 0.44$ (SE = 0.14), both for the 1950 cohort. Similarly, for the 1952 cohort, the authors report $\beta = 0.33$ (SE = 0.1) using the uncorrected exposure and $\beta = 0.54$ (SE = 0.2) for the exposure error adjusted model. For both cohorts, the error correction increased the risk estimates by about 60%. The authors found a submultiplicative relationship between radon exposure and smoking, both with and without error correction. The authors also observed an exposure-rate effect; this, however, diminished after correction for exposure measurement error. With their simplified BEIR VI model, the effect of low exposure rate (0–15 WL) was essentially the same with and without measurement error correction.

175. Gilliland et al. [G4] reported a study of the exposure to RDPs and lung cancer risk in non-smoking uranium miners. The authors used case–control methodology and conditional logistic regression analysis to investigate the relative risk of death as a function of cumulative exposure to RDPs. Their findings are in close agreement with a parallel analysis of miners reported by Lubin et al. [L10]. The authors concluded that non-smoking miners were indeed at increased risk of developing lung cancer.

176. Luebeck et al. [L16] noted that with their biologically based model and parameters applied to the Colorado Plateau miner cohort, an inverse dose-rate effect was not seen with levels of exposure typical in residences. The ERR was estimated to be about 0.0078 (95% CI: 0.0036, 0.0165) per WLM for 25 years of residential radon exposure at a level of 150 Bq/m^3. This is consistent with the value reported from a ratio analysis of eight epidemiological analyses of residential radon exposure [L4]. Finally, Luebeck et al. [L16] noted that the comparable risks in the BEIR VI report, expressed as lifetime risk, were higher than their own estimates by a factor of 2–4.

177. The Navajo of the south-west United States were involved in the mining and milling of uranium ores in the Colorado Plateau area from the 1940s to the 1970s. The 1995 study of Roscoe et al. [R9] updated an earlier study [A4] of mortality among Navajo uranium miners. The 1995 study reported on a cohort of 757 Navajo miners with vital status followed from 1960 to 1990 and a mean cumulative exposure of 755 WLM accumulated over an average of 8.3 years of underground work. The exposures were based on the work of Lundin et al. [L13]. A life table approach based on mortality data for non-white men in New Mexico and Arizona, direct standardization of rates and internal comparisons between the exposure categories were used to analyse the cohort. All exposures were lagged five years, to represent a reasonable minimum period for the induction of lung cancer. A Cox regression analysis was used to account for simultaneous risk factors and the use of external mortality rates. The time-dependent regressors considered in the model included cumulative exposure, log cumulative exposure, duration of exposure, log exposure rate, log cumulative pack-months of smoking, time since first exposure and others.

178. Standardized mortality ratios for a number of causes of death (heart, circulatory and digestive diseases) were lowered. Of the diseases examined, only the values for lung cancer, pneumoconiosis and "other respiratory diseases" were elevated. The mean exposure to RDPs among the 34 deaths observed from lung cancer (versus 10.9 expected) was 1,517 WLM. Smoking status for the miners with lung cancer was similar to that for the entire cohort of Navajo miners. A log-linear model and a linear model in cumulative exposure fitted the data equally well, and yielded ERRs of 13.8 and 9, respectively, for a cumulative exposure of 400 WLM relative to no exposure. Unlike the case of the white miners, smoking was not strongly associated with lung cancer risk in the Navajo cohort. The authors attributed the excess non-malignant respiratory disease (standard mortality ratio of 1.4) to be due mainly to pneumoconiosis and exposure to silica and other workplace contaminants rather than to radon.

179. Latency is an important consideration in evaluating potential lung cancer risk following exposure to RDPs (e.g. [C20, L10, N7, N11]). Langholz et al. [L39] reported on an investigation of methods to assess latency effects and an analysis of latency in Colorado Plateau uranium miners using a nested case–control methodology with 263 lung cancer deaths. Of these, 239 cases were matched to 40 controls each and the remaining 24 cases with fewer than 40 controls (all controls were used). The relative risk of lung cancer increased for about 8½ years and then decreased, reaching background levels after about 34 years. The decline in risk with increasing time since exposure was much more pronounced in persons over 60 years of age. Hauptmann et al. [H30] reported on the use of splines (piecewise polynomial functions) to analyse latency in the Colorado uranium miner cohort and reported similar results, with ERR > 0 for the period from 9 to 32 years prior to the identification of lung cancer. The ERR reached a maximum of about 0.6 for 100 WLM about 14 years after exposure and decreased to about 0.02 thereafter [H30].

6. Evaluation

180. Estimated exposure rates for individual miners in the Colorado Plateau area have large uncertainties due to: variations between workplaces within mines (even for those mines where RDP concentrations were measured); the necessity to use "guesstimates", extrapolations or estimates from other mines in the area; uncertainties in OHR mining exposures before (and possibly after) employment at uranium mining facilities; and discrepancies in the work histories of those in the sample group examined. There are significant discrepancies in the work histories of workers who worked underground part-time. Furthermore, the average WL values may not apply to this type of worker, as they may have worked in areas subject to lower ventilation rates than those for the other workers. Reconstruction of exposure histories from company records is probably not feasible, because workers tended to work in numerous mines, for which few or no records are available. However, it should be possible to study a statistically valid sample of miners and from these investigations to draw conclusions concerning the uncertainty associated with the estimates of exposure for the cohort per se.

181. Overall, notwithstanding limitations in the exposure data, the Colorado Plateau cohort of uranium miners is an extremely valuable resource for risk estimation. It provides one of the most substantial bases for risk estimation for groups exposed to RDPs, and the best information on smoking histories. The prominent strengths of this group include: the size of the cohort; the extent of follow-up; the considerable amount of exposure information for the periods of interest (prior to the mid-1960s); information on smoking; and the possibility to assess the effect of OHR mining. Although no systematic bias was identified in the estimates of the exposures for this cohort, the uncertainties in the exposures of individual miners are very large, particularly for the early years of mining.

B. Canada: Ontario uranium miners

1. Introduction

182. Uranium mining in Ontario started in the early to mid-1950s in the Elliot Lake area. Uranium production developed

rapidly to reach a peak during 1957–1960, and declined just as rapidly after 1960. To illustrate this, there were 2 operating uranium mines in 1955, 15 in 1958 and 5 in 1964. The period during which the largest exposures occurred for Ontario uranium miners was relatively short (10 years or so). In total, more than 16,000 men were employed in the Ontario uranium mines at various periods between 1955 and 1977 [M8]. By 1988, only two uranium companies, both in Elliot Lake, remained in operation, and by 1993, only one of these, Stanleigh, remained in operation. It ceased operations in 1996.

183. Muller and co-workers have carried out a number of studies (e.g. [K13, M3, M8, M15, M19, M21, M22, M23, M24, M25, M26]) on some 50,000 men who worked in one or more mines in Ontario. The Ontario miner population was subdivided into gold miners, nickel/copper miners, iron ore miners, uranium miners, other ore miners and a mixture of miners. Men who worked at the Eldorado Mining Company's Port Hope plants or in Eldorado mines in Saskatchewan or the Northwest Territories, as well as those with documented exposure to asbestos, were treated separately. This left a cohort of about 15,000 miners with exposure in Ontario uranium mines. Men were considered to have entered the study at the time of their first medical examination (between 1 January 1955 and 1 January 1978) if they reported having worked for a half-month or longer in dust exposure in an Ontario uranium mine. Miners left the study on 31 December 1981, or at death if this occurred earlier. Miners with uranium mining exposures outside Ontario and asbestos miners were excluded.

2. Radon and radon decay products

184. A certain number of workplace exposure data for operating uranium mines in Ontario became available starting in 1955, although systematic measurements were not started until 1958. According to Ham [H1], the Ontario Department of Mines issued codes in 1957 requiring that various measurements, including measurements of RDPs, be taken in the mines. Thus, however infrequent, measurements were made in each operating mine from that time on. Records of RDP levels for 1954–1955 show that average RDP concentrations ranged from 3 to 7 WL and the average exposures of full-time underground miners from 36 to 84 WLM/a. The levels varied (up and down) over time as new mines developed and new methods of mining and ventilation were incorporated. Considerable information about exposure conditions in Ontario mines is given in Ham [H1] and in Muller et al. [M19].

185. Ventilation flows changed in Ontario mines over the same time period. McCrodan [M4] reported ventilation rates for two of the Elliot Lake mines (table 11).

186. The Ontario investigators recognized that estimates of RDP concentrations involved some uncertainty, especially for the early years of mining for which relatively few reliable measurements were available. The epidemiological investigators worked together with mine ventilation engineers who were familiar with the Ontario uranium mines over the early years of operation to develop two estimates of RDP concentrations, "standard WL" and "special WL", in an attempt to bracket the uncertainty [M19]. Muller and his co-workers suggested that the standard WL estimate was more representative of a miner's exposure, while the special WL estimate was probably on the high side.

187. In 1985, a reconstruction of early underground uranium mining environments was undertaken [D7]. Mining practices during the late 1950s and early 1960s in the Elliot Lake area were reproduced in reconstructed underground mine areas. Extensive measurements were made during these tests. RDP levels were recorded under a variety of operating conditions. Under continuous ventilation with compressed air (the most favourable condition), exposures during the 1950s were likely to have been not more than double the exposures received by miners today. However, in unventilated areas, the exposure levels could be as much as 10 times higher than in mines today [D7]. While this work reduced the uncertainties associated with the concentrations of RDPs, the uncertainties in ventilation practice and in the times spent in different locations in the mine remained. The 1985 study concluded that a typical raise miner in the 1950s, relative to miners of around 1985, would have been exposed to RDP levels three times higher, about equal levels of thoron decay products and gamma radiation, and much higher levels of uranium and quartz dust. Further information concerning the atmospheres of the Elliot Lake mines and the levels of radon and thoron decay products is given in reference [D8]. The measurements made during the reconstruction also confirmed that the exposure values used for the early years (specifically for raise miners) did not overestimate the working exposures at that time.

Table 11 Ventilation rates at Elliot Lake mines (adapted from reference [M4])

Year	*Denison*		*Quirke I*	
	cfm[c]	*cfm/ton*[d]	*cfm*[c]	*cfm/ton*[d]
1957–1958	200 000	33	300 000	54
1960	–	–	300 000	70[a]
1961	350 000	59	–	–

Year	Denison		Quirke I	
	cfm[c]	cfm/ton[d]	cfm[c]	cfm/ton[d]
1965	450 000	75	–	85
1965	530 000	88		
1965	530 000	150[a]		
1968	–		250 000	125[a]
1969	–			
1971	650 000	108	400 000	200[b]
1975	650 000	108		

[a] Reduced tonnage.
[b] Mine reopened.
[c] Cubic feet (of ventilation air) per minute (1 cfm = 4.72×10^{-4} m^3 s^{-1}).
[d] Cubic feet (of ventilation air) per minute per short ton (907.19 kg) hoisted to surface.

3. Exposure estimation

188. According to Muller et al. [M19], some 131,000 measurements of RDP concentrations were made over the period 1955–1981, using the Kusnetz method. These data were obtained over a period representing some 141 mine-years of operation, which corresponds to 929 measurements per mine-year of operation. Up to 1977, there were approximately 55,000 measurements representing some 126 mine-years of operation; this corresponds to approximately 430 measurements per mine-year of operation over this period. Expressed another way, in the period 1955–1977, approximately 1.7 measurements were made per average man-year worked, or, on average, one WL measurement for every 9 WLM accumulated by the study cohort.

189. About 23% of the assigned WLM exposure of the cohort was based on extrapolated values of RDP concentrations, particularly during the early years of mining. The mean period of extrapolation was approximately 1.1 years.

The key observations concerning exposure estimations for the Ontario study taken from references [M23, M24] are summarized below:

1955: 2 uranium mines; sporadic measurements;

1958: 15 uranium mines; systematic measurements, assuming 80% of time spent in working areas (headings, stopes, raises) and 20% in travelways;

1955–1981: 131,000 RDP concentration measurements over 141 mine-years of operation (an average of 929 per mine-year, though fewer in the early years); mean extrapolation period (pre-1958) of 1.1 years (representing 23% of the total collective exposure expressed in person-WLM); "standard WL" and "special WL" by mine and calendar year, with "standard WL" considered more representative.

Table 12 shows the distribution of collective exposure based on extrapolated and measured WL values.

Table 12 Exposure (WLM) of Ontario miners based on extrapolated WL values [M23, M24]

Calendar year	Extrapolated standard WLM	Total standard WLM	Per cent WLM based on extrapolation
1954	843	843	100
1955	4 276	4 276	100
1956	20 806	20 806	100
1957	52 263	52 263	100
1958	9 736	87 048	11
1959	10 617	81 733	13
1960	12 902	55 275	23
1961–1977	0	185 079	0
TOTAL	111 443	487 323	23

190. It is not clear how the upper bounds for the exposures of uranium miners from 1955 to 1977 in reference [M19] were generated. Unfortunately, no justifications or explanations were given; however, as noted earlier, the exposures used for the early years were not likely to have been overestimated [D7].

191. The Ontario mining industry experienced frequent changes in market conditions. Mining companies and miners moved from mining one type of ore to mining another, and from one location to another [M19]. According to Muller et al. [M15], the miners were all requested to fill in detailed employment information concerning their first 60 months of mining experience so that those involved in mining other ores could be identified and excluded from the study.

192. For the period 1955–1977, full-time miners were assumed to spend 80% of their working time underground and 20% on travelways. Part-time miners were assumed to spend 50% of their time underground in areas with higher than average concentrations of RDPs. Up to the end of 1967, and in one mine up to 1 April 1968, WLM exposure values for each miner were estimated as WLM = WL × WHF × Months, where WL is the weighted average of WL measurements in stopes, raises and travelways; WHF is a work history factor introduced to account for overtime or work stoppages; and Months are the total number of actual months worked underground. The Ontario investigators set WHF equal to unity if normal working hours were maintained. The WHF was increased or decreased as appropriate if overtime hours were worked or if work stoppages occurred in a particular calendar year. After 1967, the individual exposure estimates were based on time cards, filled out daily by miners, and on WL measurements made in the particular work locations reported by the miner on his time card.

193. Smoking information on the Ontario uranium miners is very incomplete. The most recent studies of Muller et al. [M23, M24] reported the results of a 3% sample of men born prior to 1939. Smoking information was sought on 226 uranium miners who had no former gold mining experience. Smoking information was also sought on 80 lung cancer deaths that occurred between 1955 and 1981 in the cohort of uranium miners with no former gold mining experience. Of the 80 lung cancer deaths, smoking information was obtained on 73 men; of these, 72 were smokers or former smokers [M22].

194. Studies of lung cancer incidence and mortality in Ontario gold miners reported by Kusiak et al. [K11, K12] suggested that both radon and arsenic might be causative factors in lung cancer. Kusiak et al. [K12] noted that available data indicated that dust concentrations in some gold mining occupations in the 1930s and 1940s were often above 1,000 particles/mL (p/mL), decreasing over time to an average of 400 p/mL by 1959 and 200 p/mL by 1967. Geological data confirmed the "anomalously high arsenic levels where gold is found..." and "that arsenic concentrations...are regionally enriched". The authors noted, however, that no excess of lung cancer could be identified in gold miners who began mining gold after 1945 [K12].

195. According to reference [K12], no RDP concentration measurements were made in Ontario gold mines prior to 1961. RDP levels in gold mines were variable. In some mines, average WL values in inactive areas were 0.3 WL, while in other mines, levels were below 0.02 WL.

196. Increased ventilation rates and related practices introduced since the 1950s may have had less effect on ore dust concentrations than on the concentration of RDPs in the mine air. According to Ham, dust levels in Ontario uranium mines decreased by only about a factor of 2 between 1960 and 1975 [H1], while the estimated concentrations of RDPs decreased by about a factor of 5 over the same period [M21]. On the other hand, data reported by DSMA Atcon Ltd. [D7] and by Duport and Edwardson [D10] suggested that the levels of ore dust and/or RDPs in the mine air diminished by comparable amounts over the years in the Ontario mines.

197. In the Elliot Lake mines, thoron decay products also contributed an appreciable radiological exposure; their concentrations, however, were less affected by increased ventilation than the concentrations of RDPs [J9]. Data for two Elliot Lake mines reported by Chambers et al. [C2] showed thoron decay product levels ranging from 0.1 to 0.3 WL, with parallel RDP levels ranging from 0.2 to 0.5 WL. This suggests a ratio of thoron decay product exposure to RDP exposure of about 0.5.

198. A great deal of information is available concerning the working environment in the Elliot Lake mines. Some data were developed by the mining companies for engineering or regulatory purposes, while other data were developed through the various research activities of the Elliot Lake Mining Research Laboratory operated by Energy Mines and Resources Canada, the Atomic Energy Control Board (AECB) of Canada and the mining companies themselves. Data are available on various subjects, including: the effects of using diesel equipment on the characteristics of mine air [B8, K2, K3]; the composition of the mineral dust (70% quantity) [B7, K4]; particle and activity size distributions [B6, B9, D10]; gamma radiation levels [C5, C6]; and arsenic levels in Ontario gold mines [O1]. A 1986 study [S11] examined the potential to use electrostatic precipitation to reduce radioactive aerosols in underground uranium mine atmospheres. This study further summarized available data on dust loadings, particle size distribution and the attached fraction of the RDPs. Overall, detailed information is available to characterize the mine environment for the 1970s onward, but little information is available for earlier times.

4. Epidemiological analyses

199. In the Ontario uranium miner cohort, the average miner had, in 1984, 1½ years of mining experience and a median age of 39 years. Overall, there was an average of

15.1 person-years at risk per man. Muller et al. [M26] reported mean exposures for this group in the range 40–90 WLM.

200. The present cohort consists of men who worked a half-month or longer in an Ontario uranium mine between 1 January 1955 and 31 December 1977 [M19]. The exclusion of those with known asbestos exposure or with exposure in uranium mines other than in Ontario reduced the cohort size to 15,984 men. It was discovered that 66% of these miners had OHR mining experience and that OHR mining, particularly gold mining, increased the risk of lung cancer significantly [M23, M24, M26]. Exclusion of miners with OHR mining experience reduced the cohort size to 5,443 [M19]. The Ontario studies demonstrate the importance of identifying OHR mining experience, since uranium miners with previous gold mining experience exhibited excess risk of lung cancer even at zero exposure from uranium mining. In men who worked as gold miners before becoming underground uranium miners, 92 deaths from lung cancer occurred, compared with the 55.7 expected [M22].

201. In a follow-up analysis of mortality from lung cancer in Ontario uranium miners, Kusiak et al. [K13] re-examined the Ontario uranium miner cohort; mortality follow-up was extended from 1981 to 1986. An association between excess lung cancer and RDP exposure was found in the miner cohort; this was similar to that found in the same cohort with follow-up to 1981. The study found that lung cancer mortality in Ontario uranium miners who also mined gold was related to exposure from both RDPs and arsenic.

202. In a cohort and case–control analysis of Ontario miners, Finkelstein [F13] investigated the presence of silicosis as a risk factor for lung cancer. A cohort of 382 miners with silicosis and 970 controls were developed from the 68,000 workers in the Ontario database. Data were available for 94% of the silicosis cases and for 99% of the controls. In discussing his cohort analysis, Finkelstein noted that there was a significant excess of cancer (mainly lung cancer) in miners with silicosis and that men with normal radiographs had a lower cancer incidence than the Ontario average. Finkelstein used a case–control methodology and logistic regression to assess the risk of cofactors. He found that silicosis is a highly significant risk factor for lung cancer. In an analysis of cumulative risk, Finkelstein calculated a weak association between silicosis and lung cancer with OR = 1.004 (95% CI: 0.9967, 1.011), while in his model of the joint effect of cumulative radon exposure and silicosis, he found no association of lung cancer with radon (OR = 0.995; 95% CI: 0.986, 1.004) and a strong association with silicosis (OR = 6.99; 95% CI: 1.91, 25). BEIR VI [C20] and the UNSCEAR 2000 Report [U2] reviewed the available data on the effects of exposure to silica in underground miners and commented on various studies, including that of Finkelstein [F13]. Overall, there is a range of opinions on the effects of exposure to silica in the mining environment, and some uncertainty remains concerning the influence of silicosis on risk of lung cancer.

203. One of the most important observations from the Ontario miner study was the use of a “time since exposure” effect, where the risk of lung cancer decreased with increasing time since exposure [M22]. NCRP Report No. 78 [N7] previously reported a dose–response model where the excess absolute risk of lung cancer was assumed to be zero before 40 years and then, following an initial latent period of five years, to decline exponentially with time since exposure. The concept of declining risk with increasing time since exposure was subsequently incorporated in the analyses of others, including Lubin et al. [L10], BEIR IV [C19] and BEIR VI [C20].

5. Evaluation

204. The Ontario miner study is a large well-defined study cohort with considerable information available on which to base exposure estimates. Researchers have attempted to assess the effect of uncertainty in exposure through the use of standard and special working levels, which are mainly estimates. While more could be done in quantitatively evaluating the effect on dose response of uncertainty in exposure estimates, the cohort provides one of the highest-quality studies available for radon risk estimation. Future updates will further increase the value of this cohort.

C. Czechoslovak miners

1. Introduction

205. Mining in Jachymov (Joachimsthal), Bohemia (now in the Czech Republic), started at the beginning of the 16th century. Thousands of silver miners were involved. Mining for cobalt, bismuth and arsenic started later in the 16th century. In the middle of the 19th century, uranium was mined for use in the glass and porcelain industry. Between 1909 and 1925, Jachymov was devoted to pitchblende mining in pursuit of ^{226}Ra, with an average annual production of about 26 g of radium [L6].

206. Mining conditions in the early years were poor. The mines were usually damp (especially in the spring when the snow was melting) and cold. Miners had to descend ladders hundreds of metres to their working areas. Natural ventilation was provided by a gallery between all the mines, and, according to Lorenz [L6], it was generally “sufficient”, except in dead-end shafts.

207. Although high death rates among miners in their prime years caused by lung-related diseases were recognized and recorded as early as the 1550s, no detailed studies of this were performed. In 1879, Harting and Hesse (as reported in reference [L6]) became the first to conduct organized investigations on workers at the Schneeberg mines, which are across the border from Bohemia, in Saxony (Germany). They found that 75% of the deaths were caused by malignant growths in the lung, and that the incidence was greater among miners than among masons or carpenters working

in the mines [L6]. From 1869 to 1877, 150 deaths due to "miner's disease" were identified in a workforce of 650 men. The onset of the disease occurred after about 20 years of work in the mines. According to Lorenz [L6], Harting and Hesse were the first to diagnose miner's disease as lung cancer but, owing to the high levels of dust in the mines, they assumed that the inhaled arsenic and poor nutrition were the predisposing factors for the disease.

208. A more recent paper by Greenberg and Selikoff [G7] reappraises the data reported by Harting and Hesse in 1879, and of the early efforts to identify the cause of lung disease among the miners and to measure how much dust the miners inhaled. One experiment (as reported in reference [G7]) concluded, "a miner inhaled 0.231 g [sic] of dust in a 7 h shift". Unfortunately, it is not clear whether the studies of Harting and Hesse led to any improvement in working conditions. The identification of RDPs as the primary causal agent was left to later investigators.

209. More investigations followed. Rostoski and Saupe, in a 1921 study of selected miners and non-miners (507 people in all), identified lung cancer as a common cause of death among miners [L6].

210. Pirchan and Sikl [P5] observed the first case of lung cancer in Czechoslovak radium workers in 1926. In 1929, Lowy [L48] reported two deaths of Jachymov miners from lung cancer. Further study of the issue was commissioned by the Czechoslovak Ministry of Public Works. In 1929–1930, Pirchan and Sikl [P5] examined necropsies in 13 of the 19 miners who died during that period. They stated that lung cancer incidence was highly prevalent in Jachymov miners and suggested that "radium emanation" (radon) was the most probable cause. Lorenz [L6], however, later suggested that genetic susceptibility to lung cancer in miners must be unusually high.

211. Once radioactivity was recognized as a major cause of lung cancer deaths among miners, measures were taken to reduce exposures. Drilling with water wash-out was introduced around 1930 to reduce the amount of airborne radioactive dust [S24].

212. Prior to 1932, the mines were ventilated using natural ventilation alone. Beginning in 1932, this was in some cases reinforced by mechanical ventilation. After the Second World War, mining started again. As early as 1946, the Czechoslovak Ministry of Health started collecting miner mortality data to better understand the hazards of exposure to RDPs. Up until 1952, ventilation continued to be mainly by natural means; however, in the 1950s, artificial ventilation, both general and local, was systematically introduced into all mines. From 1955 onward, all mines were mechanically ventilated. From 1956 onward, auxiliary ventilation was also provided in selected areas where appropriate. In 1954, the Czechoslovak Hygienic Service started undertaking inspections of working conditions in uranium mines. Increased efforts to reduce radioactive contaminants by increased ventilation and tighter safety regulations commenced in 1966 [V1].

213. Working conditions were improved as the knowledge of the risks of lung cancer in miners from exposures to short-lived RDPs increased. According to Vesely and Sada [V1], a dramatic reduction in radon concentrations took place in Czechoslovak mines during the 1950s. Mean cumulative exposures of about 310 WLM were reported for a cohort of miners who started work in 1948–1952. The mean cumulative exposure of miners starting work in the years 1968–1972 was 40 times lower [S24].

2. Radon and radon decay products

214. Pirchan and Sikl [P5] reported three active pits in Jachymov prior to 1930. The quantity of radium emanation (radon) found in the air discharged from the Svornost (Harmony) pit (depth 500 m) was 4 Mache units, from the Werner pit (depth 476 m) 15 Mache units and from the Saxon Nobility pit (depth 120 m) 10 Mache units. (One Mache unit is approximately 10 Bq/L, see Behounek [B3]). Pirchan and Sikl realized that radioactivity levels varied throughout the mine and suggested taking measurements at various locations to assess the exposures to miners [P5]. Lorenz [L6] made a similar observation.

215. Water is a significant source of radon in underground mines. The Czechoslovak mines were no exception. Behounek [B3] reported radon in groundwater at levels of up to 426,000 pCi/L in the Jachymov mining district.

216. Systematic radioactivity measurements were not performed in Jachymov until the late 1940s. From 1949 to 1960, radon concentration measurements were recorded. Hundreds of readings for each uranium mine for each year during this period are available [S18]. The "classical" method was used to measure radon, i.e. measuring the current with an electrometer in an ionization chamber [S20]. The measurement of RDPs did not begin in Jachymov until 1960 [S20]. In 1968, personal exposure records, which took working place and work time into consideration, were established. The records were reported quarterly for each individual [S29].

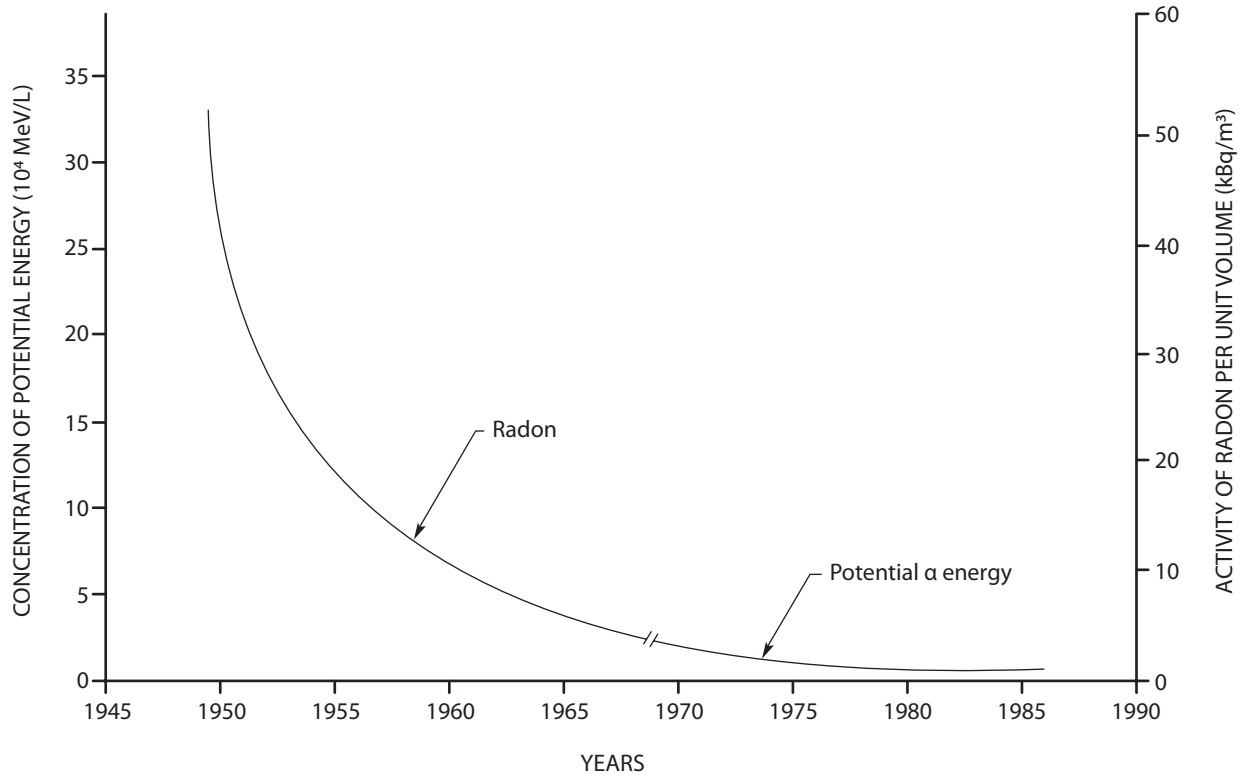
217. Data on attached/unattached fractions are sparse. Some data were collected from 1963 to 1965 by the Czechoslovak Academy of Sciences. These measurements indicated that, typically at that time, the fraction of free unattached radium A (historical name for ^{218}Po) atoms was approximately 10% in the Czechoslovak mines. The AMAD (activity median aerodynamic diameter) was estimated to be between 0.05 and 0.2 μm, with the mean at about 0.1 μm [H2].

218. In 1968 and again in 1973, there were "ventilation incidents" in underground uranium mines in Czechoslovakia [H2]. In both incidents, mechanical ventilation stopped for a period of time. During these periods, radon/radon decay product levels were measured to assess the equilibrium conditions that might have existed in the early days of mining, prior to the introduction of mechanical ventilation. On the basis of data collected during these

two incidents, an overall equilibrium factor of approximately 86% for the period 1948–1952 was estimated. It was noted, however, that in some of the newer mines, the situation was better. From 1953 to 1959, a nominal equilibrium factor of approximately 55% was assumed. For the period 1960–1966, a radon equilibrium factor of 36% was assumed; some of the older mines had higher equilibrium factors and some of the newer mines had lower equilibrium factors.

219. During the period 1948–1952, when the uranium mines in western Bohemia were naturally ventilated, 40% of the measurements indicated radon levels in excess of 1,000 pCi/L. The early high radon levels and the improvements that took place are illustrated in figure V. The break in the curve shown in figure V indicates the change from routine radon measurements to routine RDP measurements as the primary basis for the estimation of exposure.

Figure V. Evolution of the average radon concentrations, 1949–1968 (right ordinate axis), and the average potential (alpha) energy concentration, 1969–1981 (left ordinate axis) [H2, K7].



220. The free unattached fractions of RDPs shown in table 13 were obtained from a series of measurements in uranium mines in Pribram, Czechoslovakia, in 1988 and 1989 [H2].

221. Measurements made more recently in an eastern Slovak iron ore mine with high dust levels (poorly ventilated) showed low unattached fractions. In one series of measurements, 1.6% ± 0.8%, and in a second, larger series, 2.3% ± 1.3%, of (equivalent) RDPs were unattached [H2]. According to investigators, Jachymov mines were thought to have unattached fractions of 6–10% [H2].

Table 13 Unattached fraction of RDPs in Czechoslovak mines (adapted from reference [H2])

	Range (%)	*Mean (%)*
^{218}Po	12.0–43.0	27.5
^{214}Pb	4.0–15.7	9.4
^{214}Bi	0.6–10.0	5.3
Overall	5.9–23.0	14.4

3. Exposure estimation

222. Sevc et al. carried out the main Czechoslovak epidemiological studies on uranium miners. The first study was in 1971 [S17] and there have been several updates [K8, K9, S18, S19, S20, S25], the most recent being in 2004 [T40]. The study involved miners who started uranium ore mining between 1948 and 1957. Sevc et al. [S20] reported that "estimates of working levels of RDPs (WL) or radon gas concentrations were made on the basis of records of the ventilation conditions and practices, emanation rates from different types of ores, and RDPs measurements made in 1960 and later" and also "the values for working level months (WLM) were estimated on the basis of radon gas measurements and from data on the number of months of employment with each mine and within each calendar year of the whole employment period for each miner." No further details were given of how each factor was accounted for. However, the authors estimated the coefficients of variation for WL estimates to be <27%, and <30% for WLM estimates.

223. The average annual RDP concentration (WL) in each shaft was used to calculate each man's exposure on the basis of his working time. Payroll cards were available for all men in the study groups. Beginning in 1968, individual personal dosimetry cards recorded each miner's exposure. Discussions with the Czechoslovak investigators indicate that the exposures estimated using time-weighted area measurements are unlikely to differ by more than a factor of 2 from those obtained from personal dosimetry [H2].

224. Radon measurements were obtained for four types of working area: mine workplaces in close proximity to the ore (stopes), where the levels were the second highest in the mine; hallways and corridors where there was no ore; chimneys (raises), where the levels were the highest; and transport ways. Where no data existed on a working area, the average value for the entire mine in a given year was used. Typically, 20% of the total number of measurements was made in transport ways and 80% of the measurements were made in the other working areas.

225. Individual miner exposures were calculated on the basis of job descriptions recorded in personnel cards for all miners (the use of which was begun in 1948 for payroll purposes). The estimates of time spent in the workplace are thought to be reasonable, since the miners were under surveillance by a controller and a mine technician, the latter being responsible for rating the workers' pay.

226. Very few measurements of the concentrations of radon in the workplace are available for the years before 1948. Estimation of the exposures of miners before this date would require consideration of many factors, including the radon levels recorded in later years and knowledge of early mining practices and ventilation systems. However, according to Hamilton et al. [H2], Group S consists of Czech miners who began mining in the period 1948–1959 and worked for four years or more; it does not include miners with pre-1948 exposures, and therefore this aspect is not important.

227. The employment history of the miners is another very important factor in estimating exposures. Jachymov is situated in the Erzgebirge (Ore Mountains), where abundant minerals are found. As discussed earlier, there was a long history of mining prior to 1909, when pitchblende was first mined. The early miners probably had previous mining experience (e.g. [L6, P2]). On the other hand, tin mines in the Erzgebirge were not operated between 1931 and 1939, and during the Second World War, the mines employed mainly prisoners [T40]. These facts limit the possibility of previous mining experience among cohort members, and this is consistent with Sevc's claim that less than 2% of the epidemiological study group mined non-uranium ores before they mined uranium [S24].

228. By definition, a "working month" conventionally now means 170 hours of work. If a miner held more than one job with a mine in the 1920s–1950s, the working hours spent in each would need to be taken into account in order to determine his exposure. Sevc et al. [S20] stated, however, that only the number of months of employment during a year was taken into consideration. There was no evidence that the miners' actual working patterns were incorporated in the WLM estimations. In discussions held in 1988, the Czechoslovak investigators commented that the workers spent about 80% of their time in the workplace and 20% in the transport ways. In the early days, the men worked 8 hour shifts (3 shifts per day), 6 days per week. After 1968, the normal working week was reduced to 5 days. Uranium miners had a total of 5 weeks of leave per year. By 1990, retirement from uranium mining was mandatory at age 50, but some retired uranium miners often continued to work in other (especially coal) mines. After 1966, people over 40 years of age were not accepted into uranium mining as new miners. Later reports [T37, T38] on the Group S cohort gave more details on how information on jobs was used.

229. On the basis of more recent (preliminary) surveys [T29], Czech investigators estimate the exposure of the general population to RDPs in typical areas of the Czech Republic to be about 0.34 WLM/a, whereas some residents of the Jachymov area could be exposed to 3–4 WLM/a. However, preliminary evaluations suggest that correction for at-home exposure, which would have shifted miners to higher exposure categories, did not affect the risk estimates significantly. The effect of exposure away from work is subject to ongoing investigation.

4. Epidemiological analyses

230. Czech investigators have studied several groups of underground uranium miners. The most studied group is the Group S cohort. These miners represent approximately 11% of the underground miners employed in the Jachymov and Horni Slavkov mines. The cohort originally included

4,364 men [S17]. Reported differences in the size of this cohort are due to whether or not emigrated miners were included. The most recent results for the Group S cohort included 4,320 miners [C20, T37].

231. In discussing the accumulation of WLM exposure, Sevc et al. [S20] indicate that person-years at risk were assigned totally to the final WLM category reached by each individual miner, rather than being distributed across each WLM interval as they accumulated. This affected the estimation of the expected number of lung cancer cases in each exposure category. This difference was discussed by Kunz et al. [K8], who re-evaluated the epidemiological data of reference [S20] and concluded that the distortion caused by earlier methods was not large. Since 1978, all analyses [K8, K9, S25] have used the conventional approach.

232. In earlier papers (1971–1988), the observed numbers of deaths from lung cancer were compared with the numbers expected from general mortality data. This was justified by an investigation of a random group of 700 miners that showed that 70% were smokers, similar to the general male population of Czechoslovakia. However, in publications since 1991, the expected numbers were modified by incorporating a multiplicative parameter that allowed the background mortality to differ from that of the general population. This approach is close to the "internal approach" if additional stratification for age and calendar year is used [T2, T26].

233. In the past, papers on epidemiological studies of Czech miners did not usually indicate the numbers of miners involved, and therefore it has sometimes been necessary to back-calculate from reported data to obtain the number of miners in the various groups. However, in the first paper [S17] and since 1988 [S25], the numbers of miners were reported.

234. In 1988 [K7, S25], investigations of other study groups were reported (table 14). These include Group S, with subgroups A and B covering the underground uranium miners whose exposure began during 1948–1952 and 1953–1957, respectively. These two subgroups represent the main cohort for epidemiological investigations. In addition, investigations of a number of other study groups are also reported: study Group N, which comprised uranium miners who started exposure at levels lower than those in study Group S; a small study group, Group K, which comprised miners in iron mines in eastern Slovakia; and study Group L, which comprised miners from the Czech shale clay mines. Studies of non-uranium miners were completed with a Czech study of tin miners [T40].

Table 14 Czech and Slovak studies of miners exposed to radon [S25, T40]

Study group	*Type of mine*	*Location*	*Cohort size*	*Exposure (WLM)*	*Latest reference*
S* (= A + B)	Uranium	Western Bohemia	4 320	152	[T38]
N* (= C + D)	Uranium	Central Bohemia	5 622	7	[T38]
K	Iron	Eastern Slovakia	1 056	40	[S25]
L	Shale clay	Central Bohemia	916	25	[S25]
C	Tin	Northern Bohemia	2 466	54	[T40]

235. A more recent study by Tomasek and Placek [T2] investigated risks to a subgroup of miners whose exposures were restricted to lower exposure rates. This subcohort had a total of 419 lung cancers to the end of 1995. A decrease in relative risk with time since exposure and age at exposure was observed. Differences in the risk estimates for epidermoid and small cell cancers were also identified, although each had a pattern of risk similar to that of lung cancer overall. The authors found no evidence for non-linearity or dependence on exposure rate (at RDP concentrations of below 8 WL), although the average dose-rate effect was seen in the Group S cohort as a whole.

236. A subsequent study by Tomasek [T26] investigated lung cancer risk in a cohort of 5,002 miners exposed in two different periods. Exposures of the 2,552 miners in the older cohort (S) were derived from workplace radon measurements commencing in 1949. For the 2,450 miners in the newer group of miners (N), exposures were based on individual dosimetric records. For the newer subcohort (N), smoking data were available for most (about 85%) of the miners. For the older subcohort (S), smoking data were available retrospectively for 279 cases and 410 (nested-in) controls. Follow-up was to the end of 1999. The analysis was based on a relative risk model that allowed consideration of time since exposure and attained age or age at exposure. Excess relative risk (ERR) was linearly dependent on cumulative exposures received more than 5 years previously. The ERR was 0.045 (90% CI: 0.017, 0.140) per WLM among non-smokers (42 cases) and 0.02 (90% CI: 0.011, 0.035) per WLM among smokers (309 cases); the differences between the two estimates were not statistically significant. The lung cancer risk in miners who smoked was about 10.8 times that in non-smoking miners (this included those who had not smoked for the previous 20 years). The ERR was found to decrease by more than 60% per decade of time since exposure and simultaneously by more than 40% per decade of age at exposure.

5. Evaluation

237. In comparison with studies reported in BEIR VI, the S-cohort had (by 1990) the second largest number of lung cancer deaths. Exposure information in the S-cohort is among the most extensive ([C20], p. 322). Extensive measurements of radon in all shafts are available almost from the first years of exposure. In results published in 2003 [T30], only 4% of all exposure years are not based on radon measurements. The most recent results on the Czech uranium miners [T39, T40] are based on a total of 929 lung cancer cases. This combined cohort includes a large proportion of miners with exposures based on detailed personal dosimetric data and provides an opportunity to investigate the role of potential modifiers of effect.

D. Swedish iron ore miners

1. Introduction

238. Iron ore has been mined in Sweden since medieval times. Originally, there was only open-pit mining, but around 1910 underground mining started [E1, E2]. Before 1945, ventilation was entirely natural. According to Axelson [A11], some mechanical ventilation was developed during the 1940s and 1950s to prevent water freezing underground. Air was warmed by taking it through old shafts before it reached the workplaces. Snihs [S36] commented that in some mines, ventilation air was brought into the mine through crushed rock; this method, although reducing airborne dust and raising the inlet air temperatures, also picked up any radon emitted from the old shafts or crushed rock. In addition, travel time resulted in the ingrowth of RDPs.

239. According to Snihs and Ehdwall [S36, S39], the primary sources of radon in the Swedish iron mines were incoming radon-rich water and, to a lesser extent, radon from radioactive minerals. The uranium content in the waste rocks in the iron mines was of the order of 15–20 ppm [S39]; however, relatively high emanation coefficients (30–40%, measured in accordance with the procedures set out in reference [A20]) for some of the rock were a contributing factor to elevated radon levels in these mines. The radon problem having been identified in about 1968, ventilation in the Swedish mines was gradually improved. The ventilation path was changed to bypass crushed rock and incoming groundwater, thereby leading to reductions in exposure [S37].

240. Snihs [S16] summarized the status of knowledge about RDP levels in Swedish mines around 1972:

> "We measured only the radon concentration in many mines to get a rough idea of the radiation problem in the mines. To get the corresponding radon decay product concentration we then applied the factor 0.5. The reason why we measured radon only, is that the mine companies were asked to send samples (in pre-evacuated bottles) by post. By that method we were able to make the survey in a relatively short time. I agree that the error may be great but it should not be more than ±50%, which is acceptable compared to other potential sources of error, even with a very sophisticated method, as local variations, seasonal variations etc. But we have tried to make all necessary corrections for these errors too as far as we know them. The result of that survey is seen in the table.

Radon decay products	*Number of mines*	*Number of workers*
<0.1 WL	25	1 121
0.1–0.3 WL	13	1 740
0.3–1 WL	17	1 739
1–3 WL	5	133

> This was the situation in 1969 and 1970. My part of the work is to make a "qualified estimate" of the radon decay product exposure during the last 20 years, which will be rather problematic I suppose."

241. The Malmberget mine, as it now exists, is actually a combination of several mines that initially were separate. The iron ore deposit at Malmberget consists of about 20 distinct, large ore bodies that outcropped to the surface; in these ore bodies several open pits were started. Most of these mines were in line with each other, separated by low-grade iron ore formations.

242. Open-pit mining in the Malmberget mine area first began in about 1890. Even later, when the depth required for mining forced a change from open-pit mining to underground mining (by about 1930 all mines were underground), the bottoms of the mines were still above the general level of the surrounding terrain. The adits could therefore be driven from the side of the mountain to the bottoms of the underground mines, which in turn were connected to the bottoms of the open pits, thereby permitting natural ventilation of the mines. By 1955, the bottom levels of the mines reached below the level of the country surrounding the mountain, and the efficiency of natural ventilation declined as progressively lower levels were developed.

2. Radon and radon decay products

243. Early Swedish mine and miner data are summarized in annex G of the UNSCEAR 1977 Report [U9].

244. According to Snihs [S36], the first radon measurements were made in the early 1950s in the Boliden mine. However, limited knowledge about radon problems in non-uranium mines and a lack of experience in taking measurements delayed the institution of routine radon or RDP measurements. A general awareness that many non-uranium mines had significant radon levels arose in about

1968. This led to radon surveys in which the general procedure was to take 3–12 radon gas samples per mine during both winter and summer periods. Samples were collected in evacuated bottles and sent to the National Institute for Radiation Protection (NIRP) in Stockholm for analysis.

245. Each mine with RDP levels of 0.3 WL or greater was investigated further by NIRP staff. They visited the mine and took RDP samples using the Kusnetz method, as well as many additional radon gas samples. Typically, more measurements of radon gas concentrations than of RDP concentrations were taken. According to reference [S28], the equilibrium between radon and RDPs was found to vary greatly but was typically 50%.

246. To provide a basis for estimating the equilibrium factor, simultaneous measurements of radon and RDP concentrations were taken. Typically, an average equilibrium factor was applied to all working areas in a mine or parts of the mine [S37]. According to Snihs and Ehdwall [S39], the equilibrium factor varied widely, ranging from 0.15 at the air inlet of the mine to nearly 1 at the air outlet. Average equilibrium factors in workplaces were typically between 0.4 and 0.6 [S39]. Axelson [A11], however, felt that a more appropriate typical equilibrium factor was 0.7.

247. Swedish measurements of radon were typically made by the NIRP using 4.8 L conventional propane containers. The containers were evacuated by the NIRP and subsequently opened at places of interest in the mines. After sampling, they were sealed and mailed back to the NIRP for analysis in ionization chambers [S39].

248. Following the first measurements of radon, the mines were divided into zones by radon or RDP level and subsequently checked according to the following frequency:

Zone 1: <10 pCi/L (<0.1 WL), once every two years;

Zone 2: 10–30 pCi/L (0.1–0.3 WL), every year;

Zone 3: 30–100 pCi/L (0.3–1 WL), once every six months.

For areas with levels of greater than 1 WL, measurements were to be taken every three months, according to Snihs and Ehdwall [S37].

249. In 1986, Radford and St. Clair Renard reviewed the history of mining methods and of general ventilation in the mines (reported in reference [S28]). When the mines were first converted from open pits to underground mines, the underground method of extraction was by shrinkage stoping. Small pillars were left between the large shrinkage stopes. After the shrinkage stopes were drawn empty of broken ore, the hanging wall was allowed to cave in and fill the opening. Mining methods evolved from shrinkage stoping, followed by sublevel stoping, and in 1965 by sublevel caving, which allowed even larger quantities of wall rock to cave in. Until 1973, fresh air was drawn through the broken rock left above the mining areas by these two methods; while the principal source of radon in the Malmberget mine was likely to have been radon-rich mine water, it is likely that additional radon entered the mine air because of this and the method of ventilation.

250. Extensive recirculation of air was widely practised in the Malmberget mine in the 1950s and 1960s. This recirculation could have permitted the buildup of both radon and the equilibrium factor during that period. Changes in mining methods in the mid-1960s made it necessary to introduce diesel equipment, which in turn led to the requirement to improve mechanical ventilation. Overall, Swent and Chambers [S28] concluded that the pre-1969 WL values given by Radford and Renard [R2] were likely to have been underestimated by a factor of above 2, as they did not take into account: the earlier practice of recirculating air in the mine; the lower volumes of air circulated through the mine in earlier decades; periods of stagnant ventilation airflow, which occurred during the years when natural ventilation was the only ventilation method; and the pattern of decline in the incidence of silicosis in later years, confirming the improvement in ventilation.

3. Exposure estimation

251. Snihs [S36] developed his estimate of risk of lung cancer by assuming that RDP levels in Swedish mines measured since 1969 were representative of earlier years. Snihs [S36] made “qualified guesses” for exposures that may have occurred in relation to the observed mortality from lung cancer. Snihs and Ehdwall [S39] provided further discussion of the measurements of radon and RDPs that were started only in the late 1960s in Swedish non-uranium mines. These authors noted that the earliest measurements of radon in mine air at Malmberget were in 1968 but that subsequently extensive measurements of radon and radon decay product concentrations in air were made by the NIRP and the Swedish mining company LKAB. Radford and Renard [R2], noting that the new ventilation system for mines became operational in 1972, stated that the reconstruction of past concentrations depended on the measurements made during the period 1968–1972 and on knowledge of the natural and mechanical ventilation used previously. However, the authors noted that the reconstruction of Malmberget exposure data depended on the “assumption” that ventilation conditions in the mines in 1968–1972 were not greatly different from those in the past [R2].

252. Key features of the exposure estimation for Swedish iron miners in the study of Radford and Renard [R2] were:

- 1930: nominal start of the study;
- 1969: first (NIRP) radon measurements;
- Until 1973: fresh air drawn through broken rock (preheated);
- 1955: mechanical ventilation introduced to replace natural ventilation;

- Until about 1965: some air recirculation occurred;
- 1965: diesel engines introduced into the mines;
- All exposures "guesstimated".

Radford and Renard [R2] assumed constant exposures prior to 1968. This is unlikely because of the changing ventilation practices (e.g. increased volume and elimination of recirculation) and the introduction of mechanical ventilation prior to the first radon measurements.

253. As discussed above, the data available from measurements of radon and radon decay product concentrations make it possible to estimate relatively well the exposure in mines after 1970; however, few if any measurement data are available from the period before 1969, and early data must be estimated from a combination of later exposure data, reconstruction of measurements and consideration of ventilation practices, radon sources and other factors. A paper by Bergdahl et al. [B45] provides a comprehensive re-evaluation of radon exposures in the Kirunavaara and Malmberget iron ore mines. The re-evaluation suggests that radon levels were higher than in the estimate by Radford and Renard [R2], but not as high as suggested by Swent and Chambers [S28].

254. In the epidemiological study of miners in Malmberget by Radford and Renard, undertaken in 1984, the historical exposure was estimated from data from 1968–1972 [R2]. Also in this study the equilibrium factor F_{eq} was assumed to be 0.7. The same exposure estimate was used for all underground workers except those that worked in Koskullskulle (these workers seldom changed their working place; Koskullskulle was a separate mine that was not a part of LKAB until 1953). The radon exposure (expressed as WLM/a) was estimated in reference [R2] for Malmberget as follows: 1970–1972: 3.2; 1960s: 4.9; 1950s: 6.2; 1940s: 6.9; 1930s: 4.6; 1920s: 4.8; and 1910 and earlier: 4. For Koskullskulle, the authors [R2] estimated the exposure for the period 1920–1969 as approximately 2 WLM/a and for 1970–1972 as approximately 1 WLM/a.

255. Bergdahl et al. [B45] estimated the exposure during the period 1925–1972 to have been constant at between 0.8 and 17 WLM/a, depending on the ore body in which the work took place. In cases where the location of the work was unknown, the exposure was estimated to have been 6 WLM/a. In Koskullskulle, the exposure in the period 1925–1972 was estimated to have been 5 WLM/a, with values before 1925 gradually increasing from the 1910 value of 0.8 WLM/a.

256. The principal differences between the exposure matrix proposed by Bergdahl et al. [B45] and the earlier exposure estimates of Radford and Renard [R2] are that Bergdahl et al. used a lower exposure value for 1970–1972, a higher value primarily during the 1940s and 1950s, and a linear extrapolation back to 1890. Furthermore, Bergdahl et al. [B45] used substantially higher estimates of exposure for Koskullskulle than did Radford and Renard [R2].

4. Epidemiological studies

257. A number of authors reported epidemiological studies of Swedish miners, including references [A6, A7, A8, A9, A11, A13, C7, D1, D2, E1, E2, J11, J12, J13, L2, R1, R2, S21, S22, S36, S38, S39]. However, the basic reference for the study of Malmberget iron miners is the paper by Radford and Renard [R2], which included a description of the basis for the estimates of exposure to RDPs. This was a retrospective study of lung cancer mortality in a group of 1,415 Swedish iron miners. The total cohort represented 24,083 person-years at risk, with an average exposure of approximately 81.4 WLM. The study cohort included men born between 1880 and 1919, who were alive on 1 January 1970, and who had worked for more than a calendar year between 1897 and 1978. Follow-up of these miners was reported for the period 1 January 1951 to 31 December 1976. The authors estimated an ERR of 3.6% WLM^{-1}, and an excess absolute risk of lung cancer of 19 per 10^6 person-years per working level month. Miners were identified from company and union records of active and pensioned miners, which were available for the years since 1900, as well as from medical records and, in a few cases, from parish records. The Swedish Government gives every person a code at birth; this code is included in all work and hospital records. Every citizen is also required to register in a local parish of the state church. These requirements helped in locating the miners and their records. Also available from company records dating back to 1900 was the total number of man-hours worked underground each year in each section of the mine. However, the exact location of the work within the mine was not available. There is some uncertainty as to work status for miners who started or stopped work or who changed work function midway through the year. Miners were assumed to work 173 hours per month on average in the period 1890–1930; 162 hours per month during the period 1930–1950; and 144 hours per month from 1950 onward.

5. Evaluation

258. There were few radon measurements in Swedish mines and none in Malmberget mines prior to 1969. It was only after this date that investigations were initiated by the National Institute of Radiation Protection, Stockholm. Therefore epidemiological studies of Swedish miners were based on reconstruction of the radon and radon decay product concentrations to determine the exposure of miners. Since underground work began in 1932, investigators undertook this reconstruction for a period of 36 years. During this period, the conditions in the mines would have changed owing to changes in the mining methods and ventilation procedures. Two attempts were made to estimate the exposures [B45, R2]. The estimates obtained in reference [B45] for the Malmberget iron miners were higher than those used in the epidemiological study of Radford and Renard [R2]. However, to date, no epidemiological re-evaluation of this group of miners using the updated exposure data has been published. These updated exposure data represent a great

improvement in the quality of the data available on the exposure of Swedish miners and will provide an opportunity to update epidemiological analyses of Swedish iron miners.

E. Canada: Beaverlodge, Saskatchewan, miners

1. Introduction

259. The uranium mineralization at Beaverlodge in northern Saskatchewan, Canada, was discovered in 1946, and a prospecting mine shaft (the Ace shaft) was started in 1949. By 1951, the company concerned, Eldorado, had identified ore reserves sufficient to proceed with uranium production. By that time, ore bodies to the west of the Ace shaft had also been identified. In 1951, the Fay shaft was started as a production shaft serving the western ore bodies. An underground haulage way also provided access to the Ace ore body. By the 1970s, the mine was over 1.6 km deep and extended more than 5.6 km horizontally. In addition, a number of satellite mines, including two underground mines, were developed. Mining production in the area increased rapidly during the 1950s, but fell off in the 1960s as the demand for uranium declined. Eldorado recruited experienced miners from the area to work at Beaverlodge. The Beaverlodge operations were closed in June 1982. As the Beaverlodge mine developed, all three shafts were connected underground, and two winzes (shafts that do not come to the surface) were also constructed.

260. Mill construction started in 1952, and the first uranium concentrate was produced in early 1953. By this time the Verna ore body to the east of the Ace shaft was identified; work started on the Verna shaft in 1953, with production commencing in 1956.

261. Twelve small satellite mines, most being small open pits, were also developed during the 1960s and 1970s [G9]. Two underground mines, Hab and Dubyna, were also developed, both located several kilometres north and east of the Verna shaft.

262. Bloy [B11] noted that the first mining method used was shrinkage stoping. This was changed to cut and fill, using waste rock and surface sand as the fill material. In turn, this method was replaced by hydraulic tailings fill. The initial ventilation in working headings was mainly by compressed air from the drills, with some surface air being supplied by a 16 inch (~40 cm) metal vent pipe located in the shaft. Because of the lack of knowledge of the hazards of radon and radon decay products, the standards used to control the mine atmosphere conditions were the same as those used in gold mines at the time. This involved the measurement of airborne dust by the konimeter dust sampling method as well as radon measurements. An annual objective of the ventilation programme was to maintain a ventilation rate of 10–25 cubic feet per minute (cfm) of air per square foot[2] of working face.

263. In the cut and fill operation, ore was removed from the bottom, and mining advanced upward from one level to the next. The sand fraction of the mill tailings was used as backfill in the mine, with miners working off the backfill to remove the next lift. Simpson et al. [S30] investigated this activity as a possible source of radon and concluded that "no positive evidence was found that backfill was a major source of radon in the mine".

264. In addition to the Beaverlodge mine, Eldorado developed and operated a number of satellite mines. In addition, according to Garbutt [G9], there were a number of other uranium mines operating in the Beaverlodge Lake area of northern Saskatchewan. Eldorado's policy was to recruit experienced miners wherever possible. Consequently, as other local uranium mines closed and Eldorado remained in production, there were opportunities for miners with non-Eldorado working experience to migrate to the Beaverlodge operation.

265. The other (non-Eldorado) uranium mines in the Beaverlodge area operated for only a few years, and all had been shut down by the late 1960s. Most of these mines were small operations without mechanical ventilation (the exception being the Gunnar mine) and hence there was a potential in these operations for high exposures even in a short time period.

266. Estimation of individual employee exposures did not begin at the Eldorado Beaverlodge uranium mine until 1967. While some measurements of the concentrations of radon and radon decay products were made between 1954 and 1967, these were intended for monitoring ventilation rather than for personal dosimetry. Eldorado began routinely recording individual exposures in 1968, but considered that sufficient workplace data were available to assess individual exposures back to November 1966. The radon and radon decay product measurements from the earlier periods were summarized and utilized to provide exposure rate estimates by occupational grouping and year [F7]. These were point estimates, primarily based on the median concentrations recorded during the year, and were subsequently used in the original epidemiological analysis of Beaverlodge miners [H19].

267. The employee exposures were estimated by merging the annual exposure rates with information from the nominal roll. The nominal roll contained information on age, duration of employment and type of employment for Eldorado employees. The resultant exposure estimates were the basis for many epidemiological studies, including the original cohort analysis [H19], a case–control study investigating the effects of smoking and previous work experience [L17], the analysis of four underground mining cohorts performed by BEIR IV [C19], and the joint analysis of 11 underground miner cohorts in reference [L10] and BEIR VI [C20].

268. On the basis of these exposure estimates, the apparent lung cancer risk observed in the Eldorado Beaverlodge

[2] 1 cfm per square foot = 5.24×10^{-3} m^3 s^{-1} m^{-2}.

cohort was substantially higher than the lung cancer risk observed in the Eldorado Port Radium cohort [H18], where the estimated exposure rates were typically much higher than those ostensibly observed for employees who worked at the Beaverlodge mine. Given the substantially different lung cancer risks observed between the two cohorts, and the importance of epidemiology for estimation of risk from RDP exposure, the Atomic Energy Control Board (AECB) of Canada commissioned re-evaluations of the exposure rates at both the Beaverlodge [S12, S14] and the Port Radium uranium mine [S15].

269. Exposure conditions were re-evaluated for underground work areas at Beaverlodge between 1949 and 1968, when individual employee exposure estimates began. The re-evaluation of Eldorado Beaverlodge exposure rates suggested that previous estimates were underestimated by about 50% [S12]. The re-evaluation study noted that exposure rates varied significantly (i.e. by more than a factor of 10) between different areas of the mine. As a result of this workplace variability, substantial uncertainty in individual employee exposures resulted when an average mine-wide estimate was assigned.

270. A detailed investigation of Eldorado Beaverlodge records was conducted to further improve exposure estimates for a case–control group [S12]. Previous mining experience (including experience in gold and other uranium mines) was noted for several of the underground employees; however, these records were largely incomplete, and exposure estimates for this experience were not calculated. Improvements to the Eldorado Beaverlodge exposure estimates involved reviewing stope production records for the presence of individuals from the case–control group. Exposure rates specific to the area and the time where the individuals worked, rather than the mine-wide estimates, were then assigned to the identified individuals. The individual exposures based on specific mine areas were higher by a factor of 2–3 than those based on mine-wide conditions.

2. Radon and radon decay products

271. The first measurements of RDP concentrations were performed in 1954 at Beaverlodge; further measurements were made in 1956 as part of surveys of radiation levels, dust levels and general ventilation conditions [F7]. These initial surveys eventually led to a programme of radon and radon decay product measurements. Data from the 1954 survey indicated that simply turning the compressed air on or off gave rise to substantial changes in workplace concentrations. Whether a miner worked within this envelope of fresh air or in the "unventilated" region outside it was thus a very important factor in estimating his true exposure.

272. Early measurements were for the purpose of providing data for ventilation control. Originally, only the Tsivoglou method was available for RDP measurements. However, because this method is complicated, most samples taken were analysed for radon only.

273. According to Bloy [B11], a few measurements of RDP concentrations were made in the 1954 survey using the Kusnetz method. Over time, an increasing proportion of the measurements were done in terms of RDPs. By the mid-1960s, the Beaverlodge ventilation department was relying primarily on RDP measurements to assess ventilation conditions in the mine.

274. During the period 1954 to mid-1962, mining engineers also began measuring radon gas concentrations in the Beaverlodge mine as a means to determine the adequacy of mine ventilation, and not necessarily to determine miner exposures to RDPs. Radon concentrations were converted to RDP concentrations by use of equilibrium factors determined in the years 1954, 1956, 1959 and 1961 from the simultaneous measurement of radon and radon decay product concentrations. A large amount of data from the mine operating statements and radiation logbooks was captured in an electronic database by the 1991 SENES analysis [S12].

275. Prior to mid-1962, most of the radiation measurements were for radon. In the years 1954, 1956, 1959 and 1961, as mentioned above, paired measurements were taken where radon decay product and radon concentrations were measured at the same time in the same workplace. The average radon/radon decay product equilibrium factors were calculated from these data. Equilibrium factors for the early years (1954–1956) appear to be generally lower than for later years (1966–1968), and the equilibrium factors for the later years at high RDP concentrations (>2 WL) approached and sometimes exceeded the theoretical maximum value of 1.0. It was expected that the equilibrium factors for later years would be lower because of generally improved ventilation conditions. Radon concentrations without RDP measurements were multiplied by the equilibrium factor to estimate the corresponding RDP concentrations [S12].

276. The total number of radon and radon decay product measurements taken per workplace per year was generally less than 12 and frequently as low as one. The average during the period 1954–1968 was about four measurements per workplace per year.

277. The mine-wide underground estimates of exposure in units of WL produced in the 1991 SENES study [S12] are compared with the previous estimates in figure VI on the basis of various interpretations of exposure data, either WL measurements or WL inferred from radon measurements. The estimates are somewhat higher than those calculated using the medians of available exposure (WL) data, which included the estimates used by Howe et al. [H19], which in turn were based on those determined by Frost [F7]. Figure VII shows that there is considerable variation in WL values throughout the year — in winter, levels are higher than in summer — and in different years.

Figure VI. Comparison of radon decay product concentration estimates with the previous estimates for the Eldorado Beaverlodge mine [S12].

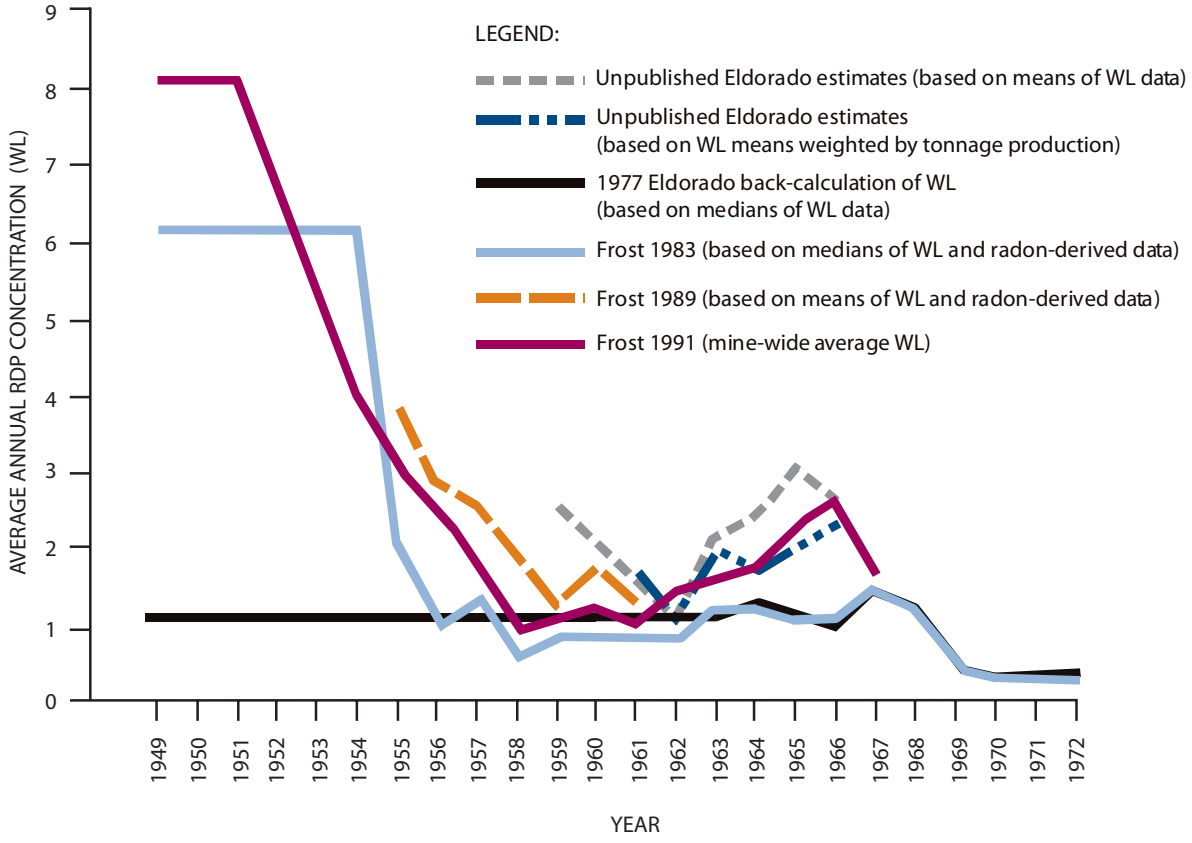
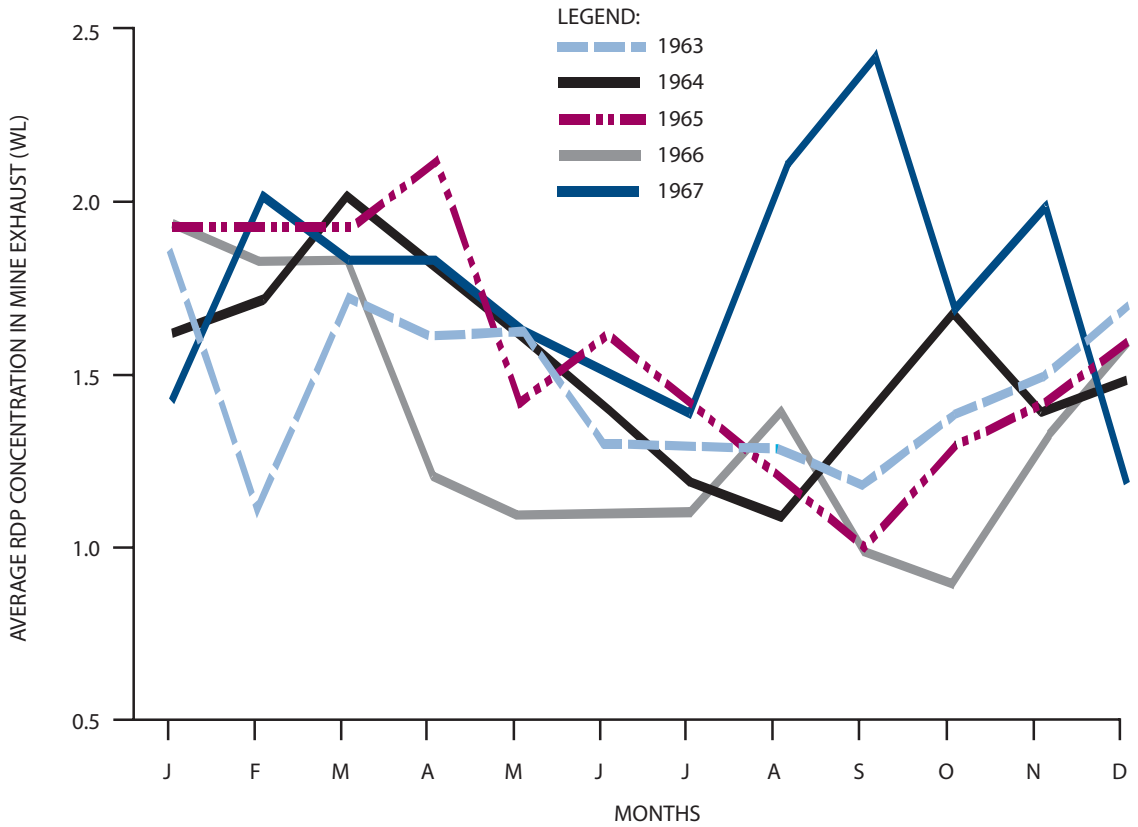


Figure VII. Seasonal variation of radon decay product concentration in Beaverlodge area mines operated by Eldorado [S12].
The high concentration in 1967 coincided with a fan failure.



278. Several factors changed over time at the Eldorado Beaverlodge mine. The calendar years from the start of operation (1949) onward can be subdivided into five periods based on a combination of the production and ventilation characteristics. The period 1949–1953 covered the development of the mine from early shaft sinking to the startup of the mill. The period 1954–1957 was a period of rapid expansion of the Eldorado Beaverlodge mine as well as of many other uranium mines in the Beaverlodge area; mine ventilation increased over the period concurrent with substantial development work. The period 1958–1962 was one of relatively little development at Eldorado, with most of the other area mines closing. The period 1963–1967 saw the development of two areas in the Beaverlodge mine that were not ventilated by flow-through methods; during this time, all other mines in the area were closed. The period from 1968 onward covers the remainder of mine operation, during which the ventilation was increased and individual personal exposure estimates were maintained.

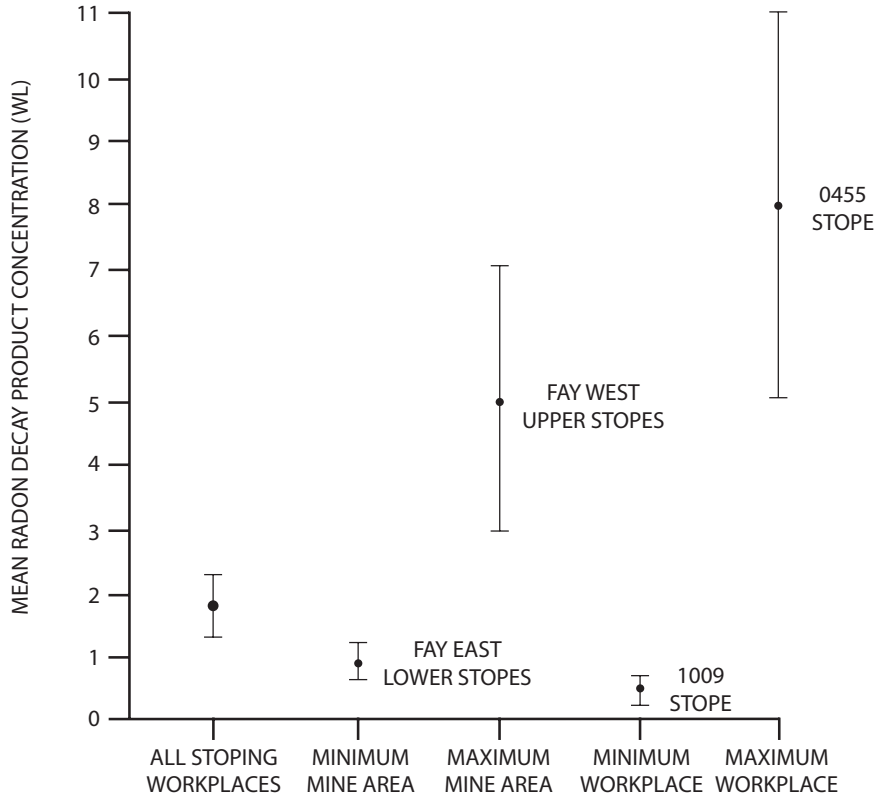
279. The 1991 SENES report [S12] discussed several sources of uncertainty in the WL estimates, including the lack of measurements in some of the early years (pre-1954). For these years, the workplace WL values were assumed to be the same as those estimated for 1954 [S12]. For the years in which no data were recorded, a linear interpolation of the data from the nearest years for which data were available was assumed. Even when measurement data are available, the measurements were focused on detecting deficiencies in ventilation as opposed to evaluating worker exposure.

280. An attempt was made [S12] to correct for these effects by excluding data for stopes and development headings where the mine operating statements did not identify any work activity. This did not mean that work was not going on in those areas. Therefore this procedure underestimated the exposures of radiation technicians and other workers who might, for various reasons, have been in those areas.

281. To correct for this, SENES [S12] weighted the reported WL values by the recorded level of activity as measured in man-shifts. However, there was oversampling in stopes relative to other areas with respect to the number of man-shifts worked. Other problems in assessing exposure were: the reporting, in early measurements, of radon concentrations rather than WL values; the uncertainty about the radon/radon decay product equilibrium factor, which led to uncertainty in the actual WL value; and the considerable variability in data within and among workplaces and with time.

282. Figure VIII illustrates, using data for the Fay area of the mine before 1963, the variability in the workplace data and the effects of agglomeration of the data. Moving from a mine-wide stope average (left side of the figure) to individual work areas, a dramatic increase in the difference between the minimum and maximum values (a factor of greater than 10) is evident. Agglomeration of data reconstruction relied on work history files from Eldorado. It was possible that the work histories of the study cohort were deficient with regard to non-Eldorado employment. This observation is important, because miners

Figure VIII. Estimates of mean radon decay product concentration (WL) by level of aggregation of stoping workplaces, for 1963, Fay area of Beaverlodge mine in northern Saskatchewan [S12].



could have worked in other mines for which no radiation exposure data were available. According to Howe et al. [H19], all exposures prior to 1 November 1966 were recorded as single lifetime totals rather than as separate annual exposures; this makes them less useful for epidemiological purposes. Howe et al. [H19] further indicated that the (annual) median value was used to describe (annual) average WLs.

3. Exposure estimation

283. In 1967, Eldorado began maintaining personal records of RDP exposure for full-shift underground workers. Workers' time cards indicated the hours spent in each workplace. These cards were consolidated into monthly printouts of manpower in each working place. These printouts, together with measurements made in the workplaces and travelways, were used to estimate monthly RDP exposures.

284. In 1970, the computer record system was expanded to include cumulative (i.e. lifetime total) exposure in the exposure summary reports. At this time, records of the previous measurements made for ventilation purposes (as opposed to the measurements made for the purpose of estimating miners' exposures) were used to back-calculate exposures to 1 November 1966.

285. In September 1971, all maintenance, technical, supervisory and other personnel who had received some RDP exposure were added to the exposure roll. Their exposures were calculated back to 1 November 1966 using mine average working levels, hours worked per year, and a factor to account for the portion of time spent underground.

286. In the mid-1970s, work started on the estimation of exposure prior to November 1966. Only RDP measurements were used in this estimate, thus excluding the early survey data and most of the measurements made during the 1950s, which had been for radon only. Because of the paucity of pre-1964 data, Eldorado used an annual averaging process. Owing to the known variability of workplace conditions, Eldorado decided to use the median, as opposed to arithmetic mean, to describe the central tendency of the exposures. On the basis of a review of work histories for individuals employed prior to November 1966, a system of 22 job categories was devised; classification into a particular category was based on potential RDP exposure. Each person was assigned to a category for each job held throughout his or her employment. An effective RDP concentration (WL) was calculated for each job category for each year based on the fraction of working time spent in each area (underground, office, etc.). These calculations [F7], recorded as single lifetime totals rather than separate annual totals, provided the basis for the exposure estimates in Howe et al. [H18].

287. SENES [S12] used the raw radon and radon decay product survey data from the Beaverlodge mine to assign WL values to individual stopes. Production data were then used to retain only those measurements that were taken when work was in progress in each area. The production data were used further to "weight" the data by the number of man-shifts worked in each area at the measured RDP concentration. The effect of this re-evaluation is shown in figure IX, which illustrates, for the case–control sample of 195 miners, revised exposure estimates plotted against the original exposure estimates. The 45º line corresponds to the situation when the two estimates of exposure are equivalent. Figure IX shows clearly that the majority of employees had revised exposure estimates that are higher than the original estimates. Although a general correlation exists between the two methods of estimating exposure, the revised estimates are substantially higher, up to an order of magnitude for some employees. For example, two employees originally in the 5–49 WLM category were reclassified to the 250+ WLM category. Table 15 shows the extent of movement from one exposure category to another due to the revision of exposure estimates.

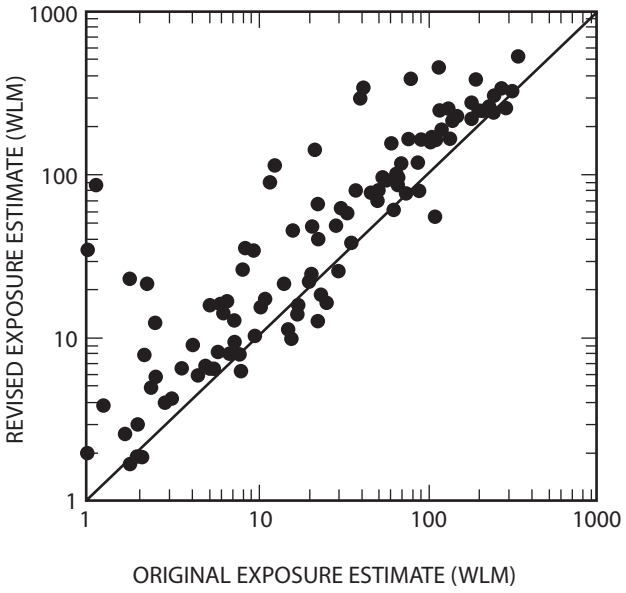
Table 15 Changes in exposure categories of Beaverlodge miners based on revised estimates of cumulative exposures [S12]

Original exposure (WLM) category	*Revised exposure (WLM) category*							*Number of employees*
	<5	*5–49*	*50–99*	*100–149*	*150–199*	*200–250*	*250+*	
<5	61 (78)	16 (21)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	78
5–49	7 (13)	36 (67)	7 (13)	2 (4)	0 (0)	0 (0)	2 (4)	54
50–99	0 (0)	0 (0)	9 (60)	2 (13)	3 (20)	0 (0)	1 (7)	15
100–149	0 (0)	0 (0)	1 (8)	0 (0)	5 (42)	5 (42)	1 (8)	12
150–199	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)	2 (67)	3
200–250	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (80)	1 (20)	5

Original exposure (WLM) category	*Revised exposure (WLM) category*							*Number of employees*
	<5	*5–49*	*50–99*	*100–149*	*150–199*	*200–250*	*250+*	
250+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)	5
Number of employees	68	52	18	4	8	10	12	172

Note: Number in brackets is the percentage of employees in the original exposure category that are also in the revised exposure category. Revised exposures were estimated for 172 of the 195 employees in the case–control group. The remainder were not traceable in company records.

Figure IX. Comparison between revised and original estimates of exposure for Beaverlodge uranium miners (note that both axes have logarithmic scales) [C17].



288. The 1991 exposure estimates [S12] did not include non-Beaverlodge mining exposure. Because of the remote location, the cost of recruiting at Beaverlodge was high; consequently, Eldorado's policy was to recruit experienced miners wherever possible. Inclusion of non-Beaverlodge exposure would probably result in further movement of the miners into higher WLM categories.

289. Beaverlodge miners were also exposed to airborne dust. Information on workplace dust levels (konimeter data) that had been recorded coincident with the WL measurements made at Beaverlodge was also entered into the new computer database [S12]. For active stopes, an analysis was conducted to determine if a correlation existed between RDP measurements and dust. The analysis revealed significant correlations between the measured values of dust, the RDP concentration and the calculated equilibrium factor. Dust levels were significantly (99% confidence level) positively correlated with radon and radon decay product concentrations and with equilibrium factors. However, the association between the dust concentration and the equilibrium factor was the strongest of the associations. A correlation coefficient of 0.31 was statistically significant at >99% level. This suggested an association of higher equilibrium factors with the higher dust levels in stopes with work activity; this seems reasonable, because high dust levels and high equilibrium factors both result from low ventilation rates. For the years where both RDP concentrations and dust levels were captured in the SENES database, the mean dust concentrations by year and type of workplace were calculated. The data indicated high dust concentrations in stopes in 1954 and 1956, with mean concentrations of more than 500 ppcc (particles per cubic centimetre) in stopes. The mean dust concentration was <100 ppcc for the years 1963–1965, and was 133 ppcc in 1966. The highest mean dust concentrations were in the raising workplaces; this is reasonable, because raises are generally more difficult to ventilate during development.

290. SENES [S12] performed a detailed review of WLM exposures for a case–control group defined by Howe. Subsequently, Howe and Stager [H16] carried out an analysis of the case–control group using the revised exposures and observed that the inverse dose-rate effect was no longer present. The revised [S12] methodology for calculation of RDP concentrations was based on estimating annual mean concentrations, taking account of the time spent by an individual employee (employee duration) at specific workplaces. Annual workplace mean RDP concentrations were weighted averages of monthly RDP averages, and the weights were estimates of employee duration based on monthly production statistics.

291. Annual mean RDP concentrations for individual workplaces were agglomerated on the basis of the hierarchy of workplace, mine area and mine-wide estimates. Annual mean concentrations for mine areas were calculated as weighted means of the annual workplace means, where the weights were representative of the estimated employee duration in each workplace. Similarly, the mine-wide annual mean concentrations were weighted means of the mine-area annual mean RDP concentrations.

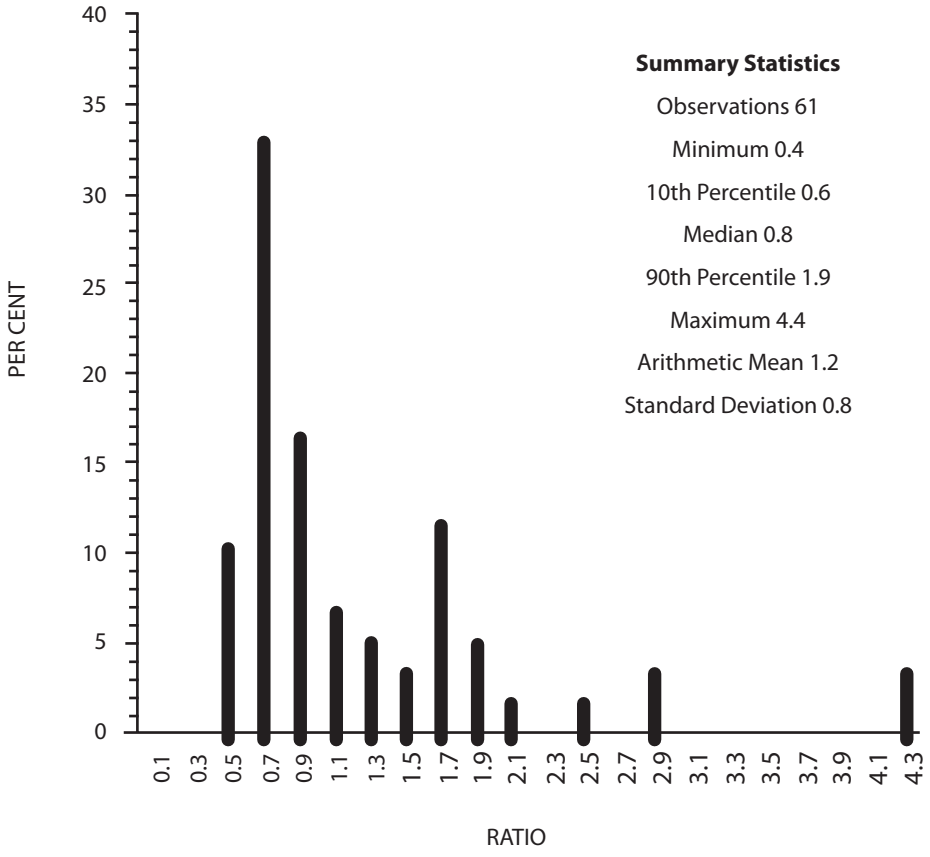
292. This methodology provided estimated annual mean RDP concentrations for individual workplaces and average mine-wide RDP concentrations. As previously noted, substantial differences in RDP concentration existed between different areas of the mine; this was related both to worker activity and the ventilation infrastructure development in those areas. Even within these mine areas, substantial differences in RDP concentrations existed. Although the method worked well for stoping workplaces, application of the

method to drifting, raising, travelways and shaft area workplace categories was not as successful.

293. Available bonus contract data were examined for the case–control group of 195 miners. Bonus contracts were available for the periods 1955–1957, May 1960 and 1963–1965. These records were searched for information on the 195 miners in the case–control group, of whom 129 had underground work experience. For 61 of these employees, information was located on specific workplace, and exposure durations were extracted from the bonus contracts. Total cumulative exposures were calculated for the 191 employees with occupations and exposure durations described in the personnel files. The total RDP exposure over 4,149 worker-months underground was 7,298 WLM for the miners with bonus contract information, based on the use of mine-wide average concentrations. When mine-area average concentrations were used instead of mine-wide average concentrations only for months with bonus contract information, the estimated exposure to the group was 7,570 WLM. A third estimate of exposure, using the ratio of mine-area to mine-wide average RDP concentrations over all months with bonus contract information, was 8,120 WLM.

294. Figure X shows the distribution for these 61 individual employees of the ratios of exposures calculated by mine-area concentrations to those calculated by mine-wide average concentrations [C17]. This distribution is indicative of the variability in exposure estimates due to RDP concentration differences between the areas in the mine for those underground employees with no bonus contract information.

Figure X. Distribution of ratios of exposures calculated by mine-area concentrations to those calculated by mine-wide average concentrations for Beaverlodge employees for whom bonus contract information was available [C17].



295. An increase in average exposure for the case–control group was seen when mine-area-specific concentrations rather than mine-wide average concentrations were used. This suggested the presence of a bias towards low individual exposure estimates when mine-wide average RDP concentrations were used.

296. For a given cohort, the total exposure calculated by summing the individual exposures that are based on workplace concentrations and durations should equal the mean exposure calculated using the total exposure duration multiplied by the duration-weighted mine-wide mean concentration, irrespective of the variation or uncertainty in concentrations between workplaces. However, this is not necessarily so for nested case–control groups, where the proportion of cases is higher than in the cohort group. This type of differential uncertainty is likely to be present in many of the other cohorts, since mine-wide average concentrations

were used for estimating the earlier (and generally higher) exposures.

297. Substantial additional RDP exposures, both from non-Beaverlodge work and from other environmental sources (particularly radon in dwellings), along with exposure to other lung cancer risk factors such as arsenic and additional radon exposure that might occur in gold mining, were considered as possible confounders. Of the 195 employees in the case–control study who joined the Eldorado company with recorded previous mining experience, 9 had worked in gold mines and 8 had worked in Beaverlodge area uranium mines. Kusiak et al. [K12] reported RDP levels of the order of 0.3 WL (or greater) in gold mines in Ontario during the 1960s, along with exposure to other risk factors, including exposure to silica and arsenic. The mean duration of recorded mining experience was 6.9 years. It is of interest to note that 5 of the employees selected from the nominal roll, the restrictions on which were to have excluded persons with work experience at other Eldorado facilities, actually had recorded work experience at Eldorado's Port Radium underground uranium mine.

298. Using the revised exposure estimates, Chambers et al. [C17] investigated how uncertainty in exposure might affect the dose–response relationship in the Beaverlodge miners. A file describing a cohort with characteristics similar to that previously studied for determining the dose–response relationship was constructed on the basis of partial information from the nominal roll of Eldorado Beaverlodge employees. This information included an employee identification code and the duration of each occupation for each time period worked at Beaverlodge. Birth year or ages were not available, nor was the vital status of individual employees. Ages at the start of employment were assigned on the basis of random sampling from a uniform distribution of ages between 20 and 40 years. The cohort was similar in size and was assumed to be similar in characteristics to the cohort studied in the epidemiological study [H19]. RDP exposures and doses were assigned to underground employment up to 1967 on the basis of the revised exposure estimates and the algorithm developed for estimating exposures. This provided estimates of nominal, or mine-wide average, annual exposures for each employee plus an estimate of the range of the exposures or variability due to differences in exposure rates between different areas of the mine. Exposures were calculated by multiplying the exposure for a given year by the number of months that the employee worked in that year. Exposures for other occupational groups and for post-1967 time periods were based on previous estimates of exposure rates and an assumed variability between workplaces [C17].

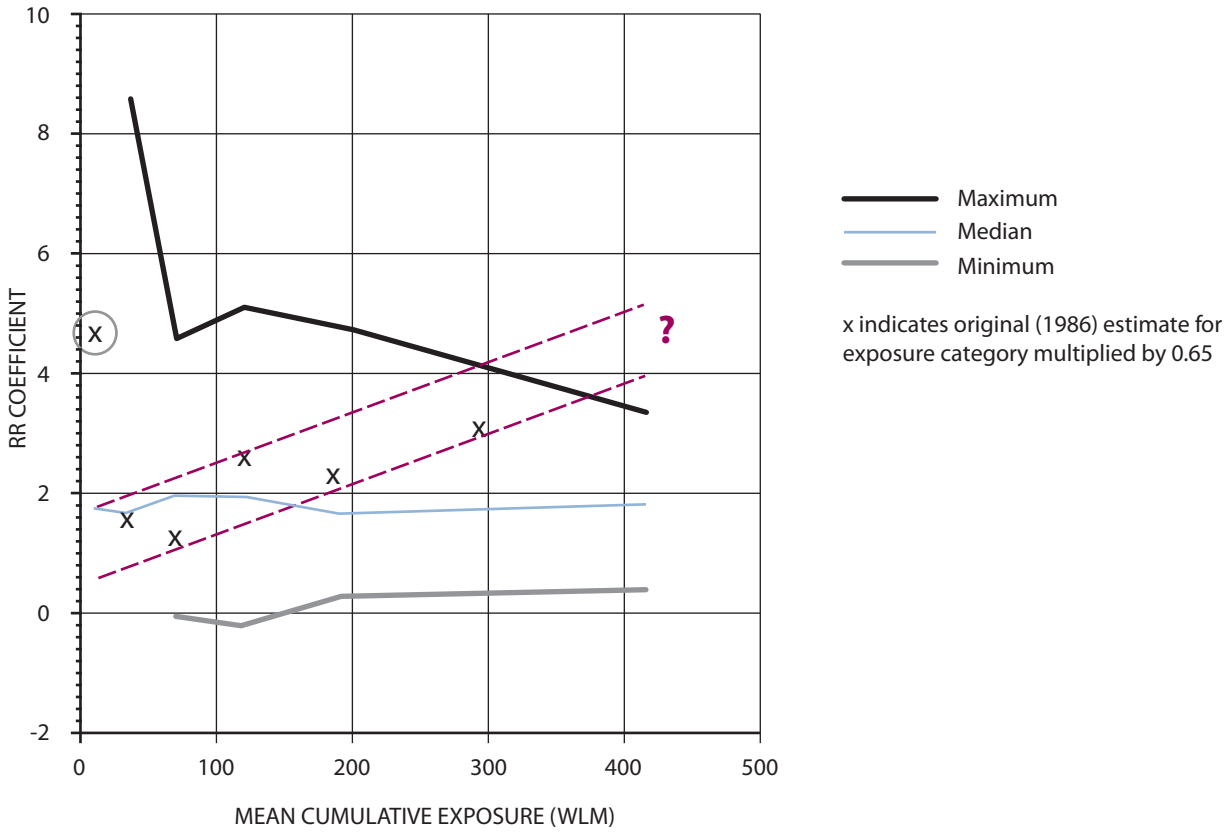
299. Expected and simulated observed numbers of lung cancer deaths were calculated using a life table with reference age-specific total mortality and lung cancer mortality rates. The probability of lung cancer during each year of follow-up was calculated for every employee in the cohort using both the reference lung cancer rates and the lung cancer rates based on the RDP exposure and the true reference (unexposed) lung cancer rate for the employee. The lung cancer status was assessed for each year of the follow-up on the basis of the probability of lung cancer for that employee during the year, conditional on that employee being alive at the start of the year [C17].

300. The exposure rates for an individual were probabilistically drawn from log-normal distributions that reflected the variability in exposure rate for that occupation and time period of employment. The mean of this distribution was equal to the mine-wide (or occupation) average conditions and was retained as a nominal estimate. The true exposure rate reflected the variation in exposure rates within the mine (or occupation). True reference lung cancer rates were based on the nominal reference lung cancer rate but included a modification based on individual employee variations in this reference rate. These variations could reflect interindividual variability in background rates or varying exposures to other lung cancer risk factors such as may have existed in other types of mine, or variability in the extent of smoking [C17].

301. Employees were assigned to the exposure categories used in the 1986 cohort analysis, and the simulated number of lung cancer deaths was determined. The expected number of lung cancer deaths was determined by summing the annual probabilities of lung cancer death during the follow-up. Relative risks (RRs) for the exposure category were determined by dividing the simulated number of lung cancers by the expected number. Relative risk coefficients were determined by dividing the excess relative risk (RR – 1) by the average cumulative exposure in the exposure category. The relative risk estimates and the relative risk coefficients were then summarized by scenario to show the variability in exposure response [C17].

302. Figure XI represents a summary of the simulated dose response based on the simple relative risk model with a risk coefficient of 2 per 100 WLM, no uncertainty in dose and no confounding factors. The three lines show the maximum, median and minimum simulated values of the relative risk coefficient, with the median relative risk coefficient following the 2 per 100 WLM value assumed to be "true" in the simulation. Any observed dose response falling within the two outer lines would be consistent with the assumed model for the scenario. The separation between the outer lines reflects solely the statistical variation in the outcome of a random process, since for this scenario there is no uncertainty in exposure and no confounding factors. For example, between 3 and 7 lung cancer deaths would typically be realized if 5 lung cancer deaths were expected from the risk model. The distance between the bounds is related to the statistical power in that either a large effect or a large study population is required for statistical significance. A mortality update, such as that reported in reference [H35], decreases the distance between the upper and lower confidence bounds, since the predicted number of lung cancers deaths is higher and the relative variability is reduced [C17].

Figure XI. Simulated exposure response for Beaverlodge miners [C17].
Shown are the maximum, median and minimum values of RR per unit exposure, based on a median RR of 2 per 100 WLM.



4. Epidemiological studies

303. The Eldorado epidemiological cohort had 19,370 work records for only 18,424 persons, meaning that up to 946 individuals worked at more than one Eldorado site [N6]. Many of these miners worked at Port Radium, which was Eldorado's first mine; therefore some miners in the Beaverlodge study are likely also to have received exposure while at Port Radium.

304. The mortality study of Ontario miners [M19] had also identified 1,430 former Eldorado employees from Saskatchewan who had worked in an Ontario mine; of this number, 726 had worked in Ontario uranium mines. Consequently, Muller et al. excluded the Eldorado employees from their analysis of Ontario uranium miners because of the lack of exposure data for the time when they worked in the Eldorado mines. Conversely, the Beaverlodge study [H16] included many of these individuals but failed to take account of their non-Eldorado (Ontario) exposure.

305. Howe and Stager [H16] reported on a study of Beaverlodge miners that was part of a larger study of some 18,000 Eldorado employees. The Eldorado epidemiology project has been followed in a series of papers published by Eldorado [A1]. Owing to the size of the study population, Howe et al. were not able to interview the Eldorado employees. The exposure reconstruction had to rely on work history files from Eldorado. It was therefore possible that the work histories of the study cohort were deficient with regard to non-Eldorado employment. This observation is important, because miners could have worked in other mines for which no radiation exposure data were available.

306. Howe [H35] reports an updated analysis of a cohort of 17,660 individuals known to have worked for Eldorado sometime in the period 1930–1999 [H35]. One of the subcohorts of Eldorado employees includes underground miners employed by Eldorado at the Beaverlodge uranium mine in northern Saskatchewan. The study design for the updated analysis was very similar to that used in the original study. The nominal roll was that used in the original study [H16] with the addition of workers who had joined the Beaverlodge operation between the cut-off of the original study (31 December 1980) and the final shutdown of the mine in 1982. A considerable effort was made to improve the quality and quantity of the data that were extracted from the nominal roll. This resulted in some deletions and additions for the pre-1980 period [F19]. Exposure estimates and estimates of gamma ray doses were accumulated for the cohort, partly from the original cohort records available at Eldorado supplemented

by records from the Canadian National Dose Registry. Also, work histories and dose records for non-Eldorado uranium mining employment were added. This included miners who worked in Ontario mines as well as a few from Newfoundland. Thus, if a Beaverlodge miner had exposure from RDPs from work at other locations (i.e. Port Radium mine, Port Hope uranium processing plant, non-Eldorado mines around Uranium City, Ontario mines and Newfoundland fluorspar mines), this exposure was added to the miner's total exposure. Exposures received up until 1999, if a miner continued to work, were also included. The updated study adds a further 19 years of mortality data for the Eldorado cohort and also includes lung cancer incidence results for 31 years, i.e. 1969–1999 [H35]. The basic characteristics of the updated cohort, including the Beaverlodge uranium miners, are summarized in table 16.

Table 16 Basic characteristics of the updated Eldorado cohort [H35]

Characteristics		*Number*	*Per cent*
Sex	Males	16 236	91.9
	Females	1 424	8.1
Site	Port Hope	3 003	17.0
	Port Radium	3 300	18.7
	Beaverlodge	10 050	56.9
	Other sites	1 307	7.4
Birth year	1900	414	2.3
	1901–1910	1 028	5.8
	1911–1920	1 803	10.2
	1921–1930	4 030	22.8
	1931–1940	3 913	22.2
	1941–1950	2 790	15.8
	1951–1960	3 118	17.7
	1960+	564	3.2

Cohort/subcohort	*Mean RDP exposure (WLM)*	*Standard deviation*
Entire cohort	48.0	182.6
Port Hope	12.5	43.4
Port Radium	174.2	369.1
Beaverlodge	23.2	81.7
Other Eldorado sites	1.9	32.9

307. Two general types of comparison were used in the analysis of the Eldorado cohort data. Firstly, observed and expected values were used to estimate standardized mortality ratios (SMRs) and standardized incidence ratios (SIRs). Expected values were derived from Canadian national population rates for mortality between 1950 and 1999 and for cancer incidence between 1969 and 1999. A second series of comparisons were based upon internal comparisons between subgroups within the cohort, i.e. with no reference to an external population.

308. The analysis of mortality rates showed that, while mortality from lung cancer was elevated, the cohort as a whole and the various subcohorts had reduced risks relative to the Canadian population for most of the other causes of death. The analysis of mortality from lung cancer among men in the cohort with respect to RDP exposure was based on 639 lung cancer deaths. (This compares with previous analyses of the Eldorado cohort where the total number of such deaths was 122.) For the Beaverlodge underground miner subcohort, there were 198 lung cancers observed compared with

120.7 expected. The SMR for lung cancer was estimated at 1.6 (95% CI: 1, 1.9) and was statistically significant ($p < 0.001$).

309. Comparisons of the cancer incidence rates between 1969 and 1999 for the cohort with those for the general Canadian population showed that the only cancer which is consistently elevated is lung cancer. For cancer as a whole and for specific cancers, incidence rates for the cohort were generally lower than those for the general population, which was considered to be a manifestation of the healthy worker effect. Howe also investigated mortality and cancer incidence for diseases other than lung cancer and found no evidence of any causal relationship between exposure to RDPs or gamma exposure and an increased risk of any other diseases [H35].

310. The application of the BEIR VI type of risk model, which allows for effect modification with time since exposure, exposure rate and age at risk, was also investigated. Using the same approach as the BEIR VI Committee [C20], Howe estimated parameters from the present study for a "full interaction model", which accounts for the influence of age at exposure, dose, dose rate and time since exposure (see table 17). The BEIR VI Committee's analysis was based on 11 studies of underground miners, including the previous analysis of the Port Radium and Beaverlodge cohorts. In this study, Howe found that the addition of both the time since exposure terms and the six exposure categories resulted in a statistically significant improvement in fit, but that the addition of age at risk terms did not. He suggested that these results may be regarded as essentially independent of the data used by the BEIR VI Committee [H35]. Howe's 95% confidence limits on estimates of time since exposure (WLM5, WLM15 and WLM25) parameters include the BEIR VI estimates.

Table 17 Parameter estimates for full interaction model and comparison with BEIR VI model estimates for males in the Eldorado cohort (1950–1999) [H35]

Parameter	*Estimate*	*95% lower limit*	*95% upper limit*	*Estimate for BEIR VI*
WLM 5	5.23	1.33	14.52	7.68
WLM 15	2.5	0.63	7.05	5.99
WLM 25	1.37	0.36	3.99	3.92
Rate(1)	1			1
Rate(2)	1.02	0.39	2.67	0.49
Rate(3)	0.49	0.2	1.21	0.37
Rate(4)	0.35	0.12	1.01	0.32
Rate(5)	0.33	0.13	0.84	0.17
Rate(6)	0.16	0.06	0.44	0.11
Age(1)	1			1
Age(2)	1.94	0.77	4.89	0.57
Age(3)	1	0.37	2.72	0.29
Age(4)	0.05	0	6 266.67	0.09

Parameters as specified below:

WLM = total WLM (per 100 WLM) lagged by 5 years
WLM 5 = WLM 5–14 years previously (per 100 WLM)
WLM 15 = WLM 15–24 years previously (per 100 WLM)
WLM 25 = WLM 25 years+ previously (per 100 WLM)
Rate (2) = WL 0.5–1.0
Rate (3) = WL 1.0–3.0
Rate (4) = WL 3.0–5.0
Rate (5) = WL 5.0–15.0
Rate (6) = WL 15+
Age (2) = age at risk 55–64
Age (3) = age at risk 65–74
Age (4) = age at risk 75+

5. Evaluation

311. The Eldorado Beaverlodge cohort was updated with the revised dosimetry [S12, S14] to add a further 19 years of mortality data and to include cancer incidence results for 31 years, i.e. 1969–1999 [H35]. The study design for the updated analysis was very similar to that used in the original study [H16]. The nominal roll was that used in the original study with the addition of workers who had joined the Beaverlodge operation between the cut-off of the original study (31 December 1980) and the final shutdown of the mine in 1982. The updated analysis found little evidence of departure from a simple linear exposure–response model.

312. For the Beaverlodge cohort, an ERR of 0.96 (95% CI: 0.56, 1.563; $p < 0.001$) per 100 WLM was reported [H35]. This can be compared with the previous Beaverlodge estimate of ERR of 3.25 per 100 WLM [H16]. The estimate of ERR for the Beaverlodge cohort has decreased substantially in the new analysis. Howe suggests that this could in part be accounted for by the time-dependent effect modifiers on the ageing Beaverlodge cohort [H35]. Extensive work was undertaken in this update to add the exposures received in non-Beaverlodge mines to those received in the Beaverlodge mines. Thus the decreased ERR could also be partly explained by the addition of these non-Beaverlodge mine exposures.

F. Germany: Wismut miners

1. Introduction

313. The Erzgebirge (Ore Mountains) of Saxony (Germany) and Bohemia (Czech Republic) have a long history of underground mining. As early as the 12th century, silver mining was performed, while later other metals, such as iron, bismuth, cobalt, nickel and tungsten, were mined. The mining of uranium started at the beginning of the 19th century in the Schneeberg area. Miners often died of what was called Schneeberger lung disease, named after the town in the Erzgebirge. By the end of the 19th century, this disease was recognized as lung cancer.

314. Shortly after the Second World War, the Wismut mining company carried out uranium mining in Saxony and Thuringia in the former German Democratic Republic (GDR). According to Jacobi and Roth [J3, J4], large-scale uranium mining started in the Erzgebirge of Saxony in 1946 and later was extended to the eastern parts of Thuringia. The Wismut mines in Saxony were high in arsenic, while the Thuringia mines were low in arsenic. Overall, Wismut produced about 220,000 tons of uranium between 1946 and 1990, making it the world's third largest producer of uranium [K15]. Mining for uranium per se ended in 1990 following the reunification of Germany.

315. Various papers discuss the potential exposure conditions in the uranium mines in the GDR [E3, E4, K5, K15, K16, L3]. Enderle and Friedrich [E4] characterized the workplace situation in the post-war years (to 1955) as compulsory labour, use of prisoners of war (almost 50% in 1947) and a high rate of illness and accidents. In August 1953, a treaty was signed to convert Wismut from a Soviet enterprise to a Soviet–GDR company, and this resulted in improvements in the working conditions [E4]. From 1946 to about 1955, the underground mine conditions in the Erzgebirge of Saxony were characterized by dry drilling, no mechanical ventilation, very heavy manual work, the absence of industrial health and safety standards, and very long working hours.

316. According to Jacobi and Roth [J3, J4], three time periods can be distinguished: the years 1946–1954, which were referred to as the "wild years"; 1955–1970, during which time, there was ongoing improvement in the conditions through the introduction of wet drilling and improved ventilation; and the period after 1970, when individual exposures were recorded and compared with ICRP limits. Some 20,000 cases of silicosis and 7,000 cases of lung cancer are reported among the Wismut miners. Jacobi and Roth [J4] noted that radiation exposures in the years preceding 1970 can only be "roughly" estimated. For 1955, on the basis of the assumptions of a mean radon concentration of 120 kBq/m^3 for drilling and ore exploration areas and 50 kBq/m^3 for other worksites, and the nominal time spent in workplaces, a nominal workplace value of about 80 kBq/m^3 was estimated. On the basis of a nominal equilibrium factor of 0.5, workplace exposure was estimated at 160 WLM/a. Prior to 1960, the concentrations of long-lived alpha activity in the air could have been higher by a factor of 100 to 1,000 than in post-1960 conditions. Finally, the mean external dose to miners in the last 10–20 years of mining were about 5 mSv/a.

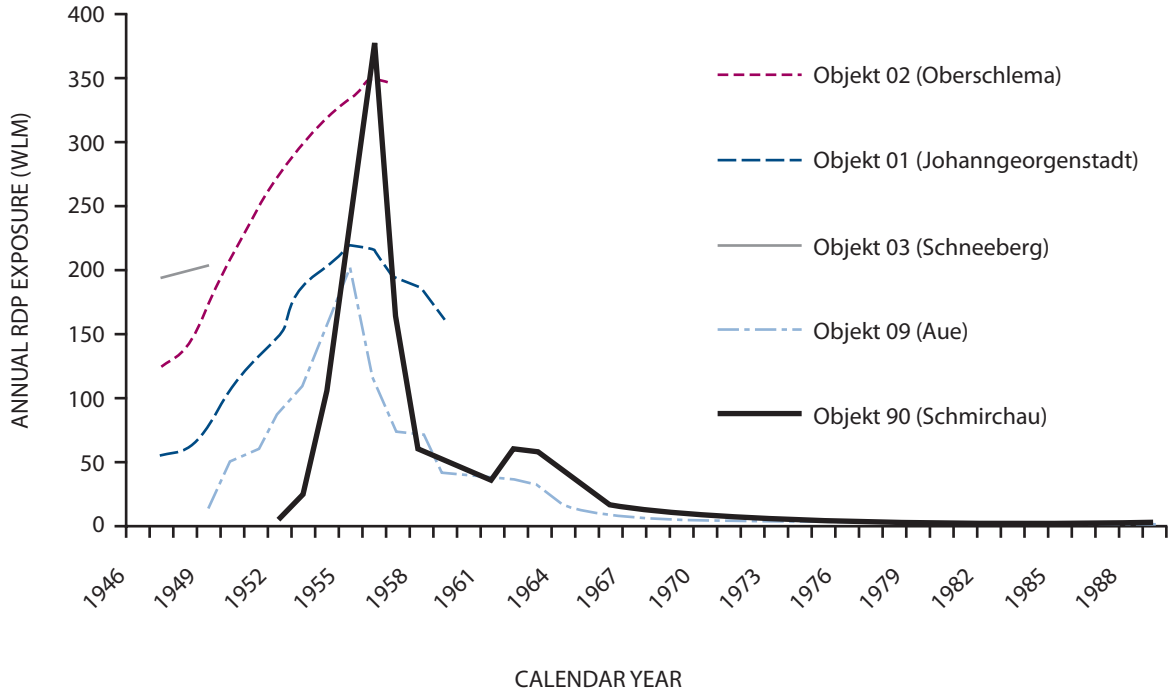
2. Radon and radon decay products

317. The first radon measurements in workplaces in the Wismut mines were carried out in 1955 [J3]. The mean of more than 2,000 measurements carried out in five mining operations was 110 kBq/m^3, which according to Jacobi [J3] showed good agreement with measurements carried out in 1937 and 1938 in the Schneeberg mines. This author [J3], on the basis of 1955 data, reported a mean annual miner exposure of 150 WLM, with a range of 30–300 WLM. (The conversion from Bq/m^3 to WLM depends on the equilibrium factor F_{eq}. Assuming an F_{eq} of approximately 0.4, exposure to a radon concentration of 110,000 Bq/m^3 for 2,000 h corresponds, very roughly, to an annual exposure of 150 WLM.)

318. As reported by Lehmann [L3], from 1946 to 1955 there was no dosimetric recording of radon by the Wismut company. In 1955, radon gas monitoring commenced. Measurement of radon and its decay products was introduced in 1966 in Saxony and in 1975 in Thuringia. Formerly, for purposes of compensation, an average annual exposure to radon decay products of 150 WLM was assumed by the Wismut company for underground workers for the period 1946–1954 [B5]. This was used independently of conditions and led to

both over- and underestimation of individual exposures to RDPs in the early years. A working group of experts was convened in 1993 to develop a job–exposure matrix (JEM) in order to improve the estimation of exposure. The results of their work were published in 1998 [L3]. Radon concentrations for 1946–1954 were estimated retrospectively on the basis of the first available radon measurements in 1955. These estimates took into account previous working conditions in the mines, mine architecture, historical measurements and data gathered by the Czech ore mining industry. Based on these estimates and the measurements available since 1955, the annual exposures to radon, RDPs, long-lived radionuclides and external gamma radiation were evaluated for each year of employment between 1946 and 1989, each mining facility and each place of work (underground, milling or processing, open-pit mining or surface mining). This evaluation (see figure XII) was performed for a reference job for each place of work (there were fewer reference jobs for underground than for above-ground workers), while the exposures received in other types of job (of which there were more than 200) were derived using weighting factors for the specific reference job (ranging from 1.0 to 0.0).

Figure XII. Annual exposure to radon decay products as estimated using the job–exposure matrix for the job of "hewer", in five mining facilities typical for the Wismut cohort [K16].



319. The improved estimates of the JEM showed that RDP exposures in the early years depended strongly on the number of old shafts in a given mine and on mining activity. In newly established mining facilities, such as Objekt 09 (Aue) and Objekt 90 (Schmirchau), radon values in the very first years of operation were rather low, while in old reopened mining facilities, such as Objekt 02 (Oberschlema), Objekt 03 (Schneeberg) and Objekt 01 (Johanngeorgenstadt), radon concentrations were already high at the beginning of the operating period. Generally the levels of radon increased with uranium mining and the area of the worked vein to a maximum in 1955–1956, and decreased later owing to the introduction of different ventilation and sealing measures.

320. The JEM [L3] provided estimates of exposures not only to radon and its decay products but also to long-lived radionuclides and gamma radiation. Owing to improved working conditions, the exposure to radon and its decay products decreased, while gamma exposures still remained relatively high. A JEM for arsenic, fine dust and quartz is being developed on the basis of estimates given in reference [B22].

3. Epidemiological studies

321. Several epidemiological studies of radiation exposures among the Wismut miners are under way. These include a large cohort study [K5], a nested case–control study on lung cancer mortality [T11] and a cohort study among the offspring of miners [T11], among others [G17, K15, K16, K29, K30, K31]. There is also an independent case–control study on the incidence of lung cancer [B23, B24, B39]. About 400,000 people worked for Wismut between 1946 and 1990. For about 130,000 of them, complete working histories, including start and end of work, job specification and places of work (with dates) are available. From these, a stratified random sample of about 64,311 people was drawn [G17,

K15, K16, K30, K31]. In order to reflect the different mining conditions in the Wismut company mines, the sample was stratified by the date of first employment (1946–1954, 1955–1970, 1971–1989), place of work and area of mining. Criteria for inclusion in the cohort study were as follows: *(a)* minimum duration of employment of at least 6 months; *(b)* date of first employment between 1946 and 1989; *(c)* year of birth after 1899; and *(d)* male. After collection and subsequent evaluation of the occupational data, which were extracted from the original payrolls, a total of 5,150 individuals were excluded from the initial cohort, because they did not meet the criteria. The final cohort for the analysis thus consisted of 59,001 men [G17, K30, K31]. On the basis of year of first employment, three subcohorts were defined to reflect the different mining conditions: 1946–1954 (subcohort A), 1955–1970 (subcohort B) and 1971–1989 (subcohort C).

Table 18 Characteristics of the Wismut cohort study [G17, K30, K31]

Characteristics	*Number of cohort members*	*Per cent*
	59 001	100.0
Year of start of employment		
1946–1954	23 917	40.5
1955–1969	17 950	30.5
1970–1989	17 134	29.0
Year of end of employment		
1946–1954	2 720	4.6
1955–1974	19 593	33.2
1975–1984	12 963	21.9
1985+	23 725	40.2
Vital status as of 31 December 1998		
Alive	39 255	66.5
Deceased	16 598	28.1
Cause of death available	14 646	88.2
Cause of death not available	1 952	11.8
Lost to follow-up	3 148	5.3
Duration of follow-up in years		
<10	3 764	6.4
10–19	11 225	19.0
20–29	12 536	21.2
30–39	12 704	21.5
40+	18 772	31.8
Year of death		
<1960	224	1.3
1960–1969	1 255	7.6
1970–1979	3 132	18.9
1980–1989	5 368	32.3
1990–1998	6 619	39.9

Characteristics	*Number of cohort members*	*Per cent*
Cause of death[a]		
Malignant cancers (C00–C99)	4 800	32.8
Circulatory diseases (I00–I99)	5 417	37.0
Respiratory diseases (J00–J99)	1 559	10.6
Digestive system (K00–K99)	815	5.6
Injuries and poisoning (S00–S99 and T00–T99)	1 284	8.8
Others	771	5.3
Exposed to radon		
Never	8 244	14.0
Ever	50 707	86.0
Cumulative exposure to radon in WLM		
Mean (maximum)	241 (3 244)	
Median	18	

[a] Codes from the International Classification of Diseases.

322. In the first mortality follow-up, the vital status for the cohort was determined as at 31 December 1998. At that time, 66.5% were alive, 28.1% had died and follow-up was not complete for 5.3%. The mean age of subjects alive at the end of 1998 was 54 years for the total cohort and 71, 59 and 40 years in subcohorts A, B and C, respectively. A total of 2,388 lung cancer deaths occurred in the first follow-up period (1946–1998), which comprised 1,801,626 person-years. The general characteristics of the Wismut cohort are summarized in table 18 [K15, K16].

323. Data on smoking habits were available for about a third of the total cohort. This proportion was considerably lower for subcohorts A (20%) and B (33%) than for subcohort C (64%), reflecting the fact that smoking habits were recorded only after 1970. More than 50% of the miners with known smoking habits were heavy smokers, while the proportion of non-smokers was about 26% [K16].

324. Within the cohort study, a nested case–control study on lung cancer deaths was conducted that included individuals born after 1927. Two controls per case were matched by date of birth. Controls could be either alive or deceased, Questionnaires were sent either to next of kin or, in the case of the controls, to the miners themselves, if they were still alive. Information was gathered on smoking habits and occupational exposures outside the Wismut facilities, which might be related to lung cancer. Next, data were abstracted from the Wismut health archives in relation to smoking habits, medical radiation exposures and jobs prior to Wismut employment [G14, T11].

325. From the cohort, a subsample of 6,000 miners was drawn as the basis for an offspring cohort study. The lifetime exposure of the 6,000 miners to radon and radon decay products varied between 0 and >3,000 WLM. The first stage of the study, which is in progress, was to identify the children of miners to be included in the study and to investigate their health status, life expectancy and causes of death. The most important outcome variables are genetic anomalies, infant mortality and childhood cancers. The offspring cohort consists of 7,855 children.

326. Another case–control study on lung cancer incidence among former Wismut employees was conducted between 1991 and 2001 [B23, B24, B39]. Patients with histologically confirmed primary lung tumours were recruited from several study hospitals in Thuringia and Saxony. Controls were randomly selected from the personnel files of the Wismut company and were frequency-matched to the cases according to birth year in 5-year groups. Inclusion criteria for cases and controls were: male workers; employed underground at the Wismut Company at some time between 1947 and 1990. Occupational exposure to radon, its decay products, gamma radiation and long-lived alpha emitters was estimated by using the JEM described in reference [L3]. All subjects were personally interviewed about occupational and smoking history. Lung cancer risk was calculated by using the ERR model and conditional logistic regression. A total of 505 cases and 1,073 controls were included in the study. The cumulative exposure from RDPs ranged from 1 to 2,911 WLM (an average of 552 WLM for the cases and 420 WLM for the controls). The exposure rate ranged from 0.1 to 31.4 WL (an average of 8.2 WL for the cases and 7.2 WL for the controls). The odds ratios (OR; adjusted for smoking, year of birth and asbestos exposure) in the two highest categories, compared with the reference category of 50 WLM, were significantly increased: ≤800–1,599 WLM, OR = 2.08 (95% CI: 1.40, 3.08); and 1,600–2,911 WLM, OR = 3.68 (95% CI: 1.92, 7.03). More than half of the study subjects had been exposed more than 35 years earlier. Assuming a linear exposure–response relationship, there was a significant increase in the

relative risk of 0.10 (95% CI: 0.05, 0.17) per 100 WLM after adjusting for smoking and asbestos exposure. After correcting, in a sensitivity analysis, for the fact that the controls of this study had a higher average exposure than the population of Wismut workers from which they had been recruited, the ERR increased to 0.24 per 100 WLM. For those still smoking, the increase in relative risk was lower (0.05 per 100 WLM), whereas it was higher (0.20 per 100 WLM) among non-smokers and long-time ex-smokers. Lung cancer risk declined with time since exposure, except for those miners who had been exposed 45 or more years in the past. No inverse dose-rate effect was observed.

327. More recently, the autopsy data in the archives of the Central Institute of Pathology of the Wismut Company were analysed for 19,271 persons; these included 12,926 uranium miners (WLM > 0, group 3), 1987 control cases of non-exposed Wismut workers (WLM = 0, group 2) and 4,358 control cases most likely never employed by Wismut (group 1) [W17]. The mean age at the start of exposure was 33.5 years, mean duration of exposure 11.8 years and mean age at death 62.7 years. The autopsy data investigated comprised about 152,300 person-years of uranium mining work with radon exposure. Mean RDP exposure was about 725 WLM, with a maximum of more than 3,000 WLM. Mean exposure to long-lived radionuclides (LRN) was about 7.3 kBq h m^{-3} (^{238}U), with a maximum of more than 50 kBq h m^{-3}. On the basis of the main cause of death or concurrent diseases, 8,882 cases of malignant tumours were found (6,403 for group 3,889 for group 2 and 1,581 for group 1). For primary malignant lung tumours, a higher incidence was found for exposed Wismut workers (4,526 (35%) in group 3, 377 (19%) in group 2 and 2,472 (11%) in group 1). For primary malignant lung tumours, a significant correlation between OR and WLM category was shown. Depending on the chosen category, the OR for groups with high exposure increased to 10. In categories of high LRN exposure, the OR increased to 8.4 (95% CI: 6.19, 11.56). Below the 400–599 WLM category, no significant difference of OR related to the leading histomorphological tumour types could be determined. In higher WLM categories, small cell carcinomas and squamous cell carcinomas showed an OR nearly twice as high as that of adenocarcinomas. For non-exposed Wismut workers, 6% of non-smokers and about 19% of smokers showed lung tumours, and for exposed workers, 18% of non-smokers and 34% of smokers had developed lung tumours. The relative share of small cell carcinomas and squamous cell carcinomas was higher for smokers than for non-smokers. In turn, the relative share of adenocarcinoma was lower in smokers than in non-smokers. According to autopsy diagnosis, the relative share of silicosis cases increased with the WLM category. This mirrored, at least for the mining sites in Saxony, the parallel exposures to quartz-containing dust and ionizing radiation. The available data gave no indication of a causal connection between silicosis as the cause of death and primary malignant lung tumours for the Wismut workers. Molecular biological investigations revealed no evidence for repeated mutations of codon 249 of the *p53* tumour suppressor gene in the Wismut workers, as had been shown for uranium workers of the Colorado Plateau in reference [T1].

328. Pathological findings on 243 Wismut uranium miners with lung cancer, recruited between 1991 and 1995 into the case–control study on lung cancer incidence [B24], are reported elsewhere [K14]. The frequencies of all tumour cell types were found to be associated with increasing RDP exposure, but high radiation exposures tended to increase the relative proportion of small cell lung cancers and squamous cell carcinomas [K14]. This effect was more pronounced among those who had stopped smoking or had never smoked, and it seemed to be masked among those still smoking [K14]. The first evaluation of the pathology archive of the Wismut company showed a shift from small cell lung carcinomas as the predominant cell type in the first follow-up years to squamous cell carcinomas in the later years [W4]. At present, 5,215 lung carcinoma cases have been identified among former Wismut employees, showing extremely high proportions of small cell lung carcinomas (69%) in the early years (1957–1965), which declined to 34% up to 1990 [W4].

4. Evaluation

329. A number of reports have presented information on the predicted numbers of lung cancers in former Wismut miners [B5, J3, J4]. On the basis of a sample of 3,654 persons drawn from the Wismut database, which resulted in a final data set consisting of 2,282 men, and using 1985 death tables for the German Democratic Republic and various risk projection models, one analysis [B5] predicted a further 1,700 to 4,800 additional lung cancer cases from 1995 onward. The peak incidence was predicted to occur between 1985 and 1991. Another study on pathological findings among Wismut uranium miners was published in 2006 [T50].

330. The main strengths of the Wismut cohort study are its size, a wide range of exposure levels, a long duration of exposure and a large number of cases of lung cancer and other diseases, as well as the availability of information on dust and arsenic exposure. A joint analysis of 11 miner cohort studies [L10] was based on a total of 60,606 exposed miners, including 2,674 with lung cancer, and a mean radon exposure of 164 WLM. Data were combined from different cohorts of miners around the world. Heterogeneity with respect to the quality of exposure assessment, the presence of relevant covariates such as arsenic, dust and tobacco smoke, as well as lifestyle and genetic factors, was likely to be present in this combination of cohorts. The Wismuth cohort provides a data set for analysis that is similar in size (59,001 miners and 2,388 lung cancer cases) to the data set used in the combined analysis, yet it is more homogeneous with respect to data collection and estimation of exposure. Therefore it represents a unique opportunity to verify the results of the combined analysis in an independent data set. The potential limitations of the Wismut cohort are the limited information on smoking and the limited validity of the exposure assessment, particularly in the years before 1955.

331. A number of reports have already presented data on the feasibility of epidemiological studies of Wismut miners and have provided some early results [K5, K15, K16, T11]. Recent observations suggest a linear smoking-adjusted ERR of about 0.10 (95% CI: 0.05, 0.17) per 100 WLM [B24, B39]. As noted in reference [T11], the results of future epidemiological studies will depend greatly on the quality of the exposure assessment and the information on vital status and causes of death.

G. Canada: Port Radium miner study

1. Introduction

332. The Eldorado Port Radium mine has a long history extending back to 1930, when a prospector by the name of Gilbert LaBine identified pitchblende on the north shore of the Great Bear Lake in Canada's Northwest Territories. Open-pit mining started shortly thereafter, and the first pitchblende was shipped in 1931. Underground operations were at a high level of activity by 1932. Mining continued until 1940, when a decreasing demand for uranium ore led to the mine being shut down. In 1942, the Port Radium mine was reopened at the government's request, and it continued in production until 1960, when it was shut down. Annual average ore grades in the 1940s were 0.5–1.2%, with occasional pockets of high-grade pitchblende being encountered.

333. Bloy [B11] noted that ventilation in the Port Radium mine was somewhat unusual. At the start of underground work, the mine had only natural ventilation; this was greatly reduced in winter, as all openings to the surface were kept closed because of the extremely cold temperatures. Openings to the surface other than the shaft, such as raises from stopes through the surface pillar, were covered and tightly sealed in the winter. The only air entering the mine, in winter was compressed air used to power the drills [T3]. Thompkins conducted a ventilation survey at the Port Radium mine in 1945 [T4]. There was only a relatively small amount of air circulating through the mine. This condition, together with there being no definite routing of the air currents and the lack of dust-reducing features on mine machinery, resulted in generally high dust concentrations in the mine. As part of the 1945 ventilation survey, Thompkins [T4] estimated the balance of the air entering and leaving the Port Radium mine by natural means. Airflow from the surface entering through the 921 Raise and the 722 Raise was estimated at 455 and 1,500 cfm,[3] respectively. Compressed air entering the mine was estimated to be 1,400 cfm. Therefore the total volume of air entering the mine was approximately 3,350 cfm. The volumes of air leaving through the shaft and the manway/pipe raise were estimated as 2,350 and 850 cfm, respectively, for a total of 3,200 cfm. Only 150 cfm of the air that entered the mine remained unaccounted for [T4].

334. By 1944, the need for heated ventilation at the Port Radium mine was recognized. A ventilation unit and steam plant for heating air going into the mine were installed in 1946 and were in operation by early 1947.

335. By 1956, ore zones at the Port Radium mine had reached a stage where it was necessary to relocate the mine surface ventilation unit and steam heating unit to accommodate active workings. Owing to the apparent short life expectancy of the mine, driving a complex ventilation raise system was deemed impractical. As an alternative, old manways and workings were used to bring the air to active areas. Air was forced into the mine down 136 Raise into 136 Stope and down to the first level through the stope raises. From there, the air was channelled to the lower levels through a series of old raises. The air on the levels was controlled by a series of vent doors [B14]; there were, however, substantial air losses between the first and fifth levels as a result of air escaping through old stopes filled with broken material. For instance, at the first level, a loss of 7,000 cfm was noted. Bloy [B14] concluded that it was virtually impossible to adequately ventilate the winze section with the existing set-up, since air volumes were substantially reduced and any air reaching the area was highly contaminated.

336. In 1956 and 1957, the main fan on the surface was stepped up from 22,000 cfm to 35,000 cfm. Heating facilities were increased to handle the extra airflow in winter. By then, a definite air route was established underground. However, when the installation was completed and the air volume checked, only 6,000 cfm of the approximately 35,000 cfm of air put into the mine actually reached the active workings, and this was contaminated with radon and dust. The rest of the air escaped through leaking doors, bulkheads and coarse backfill. To solve this problem, the airways were lined with two types of plastic: polyethylene plastic sheeting of about 4 mil thickness (0.1 mm) and a liquid spray plastic called cocoon [B12].

337. The plastic sheeting lined the raises from the first level to the surface fan inlet. Although the sheeting worked very well, it was difficult to handle and tore easily. By the time these raises were completed, the liquid spray plastic method (cocoon) was being tried, and the results were so encouraging that the system was used for all remaining airways [B12].

338. An auxiliary fan delivering approximately 11,000 cfm was located on the 11th level to ventilate the winze area, thus supplying this area with much better ventilation than had been found in the 1956 survey. A raise system was also completed in the winze area, which greatly improved the ventilation [B13]. In addition to the cocoon method, three additional auxiliary fans were added in the winter of 1957–1958, resulting in a significant lowering of radon concentrations by the spring of 1958 [F5]. No ventilation data for the periods after 1958 were found in the records of Cameco, the later owner, or the literature review.

339. Kupsch [K6] provided a detailed history of the Eldorado company, including its Port Radium mine. Additional interesting information on the history of the Port Radium mine was provided by McNiven [M6]. He reviewed the

[3] 1 cfm = 4.72×10^{-4} m^3 s^{-1}.

operations at Port Radium from the reopening of the mine in 1942 to its final shutdown in September 1960.

2. Radon and radon decay products

340. Port Radium was the first mine in Canada where radon and radon decay product sampling was performed. According to Bloy [B11], the first radon samples at this mine were taken in February 1945. The reason for the study was to investigate the "suffocating gas" noticed by the miners. (The suffocating gas turned out to be simply oxygen deficiency.) Radon levels were found to range from 13,000 to 47,000 pCi/L.

341. Representatives from the Chalk River Nuclear Laboratories visited the Beaverlodge and Port Radium operations and carried out preliminary surveys for radon in 1951 and 1952 [S15, S30]. The surveys established that both Beaverlodge and Port Radium had serious radon problems. By 1954, Chalk River Nuclear Laboratories had designed and built new radon sampling equipment which they used in another survey of the Port Radium mine [B11].

342. It was the practice in those years to perform konimeter dust sampling from time to time in parallel with the radon and radon decay product sampling. In addition, an external gamma radiation survey of the Port Radium mine in 1952 showed gamma exposure levels in a few areas of 95 mR (approximately 0.95 mSv) per shift [B11].

343. The radon and radon decay product data were discussed by Frost [F6]. Initially, only radon was measured. By the mid-1950s, both radon and radon decay product concentrations were measured (the Kusnetz method was used for the latter). The equilibrium factor between radon and its decay products was found to be approximately 30% in 1957. Frost [F5, F6] concluded that the equilibrium factor was likely to have been higher in the early years of mining when there was no forced ventilation.

344. Average RDP concentrations were estimated by Frost [F5] to be of the order of 77 WL in 1945, 38 WL in 1952, 10 WL in 1956 and 8 WL in 1957, on the basis of the available measurements and assuming an equilibrium factor of 30%. Unfortunately, these data and the corresponding estimates of miner exposure have not been published.

345. In a 1996 re-evaluation [S15] of RDP concentrations, radiation measurements recorded by Eldorado staff were compared with the duration of employee exposure for each radiation measurement. All but a few of the radiation measurements were solely for radon concentrations, as the three paired measurements of radon and radon decay product concentrations in 1957 did not provide enough information to estimate equilibrium factors. Thus a modelled equilibrium factor based on the ventilation rate was used to convert radon levels to WL RDP concentrations.

346. WL estimates for individual workplaces were aggregated as a function of the workplace classifications to which the 171 employees selected for this study were assigned by Howe [S15]. At each level of aggregation, efforts were made to provide a consistent estimator of WL. Since there were no individual measurements of RDP concentrations, individual WLM exposures could not be calculated directly. An estimate of the exposures of individual employees was based on the mine-wide average.

347. The duration-weighted arithmetic mean was chosen in this analysis as the estimator to characterize RDP concentrations. This statistic facilitated the calculation of a mine-wide average for a variety of employee classifications. This mine-wide average (mine index) provided a WL value that would be the expected WL concentration over all the individuals in the classification. From a preliminary review of the data for Port Radium provided by Cameco, the amount of downcast ventilation into the mine varied substantially (by about a factor of 2) by season of the year, especially from 1947–1957. This was because air needed to be heated in the winter to prevent the upper portion of the shafts from freezing. The limited capacity of the heating plant required the ventilation volume to be reduced during the colder winter months (mid-December to mid-March) to avoid freezing the workings.

348. On the basis of the ventilation characteristics, the entire operational period of the mine was divided into three subperiods: pre-1947, 1947–1955 and post-1955. Table 19 summarizes the available radon data for shaft stations. Similar data are available for active stopes and other underground workplaces. A large winter/summer difference is evident.

Table 19 Radon concentration in shaft stations of the Port Radium mine [S15]

Period	*Season*	*Radon concentration (pCi/L)* [a]
Pre-1947	Summer	8318 (6)
	Winter	8318 [b]
1947–1955	Summer	2084 (10)
	Winter	4760 (6)
Post-1955	Summer	3434 (16)
	Winter	593 (4)

[a] Arithmetic mean of measured radon levels with number of observations given in round brackets; 1 pCi/L = 37 Bq/m^3

[b] Indicates an estimated value, since measurements were not available.

3. Exposure estimation

349. Information provided by Howe allowed a comparison of exposure duration and exposure (WLM) for a 171-member case–control group. There were significant differences in the estimates of months worked between the 1996 re-evaluation and the original epidemiological study [H15]. Estimates of total WLM for the case–control group as a whole are not significantly different between the two approaches. However, as for exposure duration, individual differences in exposure (WLM) could be very large. To assess the implications of this re-evaluation for the epidemiological study, it would be necessary to look at the differences in the individual miner exposures between the two studies. It is not known from the evaluation whether the cases follow the general pattern or if a difference exists between the cases and the controls (which was the expectation of the authors).

4. Epidemiological studies

350. Since early miners had a potential excess risk of lung cancer, Eldorado sponsored a pilot epidemiological study of Port Radium workers. This study [G5] found an excess of lung cancers in miners who had 5 years or more of underground experience.

351. Consequently, Eldorado initiated a more detailed epidemiological study [A1] that included the radiation exposure data, and that involved Statistics Canada and the National Cancer Institute of Canada, which performed the actual epidemiological analyses.

352. This study by Howe et al. [H18] investigated some 2,103 miners employed between 1942 and 1960. In this group, 57 lung cancer deaths were observed compared with 24.73 expected. Employment records were not available before 1940 and hence exposures before that date were not estimated. As a consequence, the exposures of the Port Radium miners may well have been underestimated. Risk coefficients estimated from the Port Radium analysis should therefore be regarded as upper limits [H18].

353. Radon gas samples were collected for seven of the years between 1945 and 1958, with between 9 and 71 samples per year and a total of 251 samples. The range of concentrations was reported as 50–300,000 pCi/L. Howe et al. [H18] cite Frost [F5] as the source of their RDP exposure data. The Port Radium study, unlike the Beaverlodge study by the same investigators, in at least some circumstances, used the annual average rather than the median as representative of workplace RDP levels. While radon gas samples were made as early as 1945, early data are sparse and the uncertainties in the exposures are likely to have been very large. Howe et al. [H18] indicated that weighted average equilibrium factors were calculated on the basis of the known labour distribution and type of workplace. The highest factor used for many work groups was 0.5, although it was discovered that there could have been substantially higher values. Working time was based on a 40-hour week and 48 weeks per year. Howe et al. [H19] acknowledged that there were many potential sources of error in the procedure for estimating RDP exposure.

354. The average exposure of the 2,103 miners was 183.3 WLM. About 42% of the person-years at risk were in the <5 WLM exposure category. The ERR was estimated to be 0.27 per 100 WLM and the excess absolute risk was estimated to be 3.1 cases per 10^6 person-years per working level month [H18].

355. The ERR per 100 WLM for Port Radium from the updated study [H35] is essentially unchanged from the previous assessment [H18], whereas the ERR for Beaverlodge has decreased substantially. Howe suggests that this could be accounted for in part by the early exposures of Port Radium workers, for whom the time-dependent effect modifiers (time since exposure and age at risk) may be of less importance than for the younger Beaverlodge subcohort [H35].

356. More recently, the Eldorado cohort was updated to add a further 19 years of mortality data (1950–1999) and to include cancer incidence results for 31 years (i.e. 1969–1999) [H35]. The study design for the updated analysis was very similar to that of the original study [H18]. The updated estimate of the ERR for Port Radium miners was 0.37 (95% CI: 0.23, –0.56; $p < 0.0001$) per 100 WLM, which may be compared with the ERR of 0.27 per 100 WLM from the 1980 mortality analysis [H18]. As noted previously, the current estimate of ERR for Beaverlodge is 0.96 per 100 WLM [H35], compared with the previous estimate of 3.25 per 100 WLM [H16].

5. Evaluation

357. During the early years of mining (pre-1947), WL values in the Port Radium mine were of the order of 60, and annual exposures of miners were likely to have been of the order of 600–1,000 WLM. By the end of mining (around 1959), the calculated WL values declined to 2–3, and hence annual exposures were likely to have been of the order of 20–40 WLM. The estimates have large uncertainties, perhaps a factor of 10, for both pre-mechanical-ventilation and post-mechanical-ventilation periods [M5]. Few data are available for their estimation, and the quality of the sparse data available is suspect.

358. Exposure estimates were particularly uncertain for a number of work types where the proportion of time spent underground was unknown. This included mechanics and electricians who may have worked underground for extended periods. The uncertainty in risk estimates if these employees are included in an epidemiological analysis could be substantial, and exclusion of these workers should be considered.

359. In addition to the sparsity and limitations of radiation and ventilation data for the early years, there exist other

recognized sources of uncertainty. Based on the sample of 171 miners provided by Howe, many pre-Port-Radium employment histories are incomplete. Many Port Radium miners are likely to have worked in other mining environments (notably gold mining), which provided a further possible risk factor. Epidemiology for Port Radium miners only included miners' work after 1941, as work records prior to that time were not available. Prior, unrecorded experience at Port Radium may be a risk factor in some instances. The Port Radium ores contained significant concentrations of other elements, including arsenic, nickel and cobalt. The degree of confounding arising from these elements in workplace dust is not known. Overall, the Port Radium cohort provided evidence of the risk of exposure to RDPs. The 1996 exposure re-evaluation [S15] represents the best available data for epidemiological assessment of the Port Radium cohort. However, the exposure uncertainties are very large, and quantitative estimates of dose–response relationships must be viewed as having substantial uncertainty.

360. Notwithstanding the re-evaluation of miners' exposures, the Port Radium data set provides a much weaker basis for dose–response investigation than, for example, the Beaverlodge cohort, and is of lower reliability for use in a quantitative assessment of risk.

H. French uranium miners

1. Introduction

361. Uranium prospecting began in 1947 in France, and the production of the first tonne of uranium occurred in 1949. Extraction was located in four main mining divisions: Crouzille (Limousin) and Forez from 1947, Vendée from 1953 and Hérault from 1977. It continued up to 1999, when the last mine closed.

362. The first radon measurements were taken in 1953. In 1956, forced ventilation was introduced in the mines, leading to a sharp decrease in exposure levels, and systematic control of individual exposures began to be applied in the mines. Individual exposure was assessed for each miner on the basis of ambient measurements. Monthly individual records of RDP and gamma exposures were kept in the mining divisions. By 1958, a regulation decreed limits for internal and external exposures. From 1959 onward, systematic records of uranium ore dust exposure were kept for each miner. After 1983, the system of assessment of exposure was replaced by the use of personal alpha and gamma dosimeters [Z10]. Pradel and Zettwoog [P8] and Bernhard et al. [B46] describe the radiation protection practices in French uranium mines.

2. Radon and radon decay products

363. A 1955 paper by Jammet and Pradel [J7] provided insight into the early conditions in the French uranium mines. This paper reported radon concentrations of 100–10,000 pCi/L in mine air (based on 40 samples). Groundwater from mineralized zones was an important source of radon. The paper noted that, in one mine, a cross-cut was driven into barren rock and air was blown through the cross-cut. The air came into contact with radon-rich water, and within a distance of 300 m exceeded the workplace concentration limit for radon, referred to as the tolerance level (50 pCi/L of air in French mines at that time). The paper also noted high levels of radon in the smoke generated by blasting (levels of as high as 50,000 pCi/L).

364. The 1974 paper by Pradel and Zettwoog [P9] showed that RDP measurements started in 1955 with about 65 samples being taken per mine per year. Beginning in 1955, miners were required to wear an individual dosimeter to record their exposure to gamma radiation. Monthly exposures to RDPs and long-lived radioactive dust were calculated, and all results of exposure were reported on personal cards. Annual and lifetime exposures for all three components of radiation dose — RDPs, gamma radiation and long-lived radioactive dust — were obtained by summing monthly exposures over time. From Tirmarche et al. [T9, figure 6], about 2,500 person-years of exposure in the 1947–1956 period had to be reconstructed, about 11% of the total person-years.

365. Pradel and Zettwoog [P8] commented that, prior to 1953, only 40 measurements of radon had been made. Large numbers of radon measurements were made later, and this enabled a close approximation to be made of the inhaled quantities of radon. The authors also discussed the possibility of relatively elevated exposures for short periods of time when mining was taking place in high-grade-ore areas. Data from Duport [D9] showed that, in the period 1956–1982, there were typically more than 30 radon measurements per person-year. This is very high compared with the number available for the other miner cohorts used for risk assessment.

366. After 1983, the system of exposure monitoring based on area measurements was replaced by personal alpha and gamma dosimeters. The portable device is described by Zettwoog [Z10]. It comprises an active dosimeter with a spectrographic head for the measurement of alpha radiation (ionograph track detection) and a thermoluminescent dosimeter for the measurement of external gamma radiation. It allows the measurement of the number of ^{222}Rn atoms inhaled, the energy of the alpha particles emitted by the three short-lived decay products of radon and thoron (^{218}Po, ^{214}Po, ^{212}Bi), the alpha activity of the five long-lived alpha emitters (^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Po) contained in ore dust (after decay of the short-lived products) and the gamma radiation dose.

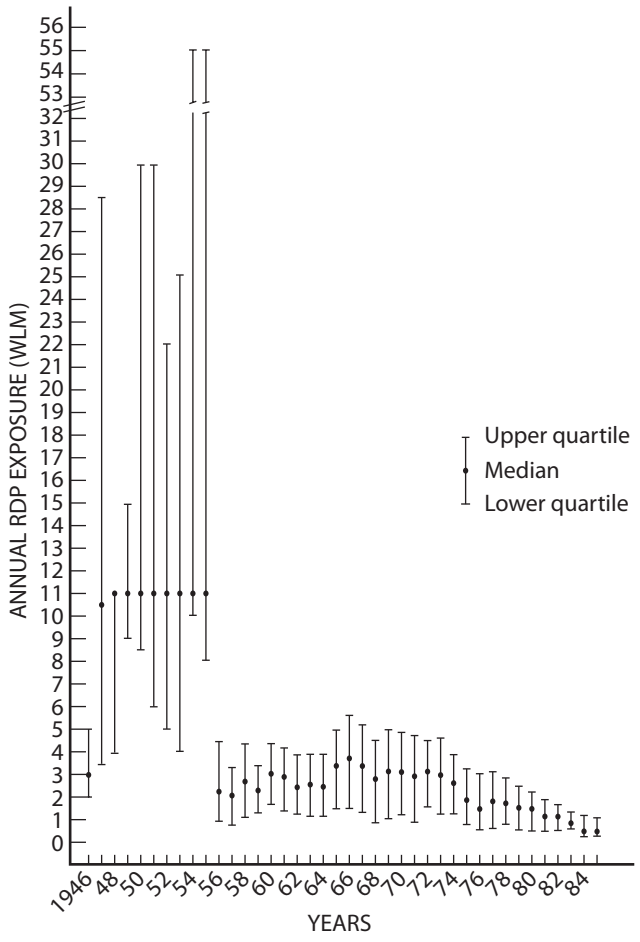
3. Exposure estimation

367. Information about RDP exposure in the initial cohort of French uranium miners is found in references [L10, T5, T6, T7, T9]. This group includes 1,785 miners who began underground work between 1946 and 1972 and who were exposed

for at least 2 years. The mean cumulative RDP exposure was relatively low (70 WLM). The reports describe the reassessment of the RDP exposure of miners by an expert group, notably for the period 1947–1956, for which retrospective estimation was necessary. Working conditions changed dramatically in 1956, with a large reduction in the exposure of miners [T8]. Tirmarche et al. [T7, T8] provided a distribution of individual annual exposure (WLM). Prior to 1956, median annual exposures were estimated to be of the order of 10–11 WLM. After 1956, annual exposures were in the range 1–3 WLM until 1980 and were <1 WLM thereafter, as illustrated in figure XIII. The figure also shows that exposures prior to 1956 had a 5-fold range of interindividual variability.

368. Rogel et al. [R10] and Tirmarche et al. [T30, T31] provided an update of the exposures of the enlarged cohort of French uranium miners. This group included 5,098 males, employed as miners by CEA-COGEMA for at least 1 year between 1946 and 1990. Among the 4,134 miners who were exposed to RDPs, the mean cumulative exposure was 36.5 WLM (with a range of individual exposures of 0.1–960.1 WLM) over a mean duration of 11.5 years (with a range of 1–37 years). The mean annual exposure was 23.9 WLM before 1956, whereas after 1956 it was only 1.5 WLM [R10].

Figure XIII. Distribution of annual exposures to radon decay products by calendar year in the initial cohort of French uranium miners [T7].



4. Epidemiological studies

369. The initial cohort included 1,785 uranium miners who began underground work between 1946 and 1972 and were exposed to RDPs for at least 2 years. Tirmarche et al. gave a status report on the epidemiological follow-up of these uranium miners in a 1985 paper [T9]. A first analysis of this cohort, based on follow-up to December 1985, was published in 1993 [T6]. Compared with national rates, significant excesses of deaths from lung cancer (observed = 45, expected = 21) and cancer of the larynx (observed = 17, expected = 7) were observed.

370. For lung cancer only, a linear dose–response relationship was described with respect to the cumulative exposure to RDPs [T6]. The ERR coefficient was relatively low ($0.35\ WLM^{-1}$) in comparison with those found in other miner studies, but as the number of lung cancer deaths was also low (n = 45), the CIs of this coefficient included most of the values of the other studies, as well as estimates from international committees [L10]. The authors cautioned that, since the mean age of the cohort was only 56 years, the cohort was too young for full expression of the lung cancer risk.

371. Laurier et al. described the results obtained from the initial cohort after extension of the follow-up to 1994 [L44]. The mean age at study exit was then 63 years. Causes of death were obtained from the National Mortality Database, which collects information from all death certificates in France. Compared with previous analyses, the use of the National Mortality Database as the principal source of information on the causes of death allowed a reduction in the potential bias in the calculation of standardized mortality ratios (SMRs). The analysis showed, however, that this had little impact on the relationship between RDP exposure and lung cancer risk. Compared with the earlier study [T6], the number of person-years was increased by 25% (n = 56,372) and the number of deaths by 74% (n = 612). The analysis confirmed the existence of an excess risk of death from lung cancer among French uranium miners (85 observed deaths, SMR = 1.9; 95% CI: 1.5, 2.3), and an increase of this risk with cumulative RDP exposure (ERR = 0.6 (95% CI: 0.1, 1.2) per 100 WLM). An excess risk of laryngeal cancer, noted in the 1993 paper by Tirmarche et al. [T6], was not confirmed in the later study (12 observed deaths, SMR = 1.1; 95% CI: 0.6, 1.9) [L44, T5].

372. The French cohort of uranium miners was enlarged by the inclusion of additional miners with lower radon exposures, and the follow-up was extended to December 1994. The enlarged cohort is described in references [L40, R10, T30, T31]. The study was limited to males who had been employed as miners by CEA-COGEMA for at least 1 year between 1946 and 1990. The main characteristics of the enlarged French cohort of miners are summarized in table 20. This cohort comprised 5,098 miners followed-up from 1946 to 1994, with a total of 133,521 person-years. The percentage of miners lost in the follow-up was 2.3%. The average age at the end of the study was 55 years. A total

of 1,162 deaths were observed. The cohort included a group of 964 non-exposed individuals (but who had the status of a miner and were working in the same mining divisions as the miners). Among the exposed miners, the mean cumulative exposure was 36.5 WLM, accumulated over a mean duration of exposure of 11.5 years.

Table 20 Characteristics of the enlarged French cohort of uranium miners [R10]

Period of follow-up: 1946–1994	
Number of workers	5 098
Non-exposed miners	964
Number of person-years	133 521
Average cumulative exposure (WLM)[a]	36.5
Average annual exposure (WLM)	
Before 1956	23.9
1956 and later	1.5
Average duration of exposure (years)[a]	11.5
Person-years by lagged cumulative exposure (WLM)	
0	49 408
0–10	35 817
10–50	27 778
50–100	11 358
100–200	6 213
>200	2 947

[a] Among 4,134 exposed miners.

373. The total number of lung cancer deaths observed between 1946 and 1994 among the enlarged cohort of French uranium miners was 125. On the basis of the reference rates for the general French male population, the number of lung cancer deaths expected was 83.1. The analysis confirmed a significant excess of lung cancer deaths (SMR = 1.51; 95% CI: 1.25, 1.79). A significant excess was also observed for all cancer mortality (SMR = 1.14; 95% CI: 1.03, 1.25), but this disappeared after exclusion of the lung cancer deaths (SMR = 1.02; 95% CI: 0.90, 1.10). Thus no significant excess of deaths from any other cancer was observed [T30].

374. Rogel et al. [R10] reported on how factors such as time since exposure and exposure rate modified the lung cancer risk in the enlarged cohort of French uranium miners. The statistical analyses were based on a linear relative risk model using Poisson regression to fit the models, maximum-likelihood methods to estimate parameters and likelihood ratio tests for nested models. A linear exposure–response relationship with an ERR of 0.8 (95% CI: 0.3, 1.4; $p < 0.001$) per 100 WLM was found. No inverse exposure-rate effect was observed in the extended French cohort. The strongest modifier was the period of exposure. Analysis showed an ERR that was 10 times higher per WLM for exposures received before 1956 than for exposures received in 1956 and thereafter. This could be explained by a better quality of exposure assessment after 1956. The ERR for exposures after 1956 was 2.4 (95% CI: 1.1, 4.6, $p < 0.0001$) per 100 WLM [R10, T30].

5. Evaluation

375. The assessment of RDP exposure among French uranium miners is of good quality. Beginning in 1956, monthly records of individual exposure were systematically kept in the mining division. Furthermore, compared with the initial cohort involved in the international joint study in 1994 [L10], the size of the cohort was increased almost 3-fold, and the follow-up was extended by 10 years. Also, it is worth noting that, after 1956, the exposures of the French miners to RDPs were of the same order of magnitude as those received in some homes. Therefore the potential contribution of the French uranium miner cohort data to the estimation of the risk coefficient for lung cancer could be especially relevant to the estimation of the risk for populations exposed to RDPs in their homes. A European project that includes the French, Czech and German cohort studies is in progress [T27, T30]. The combined data, which are of good quality and relate to miners with low levels of exposure, will allow an analysis to be undertaken with a large statistical power.

376. The quantification of the relationship between cumulative RDP exposure and risk of lung cancer mortality required elaborate statistical methods. Different models were applied, and modelling was performed independently by different researchers [L10, R10, T6, T27]. All analyses confirmed an increase of lung cancer mortality with cumulative exposure. In the framework of a European programme, data from the French and Czech miner cohorts were made available to researchers involved in biologically based modelling, with the aim of comparing the approaches and results of the different models [T30]. The different biologically based solutions gave a reasonably good fit of the data and confirmed a linear increase of risk with cumulative exposure [B27, H32]. The comparison of the different approaches provided very interesting discussions regarding the biological validity of the models, the means for testing various hypotheses about the processes of radiation carcinogenesis, the selection of the best fitting model, and the comparison of these biologically based models with the empirical approach that uses a statistical model for describing the data [B37, H33, L42].

377. Data on yearly gamma and long-lived radioactive ore dust exposure are also available since 1956 and 1959, respectively, for each miner of the enlarged cohort. The information will allow all three components of radiation exposure — RDPs, gamma radiation and long-lived radioactive dust — to be considered in an analysis of the dose–response relationship. In addition, a nested case–control study investigating the joint effect of RDP exposure and smoking on lung cancer risk among French uranium miners was published [L44]. It confirms the existence of a significant effect of RDP exposure when smoking information is taken into account.

I. Canada: Newfoundland fluorspar miners

1. Introduction

378. The 1969 report of the Royal Commission Respecting Radiation, Compensation, and Safety at the Fluorspar Mines, St. Lawrence, Newfoundland [A14] provided a wealth of interesting historical data, and is the source of much of the following information.

379. For many years, St. Lawrence, Newfoundland, was an isolated fishing community. Shortly after the First World War, the community was devastated by the drop in price of salt-cured fish (the main source of income) and, in 1929, all of the fishing equipment was destroyed by a tidal wave. Mining eventually took over as the principal occupation [D4]. Fluorspar, which is used in the production of steel, aluminium and high-octane gasoline, is the only mineral resource known to be of economic quality in the St. Lawrence area.

380. The earliest mine to start operation at St. Lawrence was the Black Duck mine, which belonged to the St. Lawrence Corporation. This mine opened in March 1933. Originally, mining was by open-cut methods. By 1937, however, the open cut had reached a depth of about 90 feet. At this stage, pumps could not cope with the amount of water in the open cut, and it was necessary to sink a shaft, which eventually went to a depth of 250 feet [A14]. This mine ceased operations in 1942. In 1937, the St. Lawrence Corporation started work on a vein called Iron Springs. Originally, mining there was also by the open-cut method, but by 1938, the work had moved underground, with the mine eventually descending to a depth of 970 feet. This mine was closed in December 1956.

381. Standard underground mining procedures were adopted with the first underground mine in 1936. Wet drilling (which resulted in reduced dust levels) was generally adopted in 1942. Shrinkage stoping and cut-and-fill methods were not practised until after 1964. The underground mines were in general very wet. Ventilation was mostly provided by natural means. Except in one case, supplementary blowers were not used until 1946 [D5].

382. The report of the Royal Commission [A14] noted that the St. Lawrence Corporation had 16 veins of fluorspar on its mining properties, and at one time or another most of these were mined by the company. Practically all miners employed by the St. Lawrence Corporation who worked underground prior to 1960 had at some point also spent time working in the Iron Springs mine. According to the report of the Royal Commission [A14], the working conditions in the Black Duck mine were unpleasant. The Commission noted, that prior to 1942, drilling was done with a dry hammer, and that dust and smoke were always such that the driller could only be seen at close quarters. “He was always like a snowman and also had to shut off his machine to clear out his eyes and nostrils.” Clearly, the mines were poorly ventilated, and any radon brought into the mines with mine water would likely remain in the mine for a long period of time, resulting in high radon/radon decay product equilibrium conditions.

383. Until 1942, there were three shifts working underground at the Iron Springs mine. Late in 1942, the mine was put on two shifts. This change was most beneficial: the four hours between shifts could be used for blowing out the smoke with compressed air. There was practically no forced ventilation in any of the mines before 1960, natural ventilation having been the only source of ventilation until that time.

384. Mines were inspected annually by inspectors brought in by the Newfoundland government; however, it was not until 1951 that mining regulations were in place. Consequently, prior to this date, the inspectors had to rely solely on persuasion to achieve improvements in the conditions in the mines.

385. Morrison et al. [M16, M17] noted that, although more than 40 fluorspar veins were located in the St. Lawrence area, most of the ore produced came from only two mines, namely the Iron Springs and the Director mine, mined by the St. Lawrence Corporation and the Newfoundland Fluorspar Corporation, respectively. According to Morrison et al.

[M16, M17], the Iron Springs mine was believed to have had the worst ventilation of any mine in the St. Lawrence area. In contrast, the Director mine, from 1955 onward, employed some forced ventilation. Both mines were extremely wet.

386. Morrison et al. [M16, M17] postulated that, since operations were converted to underground mining procedures in the mid-1930s, in the middle of the Depression, work was done with antiquated equipment, and it was possible that the health and safety conditions were poor as a result of the financial situation at that time. From the mid-1950s, it was clear that miners in St. Lawrence were suffering from various respiratory troubles, some of which had been diagnosed as silicosis. For this reason, the Newfoundland Department of Mines requested the federal Department of National Health and Welfare to carry out a survey of dust conditions in the St. Lawrence mines. Subsequently, the Industrial Hygiene Division of the Department of National Health and Welfare (now Health Canada) conducted a dust survey in the period 1956–1957. By the end of 1957, it was clear that the miners of St. Lawrence were suffering from a respiratory ailment that was not caused by the excessive quantities of siliceous dust. The dust survey was therefore expanded into a broader epidemiological study. By the end of 1959, Windish and Sanderson had completed two brief radiation surveys in the mine [W6, W7]. The results of these surveys established that airborne radioactivity in the form of radon and radon decay products was present in the two mines surveyed in excess of the maximum permissible concentration (indicated in the Royal Commission report to be 1 WL). One of the suggestions made by Windish [W5] was that radon was carried into the mine by mine water and then released to mine air.

387. As described in Aylward et al. [A14], the Occupational Health Division of the Department of National Health and Welfare began a detailed clinical investigation of the St. Lawrence miners in August 1960, under the direction of Dr. A.J. de Villiers. Current epidemiological studies have evolved from this foundation. It should be noted that, prior to 1959, there were no measurements of radon or radon decay product concentrations in the Newfoundland fluorspar mines.

2. Radon and radon decay products

388. On the basis of work done described in the report of the Royal Commission [A14], the source of radon was eventually identified as the water that poured into the mines [D5], the radon itself apparently originating from the host granite. The report of the Royal Commission ([A14], table II) reported levels of radon of 300–13,000 pCi/L in mine water.

389. Interestingly, high radon levels in water were not limited to the mine. According to data of the Royal Commission [A14], measurements of municipal water supplies in the St. Lawrence area revealed radon levels ranging from 1,800 to 14,370 pCi/L. Radon levels in other water supplies in the St. Lawrence area ranged from 6,000 to 12,000 pCi/L. Radon levels in neighbouring communities close to St. Lawrence were found to range from 1 to 1,140 pCi/L.

390. Morrison et al. [M17] noted that in 1960, because of the high levels of radon identified, mechanical ventilation was introduced into all levels of the mine that were still operating, and the RDP levels subsequently fell below the then current limit of 1 WL. In 1978, mining operations in St. Lawrence ceased, and the last fluorspar mine was closed. By this time, 78 cases of lung cancer had already been identified [C12].

391. Surveys conducted by Windish and Little in 1959 and 1960 collected 17 radon and 80 RDP readings (the Kusnetz method was used for measuring the RDP concentrations) and several gamma radiation readings (reported in reference [A14]).

392. A retrospective study of early mining conditions and working level exposures to RDPs was carried out by Corkill and Dory of the Atomic Energy Control Board (AECB) of Canada [C12]. On the basis of a detailed study of each mine, including measurement data, mining records, ventilation data, interviews and simulation studies, every mine was assigned a high, medium or low RDP exposure level for each operating year (1933–1960). No RDP measurement data were available prior to 1959. Before 1960, when additional ventilation and control measures were introduced, miners were likely to have been exposed to average RDP concentrations of 2.5–10 WL, depending upon the type and place of work. From 1960 onward, estimates of RDP exposures were available for miners by calendar year [M14].

3. Exposure estimation

393. Sources of RDP exposure data include: values estimated and assigned to each mine and each calendar year for 1933–1960 in the retrospective study by Corkill and Dory [C12]; survey data by Windish and Little in 1959 and 1960 (as reported in reference [A14]); and personal exposure data starting in 1960. The data (80 samples collected at 50 different locations) in Windish and Little's survey formed the basis of de Villier's epidemiological studies in 1964 and 1971 [D4, D5]. In the 1964 study, mortality among fluorspar miners was compared with normal mortality in the same geographical region and with that of uranium miners.

394. The WLM method of exposure measurement for individuals was not used until de Villier's 1971 paper [D5]. In the 1971 study, analyses were performed on miners, drillers and muckers. Pre-1960 exposures were included, since the mortality analysis started in 1933; however, no explanation of how they were accounted for is given. The only exposure data mentioned were those given in the Windish survey.

395. The retrospective study of Corkill and Dory [C12] and the study of Morrison et al. [M14] provided more comprehensive data for miners employed during the period

1933–1978, including mortality data from 1933 to 1981. In the analysis by Morrison et al. [M16, M17], the cohort consisted of 1,772 miners employed either by the St. Lawrence Fluorspar Company or Newfoundland Fluorspar Limited. Morrison et al. [M16, M17] described the databases, personal identifying information and occupational histories. The exposure estimates in WLM used in this analysis were calculated on the basis of year, mine and occupation for the period 1933–1960, based on data provided by the AECB [C12].

396. According to Dory and Corkill [D6], from 1961 onward, estimates of RDP concentrations in WL and/or RDP exposures in WLM were available from the miners' individual files. The environmental conditions in the early years of mining were reviewed. Exposure re-evaluations were carried out on the basis of an assessment of the environmental conditions determined by a review of mine maps, inspectors' reports, Royal Commission hearings, anecdotal information from former workers and the authors' own experience. Comparison was also made with the conditions in the mines in later years for which radiation measurements were available. Dory and Corkill [D6] also used computer modelling to simulate expected work environment conditions for each mine for each year of its operation. Most importantly, the authors recognized the uncertainty in the estimation procedure and presented a range of RDP concentrations for various workplaces taking into account the degree of wetness and the degree of ventilation assumed to be present. Corkill and Dory [C12] noted that the ranges they presented do not represent extreme concentrations but are "average workplace concentrations for high, medium and low areas".

397. Corkill and Dory [C12] considered it possible to place job types within certain average concentration ranges. Their exposure re-assessment substantially improved the estimation of RDP concentration (WL) and RDP exposure (WLM) for the Newfoundland fluorspar miners. Nevertheless, as in all attempts at reconstruction, there are large (and probably irreducible) uncertainties in the actual conditions that existed in the workplaces for the period 1933–1960.

398. Detailed occupational histories were obtained from company records. The records for the 1933–1936 period were reconstructed from census data, interviews with company officials and company report reviews [D4].

399. Workers' occupational histories, by accumulated hours of exposure and type and place of work, were prepared for all men on the employee list [D5]. Occupational history was particularly important for fluorspar miners, since epidemiological studies included surface workers as well as underground miners.

400. Corkill and Dory [C12] assumed that the type of job dictated where a man worked, and thus the concentration of RDPs to which a worker was exposed. For example, development miners were assigned the high average concentrations, stope miners the medium average concentrations, and miners working in an established area near an air circuit the low average concentrations. The high and low averages often differed considerably (by a factor of from 2.5 to 10). To use these averages, good knowledge of each worker's duties was developed.

401. No mention of the effect of job mobility was made in Corkill and Dory's study. This might have been taken into account, at least to a certain degree, since the payroll records used to construct the occupational history were recorded every two weeks.

402. No mention was made of whether previous hard rock mining experience had been taken into account. However, since St. Lawrence was an isolated fishing community, especially in the early years, miners were most likely to have been fishermen before they started work in the St Lawrence mines.

403. A working month was considered to be 167 hours by de Villiers and Windish [D4] and 170 hours by Morrison et al. [M16, M17] in the WLM calculations. Accumulations of working hours were likely to have been reasonable, since they were compiled from payroll records (on the basis that miners were paid on an hourly basis).

4. Epidemiological studies

404. Early studies of the St. Lawrence fluorspar miners included those of de Villiers and Windish [D4], Parsons et al. [P1] and Wright and Couves [W10]. However, the first attempt to examine the RDP exposure–response relationship was given in a paper published in 1971 by de Villiers et al. [D5]. The number of hours worked underground was used as a surrogate for actual exposure to RDPs. A plot of lung cancer deaths versus number of hours worked revealed an exponential relationship. To adjust for the variable radiation exposure of differing occupations, hours worked were weighted according to occupation. Drifters and stope workers were assigned an RDP concentration of 8 WL, muckers, trammers, and chute operators were assigned a level of 4 WL and shaftmen were assigned a level of 2 WL. With the assignment of these weights, the exposure–response relationship became linear. This procedure was followed by Morrison et al. [M18], who extended the mortality follow-up first to 1978 [M18] and then to 1981 [M14]. In the latter study (published in 1985), the expected number of deaths was calculated from data for an internal control group of unexposed surface workers, with an attempt to account for cigarette smoking and latency period. These analyses, like previous ones, used a modified person-years approach.

405. In 1988, Morrison et al. [M16, M17] modelled the exposure–response relationship using an external control group, and estimated the attributable and additive relative risk coefficients. The radon exposure estimates developed by Corkill and Dory [C12] were used. Attributable and relative risk coefficients were examined by attained age, age when

first exposed and smoking status. In addition, lifetime risk of lung cancer mortality was assessed using both the relative and the attributable risk model. The cohort consisted of 1,772 miners employed by either the St. Lawrence Fluorspar Company or Newfoundland Fluorspar Limited. Morrison et al. [M16, M17] described the databases, personal identifying information and occupational histories. Estimates of exposure in WLM were calculated on the basis of year, mine and occupation for the period 1933–1960, and the calculations provided by the AECB [D6].

406. A 1995 cohort study of the Newfoundland fluorspar miners by Morrison and Villeneuve [M20] and Morrison et al. [M27] examined the mortality experience (1950–1990) of 1,744 underground miners and 321 millers or surface workers. As in the 1988 study, exposure estimates in WLM by year, mine and occupation for 1933–1960 were provided by the AECB [C12]. RDP exposure during the 5 years preceding lung cancer was assumed to be unrelated to lung cancer risk. Overall, 60,000 person-years of follow-up were noted, with a mean cumulative exposure for underground workers of 382.8 WLM over an average of 5.7 years of exposure. Smoking information was available for 65% of the exposed cohort.

407. Values of relative risk (for exposures estimated on the basis of the category of work), adjusted for attained age and period since exposure, increased with cumulative exposure and were statistically significant for cumulative exposures exceeding 200 WLM. On the basis of Poisson regression of the ERRs and the exposure estimates, and through the use of a constrained intercept, the ERR was estimated to be 0.66 per 100 WLM, with a standard error of 0.17%. The effect of cell killing at high WLM was not statistically significant at the $p = 0.05$ level. The attributable risk coefficient for continuous exposure was estimated to be 6.3 deaths (standard error of 0.74) per working level month per 10^6 person-years, with a multiplicative correction estimated from the cohort.

408. The ERR per unit exposure increased with duration of exposure, suggesting that those exposed over longer periods of time had a greater risk than those exposed over a shorter period at the same exposure level.

409. The ERR per unit exposure decreased with increased duration of exposure in a previous 11-cohort study by Lubin et al. [L10]. While this is biologically plausible, these authors presented an alternative explanation for the inverse dose-rate effect, namely that the finding is an artefact resulting from a greater non-differential exposure misclassification at higher exposure rates than at lower exposure rates. Because exposure rates were extrapolated for the period prior to 1960, when they were high, these authors suggested that there may be a much higher degree of miscalculation of these exposures than of exposures from 1960 onward. The effect of non-differential misclassification would be to bias the risk estimates towards lower values, resulting in a greater reduction in risk estimates at high dose rates than at low dose rates. If the inverse exposure-rate effect is indeed the result of bias, then it follows that the ERR would have been an underestimate, since the significant random exposure misclassification that constituted the basis for the bias should also bias the overall ERR towards lower values at higher exposure rates.

410. The 1995 analysis [M20] found an ERR for lung cancer of 0.66 per 100 WLM, slightly lower than that observed by Lubin et al. [L10] in their analysis of the fluorspar cohort. The difference with the more recent analysis was that it was based on a later follow-up time (1990 versus 1984 for reference [L10]). However, the ERR of 0.66 per 100 WLM was similar to that noted by Lubin for all 11 mining cohort studies combined (0.49 per 100 WLM). Although statistically significant differences in ERR were detected between smokers, non-smokers and former smokers, the joint effects of exposure to RDPs and smoking could not be assessed.

411. A more recent report described an 11-year updated analysis of the mortality experience (1950–2001) of the Newfoundland fluorspar miners [V4]. The new study reports on an analysis of 328 miners who worked exclusively on the surface and 1,742 individuals exposed to RDPs from working underground. When compared with Newfoundland males, the fluorspar miners had significantly increased numbers of deaths for lung cancer, silicosis, and accidents, poisoning and violence. In total, 206 lung cancer deaths were identified, 191 of which occurred among individuals who had at some stage worked underground, the other 15 occurring among miners who had worked only on the surface.

412. Villeneuve et al. [V4] found a strong association between cumulative exposure to RDPs (WLM) and lung cancer risk. Workers with estimated cumulative exposures exceeding 2,100 WLM had relative risks more than 20-fold higher than unexposed miners. After adjusting for age and calendar period, the linear ERR among underground and surface miners (combined) was estimated to be 0.47 (95% CI: 0.28, 0.65) per 100 WLM. The relationship between cumulative exposure in WLM and lung cancer risk was modified by time since last exposure, duration of exposure and exposure rate. In contrast, age at first exposure was not a statistically significant determinant of lung cancer risk. After 35 years since the time of last exposure, lung cancer mortality rates among exposed miners dropped to levels experienced by those who worked exclusively on the surface. Morphology was available for 88 of the 191 lung cancer deaths among those who worked underground. The histology included squamous cell carcinomas (28), adenocarcinomas (8), small cell carcinomas (7) and other carcinomas (45). Owing to the small number of cases, it was not possible to determine the ERR per unit exposure by histological type.

413. Twenty-eight lung cancer deaths occurred among men who started working after 1960 (when ventilation was introduced). There was no significant variation in the ERR per unit exposure between those who started work before and after 1960. However, the evaluation of cancer risk among those who started mining after 1960 is based on younger

men. Thus few cancer deaths were identified, so the statistical power to detect an association in these workers was limited [V4].

414. Some data on smoking were available for 1,107 of the 2,070 miners (53%). There was no statistically significant difference in the ERR per unit exposure between those who had smoked and those who had never smoked. However, strong associations between cumulative radon exposure and lung cancer risk were noted among individuals who had smoked different numbers of cigarettes daily ($p < 0.05$). Specifically, the ERR was 0.31, 0.46 and 0.94 per 100 WLM among individuals who reported smoking <15, 15–<30 and 30 or more cigarettes per day, respectively. An evaluation of the joint effect of exposure to RDPs and smoking (as measured by the number of cigarettes smoked daily) could not adequately discriminate between additive and multiplicative models. However, the data were suggestive of an intermediate relationship (between additive and multiplicative). The evaluation of the joint effects of smoking status and radon was severely limited by the small number of lung cancer deaths that occurred among miners who never smoked ($n = 8$) [V4].

5. Evaluation

415. One possible advantage of the Newfoundland fluorspar cohort over most other studies of radiation-exposed mining populations is that the source of RDP in the fluorspar mines was from groundwater and not from radioactive ore. Thus it was possible to exclude the effects of gamma radiation, thoron and radioactive dust. The cohort was, however, exposed to silica. Another advantage is that fluorspar miners were almost without exception local men with no previous mining experience. Upon ceasing to mine fluorspar, the people went back to non-mining professions. Unfortunately, all the exposures in the period of high exposure rates (1933–1960) were estimates only and are subject to large uncertainty. While the exposure data are weak, the availability of smoking histories and the ability to investigate the effect of changes in individual smoking histories over time are strengths of the cohort. The study of Villeneuve et al. [V4] confirms the strong association between cumulative exposure in WLM and lung cancer incidence.

J. Chinese miners

416. Uranium prospecting and uranium mining started between 1955 and 1958 in China, and routine monitoring of radon was carried out after 1959. The first uranium mine was established in Hunan province in August 1958; very high levels of radon were measured because of poor ventilation. Radon levels decreased rapidly after 1960. Comprehensive studies of radon exposure were completed in 1993 for 11 uranium prospecting teams and four uranium mines. A total of 27,172 and 108,744 person-years were accumulated for the prospecting teams and the miners, respectively, during the follow-up period 1971–1985. Over this period, the average RDP concentrations were 0.3 WL for the prospecting teams and 1.0 WL for the miners, resulting in average cumulative exposures of about 80 WLM for each group. In total, there were 28 lung cancers. ERRs of 1.19 per 100 WLM and 1.09 per 100 WLM were estimated for the prospecting teams and the miners, respectively [S52].

417. There are some English language papers (e.g. [L11, L27, S26, S27, S50, S51, S52, S53, S54, S58, T32, Z6]) and many Chinese papers that discuss the lung cancer experiences of miners who worked in the Yunnan tin mines in China. Qiao et al. [Q1] reported on an investigation of risk factors and the early detection of lung cancer in a cohort of Chinese tin miners. They described a dynamic cohort using an ongoing lung cancer screening programme among tin miners exposed to arsenic and RDPs. The investigation noted that about 6,000 tin miners are screened per year with sputum cytology, chest X-rays and personal interviews. The authors calculated SMRs and 95% CIs. They also calculated relative risk and 95% CIs for lung cancer risk factors from a proportional hazards model. Exposures to RDPs and arsenic were the predominant risk factors, but silicon and smoking were also lung cancer risk factors in their cohort.

418. Chen and Chen [C36] reported a nested case–control study of 130 male lung cancer cases and 627 controls from a cohort of 7,855 miners employed for at least 1 year between 1972 and 1974 in any of four tin mines in China. The Maentel-Haenzel OR was used to measure the association between lung cancer and various risk factors. Unlike Qiao et al. [Q1], these authors did not find that silica exposure was related to the risk of lung cancer. However, the authors did find a strong association between risk of lung cancer and cumulative exposure to dust, cumulative exposure to arsenic and duration of dust exposure. The most recent English language summary of this cohort was provided in a paper by Shiquan et al. [S54], which indicated that the high incidence of lung cancer in miners of the Yunnan Tin Corporation (YTC) in Gejiu, South China, had attracted attention since the early 1970s. Underground monitoring of dust started in 1955 and of RDPs in 1972. Data collection from medical examinations, chemical analysis of pulmonary tissues and animal experiments started in 1975. The database for epidemiological studies was established in 1976, and reports from cooperative studies with the National Cancer Institute (NCI) in the United States began in 1998. Some of these epidemiological studies and the database provided by YTC were used by the National Institutes of Health [L10] and BEIR VI [C20] as the basis of data for the Chinese miners in the joint analysis of 11 underground miner cohorts. Lung cancer cases from the YTC, one of the largest of the 11 cohorts, made up 36% of the total cases in the NIH and BEIR VI combined analyses.

419. About 90% of the lung cancer cases at YTC had a history of working underground [S54]. Prior to 1950, the principal work involved men carrying ore on their backs in small tunnels. That working style was gradually abolished after 1953 as mechanization started to be introduced.

YTC miners represented a stable population without significant loss of follow-up. Mose miners started work before 1950, some before 1920. Most miners with lung cancer began mining as children under the age of 14, but this group's age of death and risk of lung cancer showed no prominent differences from those of miners who started mining after the age of 15 or 20.

420. The first systematic monitoring for radon was done in four YTC mines in 1972. The highest level was at the L–mine, at 28.6 Bq/L on the average. Radon concentrations decreased after the improvement of underground ventilation in 1974 [S54]. To estimate RDP exposure before 1950, 13 existing small tunnels were measured in around 1980, and showed an average RDP concentration of 2.3 WL. Measurements of ^{210}Pb in rib bones obtained after operations on miners with lung cancer provided additional data for retrospective dose assessment [L27]. Estimates of cumulative exposure to RDPs could be divided into three stages: 1950–1953, when the miners carried ore on their backs; 1953–1972, a period of modern mining but lacking radon monitoring; and after 1972, with radon monitoring.

421. Before 1965, airborne dust concentrations underground were very high at about 27–60 mg/m^3, because of dry drilling. In 1965, the dust levels decreased to 6 mg/m^3 after the introduction of wet drilling, [L27, S58]. Arsenic and iron were present as relatively insoluble compounds in YTC mines; the arsenic concentration in the rock was about 0.5–1%. Airborne arsenic concentrations measured in the 1970s were about 0.01 mg/m^3 and were thought to have been more than 10 times higher in earlier years.

422. A combined effect of exposure to RDPs and arsenic on the aetiology of lung cancer among the YTC miners was reported previously [S51, X1, Y1]. The data for arsenic (dust containing arsenic) and RDPs, the only occupational carcinogens underground, were compared to identify their relative contribution in the aetiology of lung cancer. Since cumulative exposures to RDPs and arsenic were highly correlated, both being related to the duration of underground work, comparison of the relative contributions to risk from RDPs and arsenic had to be approached from differences in lung cancer risks in miners working at different jobs (mining, tunnelling, auxiliary) and different mines (L, M and S, the three largest YTC mines).

423. Sun et al. [S53] suggested that the arsenic adjustment used by the NIH [L4] was unsuitable for use in risk projection. BEIR VI [C20] noted that adjustment for arsenic exposure was difficult because of the strong correlation between RDP and arsenic exposures. Hazelton et al. [H7] noted a high risk from arsenic exposure and an interaction of arsenic with other sources of exposure in the study of the YTC miners. These authors analysed the arsenic, radon, cigarette smoke and pipe smoke exposures using the biologically based two-stage clonal expansion model. They concluded that, of 842 lung cancer deaths among YTC miners in Gejiu, 21.4% were attributable to tobacco smoke alone, 19.7% to a combination of tobacco smoke and arsenic, 15.8% to arsenic alone, 11% to a combination of arsenic and RDPs, 9.2% to a combination of tobacco smoke and RDPs, 8.7% to a combination of arsenic, tobacco smoke and RDPs, 5.5% to RDPs alone and 8.7% to background gamma radiation.

K. Australia: Radium Hill uranium miners

424. In addition to the studies discussed earlier in this section, other miner groups exposed to radon have been discussed in the literature. For example, the BEIR VI report [C20] also discussed lung cancer in workers in the Radium Hill uranium mine in Australia [W15]. Exposures in this mine were estimated on the basis of 721 measurements of radon concentrations; however, no data on RDP concentrations were reported. Ventilation data were used to estimate mean residence time in the mine and subsequently the exposure of miners in WLM. Overall exposures of this cohort were very low, with a mean exposure of 7 WLM. The authors reported an excess of lung cancer (4 lung cancer deaths) in miners whose estimated cumulative exposures exceeded 40 WLM. Thirty-six per cent of the cohort could not be traced beyond employment at the mine. Overall, while supporting the findings of an association between RDP exposure and increased lung cancer, this study provides no useable information for the evaluation of an exposure–response relationship.

L. Overall evaluation of miner studies

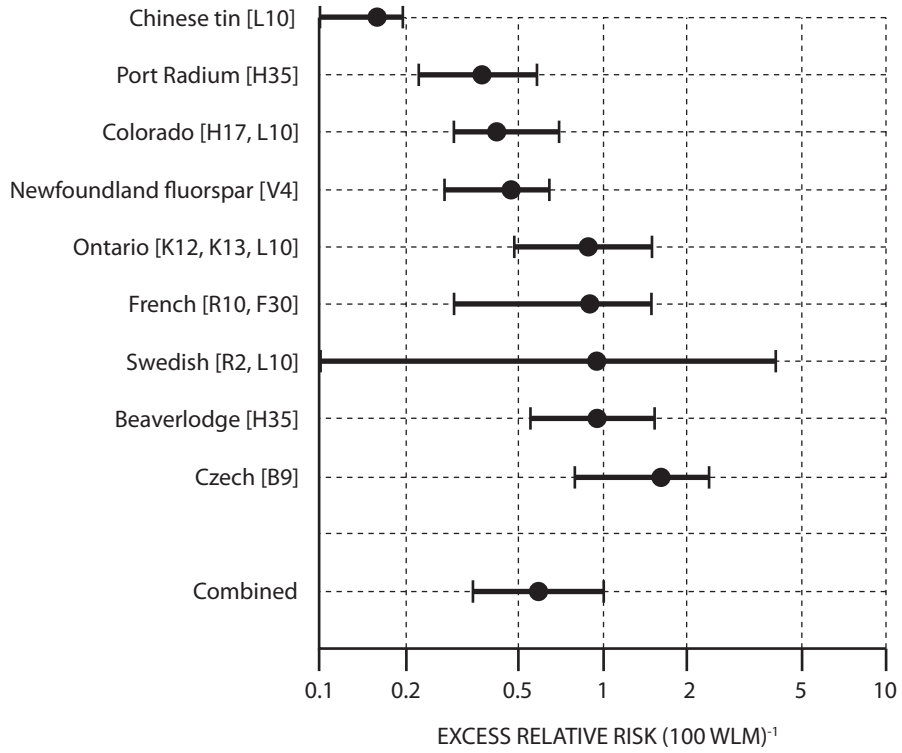
425. All of the miner studies reviewed in this section involve retrospective evaluation of exposures to RDPs. In some cases, such as the Newfoundland fluorspar miners and the Port Radium miners, almost all of the exposures were estimated, whereas for the Czech and Wismut cohorts (starting after about 1971), relatively less speculation was needed. Studies of miners also differ by type of mine (e.g. uranium, iron, tin, fluorspar), exposure rate (i.e. WL in the workplace), size of study (e.g. number of subjects, number of lung cancers) and other factors. All of the miner studies described in previous sections confirm the risk of lung cancer from exposure to RDPs. However, not all of the studies are of the same "quality". It is evident that the studies summarized in table 21 vary widely with respect to factors that affect the determination of the exposure–response relationship and factors that modify that relationship, including, for example, the number of excess lung cancers, the quality of the exposure data (both the range of exposures and the uncertainty in exposures) and confounders such as smoking and exposure to arsenic. A qualitative overall evaluation of such considerations is provided in table 21.

426. Table 2l is a summary of some of the key features of the various miner studies discussed in this section. The table also provides the average ERR per unit exposure estimated for a simple linear ERR model for each of the studies. The ERR per unit exposure ranges over approximately a factor of 5. On the basis of an analysis of 11 miner cohorts, Lubin

et al. [L10] reported an ERR of 0.49 (95% CI: 0.2, 1.0) per 100 WLM.

427. In a manner similar to that adopted by Lubin et al. [L10], the estimated ERRs reported in table 21 were combined using a generic inverse variance method and assuming that random effects were present between studies. The results are illustrated in figure XIV, which shows a combined ERR of 0.59 (95% CI: 0.35, 1.0) per 100 WLM, comparable to that reported by Lubin et al. [L10].

Figure XIV. Estimates of ERR per unit exposure from studies of miners.
Combined ERR of 0.59 (95% CI: 0.35, 1.0) per 100 WLM; 95% CI developed with random effects model.



428. It is of interest to compare the risks based on miner studies with those from the more recent pooled residential radon studies. Assuming for purposes of illustration a nominal indoor equilibrium factor of 0.4, 35 years of exposure to indoor radon at 100 Bq/m^3 for 7,000 h/a, an exposure of about 19.5 WLM can be estimated. If an ERR of 0.59 (95% CI: 0.35, 1.0) per 100 WLM based on miner studies is assumed (see para. 427), an ERR of about 0.12 (95% CI: 0.04, 0.2) per 100 Bq/m^3 can be estimated, a value which compares remarkably well with values from pooled residential radon studies, as discussed in the next section. The miner studies show that the ERR decreases with increasing time since exposure. This is an important consideration in evaluating potential risks from lifetime exposure. Finally, as evidenced by the updates of the Port Radium and Beaverlodge cohorts [H35], continued follow-up of miner studies is important, because the ERR and other outcomes of the epidemiological analyses may change as the cohort ages.

Table 21 Excess relative risk of lung cancer for exposure to radon in mines

Study	*Observed cases*	*Expected cases*	*Mean cumulative exposure (WLM)*	*Mean follow-up time (years)*	*Person-years*	*Main potential confounders*	*Average ERR per unit exposure (per 100 WLM) (95% CI in brackets)*	*Overall evaluation (subjective)*
Colorado Plateau uranium miners [H17, L10]	327	74	807.2	24.6	75 032	Exposure uncertainty; other hard rock mining; smoking	0.42 (0.3, 0.7)	Medium
Ontario uranium miners[c] [K12, K13, L10]	282	221	30.8	17.8	319 701	Other hard rock mining; smoking	0.89 (0.5, 1.5)	High
Czech uranium miners [T39]	915	240.8	70	23.2	261 428	Exposure uncertainty in early years; smoking	1.6 (1.2, 2.2)	High
Swedish iron miners [L10, R2][a]	79	44.7	80.6	25.7	32 452	Exposure uncertainty[b]; smoking	0.95 (0.1, 4.1)	Low[a]
Beaverlodge uranium miners [H35]	279	217.8	23.2	33	285 964	Exposure uncertainty[b]; smoking	0.96 (0.56, 1.56)	High
Wismut uranium miners [K27, K28, K29]	2 328	Not available	242	30.5	1 801 626	Exposure uncertainty (especially prior to 1966); smoking; arsenic; asbestos	0.21 (0.18, 0.24)	Potentially high
Port Radium uranium miners [H35]	230	142.7	174.2	>44	111 222	Exposure uncertainty[b]; smoking	0.37 (0.23, 0.59)	Low
French miners [R10, T30]	125	83.1	36.5	26	133 521	Exposure uncertainty prior to 1956; smoking	0.8 (0.3, 1.4)	High
Newfoundland fluorspar miners [V4]]	206	–	378	>35	70 894	Exposure uncertainty; smoking; high dust levels	0.47(0.28, 0.65)	Low to medium
Chinese tin miners [L10]	936	649	277.4	10	135 357	Exposure uncertainty; smoking; arsenic; age at exposure	0.16 (0.1, 0.2)	Low to medium

a Exposures in the Malmberget iron ore miner study have been updated, and miner doses have increased by about 50% from those used in reference [R2]; however, at this time the epidemiological study has not been updated.
b Uncertainty remaining after dose re-evaluation.
c The Ontario miner study is being updated and will more than double the number of person-years.

V. EPIDEMIOLOGICAL STUDIES OF RESIDENTIAL EXPOSURES

A. Introduction

429. Over the past twenty years or so, there has been a great deal of interest in the risks arising from exposure to radon and its decay products. It is clear from studies of miners that exposure to radon and radon decay products causes lung cancer, as described in section IV (e.g. [C20, D14, D16, I2, I5, L10, N11, U2, U5]). Data from animal experiments such as described in section III also demonstrate that exposure to radon and its decay products causes lung cancer. Until recently, data from studies of underground miners, in uranium mines and other mines, formed the basis for estimating risks from exposure to RDPs and for investigating the exposure–response relationship, as, for example, was carried out by BEIR VI in their pooled analysis of 11 miner cohorts [C20]. Risks from residential RDP exposure were estimated by extrapolation from miner studies. Now, however, there are more than 20 case–control studies of residential radon exposure and lung cancer. While individual studies have limited power, pooled analyses of European [D17, D21], North American [K1, K26] and Chinese [L26] residential radon exposure studies provide a clear demonstration of the risks of lung cancer from residential radon exposure and a direct basis for estimating risk in dwellings from such exposure. This section provides an overview of the case–control residential radon exposure studies and, in addition, a short commentary on the relevance of geographical correlation ("ecological") studies. Further information on the epidemiology of radon exposure and on "ecological" studies is provided in annex A, "Epidemiological studies of radiation and cancer".

B. Case–control studies of residential radon

430. Many case–control studies published prior to about 2000 are well described by Lubin and Boice [L4], BEIR VI [C20], NCRP SC65 [N11] and the UNSCEAR 2000 Report [U2]. Lubin et al. [L8] discussed how errors in exposure assessment can affect the interpretation of results. These authors showed that information from seven case–control residential radon studies supported a wide range of risks, ranging from no excess risk to excess risks larger than those predicted using data from miner studies. These authors discussed various sources of error in the estimation of residential exposure, including various potential sources of measurement error. In addition, a number of authors (e.g. [L4, P17]) have reported meta-analyses that are based on published relative risks. However, such studies are not able to correct for smoking, a key determinant of lung cancer and therefore a matter that requires careful consideration, ideally on a subject-by-subject basis. The pooled studies [D17, D21, K1, K26, L26], based on individual data, are more informative than the previous meta-analyses.

431. Several ways to address the problems resulting from such exposure assessment errors are discussed in the literature, including the use of special films placed on glass artefacts to measure the long-lived RDPs directly [L31]. One of the primary motivations for the glass-based measurements was to take account of systematic increases in residential radon concentration due, for example, to efforts to increase the energy efficiency of homes by providing better insulation and decreasing air leakage. However, United Kingdom data [L45] suggest that, in the United Kingdom at least, any increase has not been substantial. Another motivation for the glass-based measurements was to eliminate the difficulties caused by missing measurements in the residential studies, for example in cases where a house was demolished. Much work in improving the retrospective assessment of radon exposure was conducted using glass-based detectors and other approaches (e.g. [F9, L31, M33, P15]). Bochicchio reviewed the use of nuclear track detectors in the context of residential radon epidemiology and also discussed the various sources of uncertainty in the retrospective estimation of residential exposures. Bochicchio suggests that ^{210}Po alpha activity on the surfaces of glass objects might be a better surrogate for past exposures than contemporary radon measurements. However, he noted that such data are confounded by aerosols from cigarettes, which reduce the ratio of ^{210}Po surface activity to radon concentration [B42].

432. An early case–control study of domestic exposure to RDPs and lung cancer in Port Hope, Ontario, was carried out to determine if there was an excess of lung cancer in Port Hope residents attributable to exposure to elevated RDP levels [L41]. Since smoking is the major cause of lung cancer, the study controlled for smoking. "Cases" were defined as any person who developed or died from lung cancer in the period 1969–1979 and who had lived at least 7 years in Port Hope. The 7-year residence period was selected because this was the shortest possible time between exposure and the occurrence of lung cancer. Twenty-seven cases met the criteria. There were two controls for each case. Estimates of exposure were developed by adding the cumulative exposures estimated for each house occupied by a case or control since 1933. The statistical analysis failed to demonstrate an increased risk of lung cancer from elevated domestic radon exposure, but did identify a very strong risk of lung cancer from cigarette smoking.

433. High concentrations of indoor thoron were observed in the Loess Plateau region of China (e.g. [S67, T35, W13, W20, Y8]). The assessment of the risk from RDPs is known to be affected by the presence of thoron and its decay products [P19, T17]. It was thought that the detectors used in the study by Wang et al. [W13] might have been affected by the presence of thoron [S10, T17] and thus that the measured radon concentrations might have been overestimated. A re-assessment of the exposures from radon and thoron decay products is currently under way. Moreover, the screen-type diffusion battery (SDB) measurements in underground dwellings indicated the presence of ultrafine particles of around 10 nm [Y8]. Current evaluations are based on exposures; however, since the dose conversion factor for these small particles is high, a modified contribution to dose might need to be considered in the future.

434. Several studies investigated residential radon exposure and lung cancer in China. A study carried out by Blot et al. [B25] in Shenyang City, during 1988 and 1989, included 308 females with lung cancer and 356 female controls. The median radon level measured in the homes of both cases and controls was 2.3 pCi/L (85.2 Bq/m^3). The median duration of residence was 24 years. No link between radon exposure and an excess risk of lung cancer was found, irrespective of smoking status (other than a non-significant trend in heavy smokers). A study by Wang et al. [W13] included 1,659 cases and 768 controls who lived in an area of Gansu province. Prior to 1976, many of the subjects had lived in underground dwellings (99%), although many had since moved to above-ground houses. The mean radon levels were quite high for both cases and controls, at 230.4 Bq/m^3 and 222.2 Bq/m^3, respectively. Using a linear model, the authors estimated an excess odds ratio (EOR) of 0.19 (95% CI: 0.05, 0.47) at an exposure of 100 Bq/m^3. If adjustments were made for uncertainty in exposure, the EOR increased by about 50%.

435. Tokonami et al. [T35] and Sun et al. [S63] carried out a radon and thoron survey in the Loess Plateau region of China. Their study area was located near Gansu province. Since the geological features seemed to be almost the same as in Gansu province, the characteristics of radon and thoron concentrations were also likely to be similar. The radon concentration was lower than that in the study by Wang et al. [W13], but the thoron concentration was higher. Because thoron was underestimated in the past, reassessment of risks due to radon exposure may need to take the presence of thoron into account.

436. Field et al. described a residential radon study of females in Iowa, United States [F10]. The Iowa radon study included 413 cases and 614 controls. The median residency was 33 years for cases and 31 years for controls. Some 357 cases and 200 controls were "ever smokers" (i.e. people who had at some time smoked); an additional 104 cases and 200 controls were former smokers. The radon exposure assessment involved a number of components, including 1 year of on-site radon measurements, regional outdoor radon measurements and linkage of the subject's mobility with exposure to radon indoors and outdoors. Outdoors, radon concentrations varied from 7.4 to 56 Bq/m^3, the latter being comparable to the United States national average indoor radon level of 48 Bq/m^3 (see figure I). An average of four radon detectors were placed in each home. The measurements exhibited approximately a log-normal distribution. The majority of basement measurements and a significant fraction of the measurements on the ground level and the upper level exceeded 148 Bq/m^3 (the United States Environmental Protection Agency action level of 4 pCi/L). The authors calculated cumulative exposures to RDPs that occurred 5–19 years prior to diagnosis for the cases, or prior to time of interview for the controls, as 8.6 WLM and 7.9 WLM, respectively. The odds ratios (ORs) for lung cancer in women who had smoked at least 100 cigarettes or for at least 6 months in their lifetime relative to women who had never smoked was 13.2 (95% CI: 9.5, 18.3). The authors also found a significant positive trend between lung cancer and RDP exposure. The authors estimated risks for a cumulative 15-year radon exposure of 11 WLM (taken by the authors as equivalent to an average radon concentration of 4 pCi/L) for all cases. After adjustment for age, active smoking and education, the authors estimated an EOR of 0.24 (95% CI: –0.05, 0.50) or (95% CI: 0.004, 1.81), when radon exposure was treated as a continuous or a categorical variable, respectively. A subsequent paper by Field et al. [F11] reported a slightly different CI for the EOR of 0.24; this was 95% CI: 0.05, 0.92, calculated treating radon exposure as a continuous variable. Field et al. observed a statistically significant trend for large cell carcinoma and a "suggestive" trend for squamous cell carcinoma (categorical p for the trend of 0.06). However, the linear excess odds between different histological types were not significant.

437. Tomasek et al. [T10] reported a residential radon study of 12,000 people living in central Bohemia with a total of 173 lung cancers and a follow-up period of 1961–1995. This follow-up period was later extended to 1999 [T29]. A total of 210 lung cancers were observed. The study area in central Bohemia is mostly granitoid and has radon levels considerably higher than other areas of the Czech Republic. Exposure estimates were based on measurements of the equilibrium-equivalent concentrations (EECs) of radon (i.e. the RDP concentrations) made in most (80%) of the homes in the study area. Typically, two detectors were installed for 1 year in the two most occupied rooms. To compare their results with those of other studies, the authors established a conversion factor on the basis of 652 simultaneous measurements of EEC and radon. Where necessary, mean values for a community were used to replace missing data. The mean radon concentration was estimated at 509 Bq/m^3, with 10% of the homes having indoor radon levels in excess of 1,000 Bq/m^3. The authors used a linear relative risk model, taking into account the exposures received between 5–34 years previously and estimating expected cases from national mortality data. The authors estimated an ERR of 0.087 (90% CI: 0.017, 0.208) per 100 Bq/m^3. The ERR did not change substantially after adjustment for smoking.

438. Barros-Dios et al. [B26] described a population-based case–control study in an area of north-west Spain considered to be radon-prone. The study covered 163 cases (151 men and 12 women) and 241 controls (219 men and 22 women). Radon concentrations were measured using alpha track detectors placed in the homes for a minimum of 90 days and a median of 150 days. The mean radon levels were 141.4 Bq/m^3 for the cases and 114.0 Bq/m^3 for the controls. Overall, 22% of the homes had radon levels of above 148 Bq/m^3. A multiple logistic regression analysis assessed the risk of lung cancer, taking into account a number of possible confounding factors, including smoking, family history of lung cancer, type of dwelling construction and hours per day spent at home. A total of 145 (91.8%) of the cases and 129 (54.7%) of the controls were smokers. The authors reported ORs by quartiles of the radon distribution, and observed a greater than 2-fold increase in the risk of lung cancer for exposures to radon of above 37 Bq/m^3. The authors noted that the risk of lung cancer in smokers was 46 times higher than in non-smokers exposed to radon levels of below 37 Bq/m^3.

439. Lagarde et al. [L31, L32] reported on a Swedish residential radon study based on an existing database of persons who had never smoked that had been developed to study environmental and occupational exposures to agents other than radon. The database was augmented with measurements of radon concentrations made with alpha track detectors that had been placed in the bedrooms and living rooms of residences for 3 months. The database was also supplemented with data from a nationwide Swedish case–control study for which the radon measurements were similar. On average, about 25 years of the 32-year residential history of subjects was covered by measurements. Covariates included environmental tobacco smoke and history in occupations with a risk of lung cancer. A total of 436 cases and 1,649 controls (all of whom had never smoked) were included in the risk assessment. The excess relative risk per unit of time-weighted residential radon concentration was estimated using conditional logistic regression and a linear relative risk model. The authors found a trend in relative risk with increasing radon exposure, comparable to that found in the nationwide Swedish study. The trend of increased risk with increasing radon levels was limited to subjects exposed to environmental tobacco smoke at home. For those who had never smoked, a relative risk of 1.10 (95% CI: 0.96, 1.38) was estimated, and for those exposed to passive smoking, the relative risk was estimated at 1.29 (95% CI: 0.97, 2.24).

440. Sobue et al. [S34] reported a case–control study of residential radon levels and risk of lung cancer in Misasa, Japan. The case series consisted of 28 lung cancer deaths (26 males and 2 females) between 1976 and 1996, and 36 (33 males and 3 females) controls chosen randomly from residents. Radon levels were measured using alpha track detectors in the most frequented areas of the subjects' homes for a period of 1 year. The average radon level in the study area was about 50 Bq/m^3. The residential radon value measured over the year was used as a surrogate for cumulative radon exposure over the 20 years of the study. None of the ORs calculated using regression was statistically significant. The authors attributed this in part to the small sample size.

441. The risks of lung cancer from residential exposure to radon in Devon and Cornwall in the south-west of the United Kingdom were reported by Darby et al. [D15]. The study looked at 982 cases of lung cancer and 3,185 controls, all under 75 years of age. Detailed information on the demographics of the study population, smoking characteristics and residency was provided. The investigators attempted to measure radon levels at all of the addresses where the subjects had lived in the preceding 30 years. Two alpha track radon detectors produced by the United Kingdom National Radiological Protection Board were installed (one was placed in the living area and the other in the bedroom) for a period of 6 months. For residences in which radon had been measured, the investigators calculated a weighted average radon concentration assuming that residents spent 45% of their time in the living area and 55% of their time in the bedroom. A time-weighted average radon concentration was estimated for the 30-year period of interest using the times spent at each address as the weights. Estimates of the lung cancer ERR per unit concentration in air were obtained using linear logistic regression on the assumption that the subjects' radon exposure was a continuous variable. The regressions also included interactions with the factors noted earlier and other variables. The mean seasonally adjusted radon level in the 9,448 residences was 58 Bq/m^3, with a maximum of 3,549 Bq/m^3. The ERR for lung cancer risk was 0.08 (95% CI: –0.03, 0.20) per 100 Bq/m^3 after adjustments for age, sex, smoking status, county of residence and social class. After further adjustment for the uncertainty in estimates of exposure, the authors reported an ERR of 0.12 (95% CI: –0.05, 0.33) per 100 Bq/m^3. The observed variations among different tumour types were no larger than would be expected by chance.

442. A case–control study for the period 1990–1996 in western Germany was carried out by Kreienbrock et al. [K18]. Detailed demographics and information on potential confounding factors (smoking and occupational asbestos exposure) were collected, as was detailed information on residences occupied in the previous 35 years. Radon measurements were made over 1 year using nuclear track detectors exposed in the living rooms and bedrooms of participants' current and previous homes. (Radon exposure was quantified in two ways: a time-weighted average of the living room and bedroom radon concentrations in the last residence; and an estimation of the time-weighted average cumulative radon exposure in the 5–15 years prior to the interview date.) An attempt was made to correct for home alterations and changes to home ventilation. There were 1,449 cases and 2,297 controls for the entire study area, with a subgroup in a radon-prone area consisting of 365 cases and 595 controls. In the overall study area, women constituted 235 of the cases and 432 of the controls. Among the men, 2% of the cases and 23% of the controls had never smoked. Among the women, 31% of the cases and 60% of the controls had never smoked. Occupational exposure to asbestos was identified for men

only (30.6% of the cases and 19.8% of the controls). Rate ratios and 95% CIs were calculated using logistic regression. All ORs were adjusted for age, sex, smoking and occupational asbestos exposure. The authors noted two results. In the entire study area, no rate ratios were significantly different from unity, while in the radon-prone areas, for an increase in radon concentration of 100 Bq/m^3, an ERR of 0.13 (95% CI: –0.12, 0.46) was obtained for the exposure assessment based on the last residence only, and an ERR of 0.09 (95% CI: –0.14, 0.38) was obtained for the assessment based on cumulative exposure in the 5–15 years prior to the interview date. After conducting sensitivity analyses, the authors attributed the absence of an observable risk in the radon-prone areas to inaccuracy of radon exposure assessment. The inaccuracy increases if the variation in exposure within the study population is low, which was the case in the entire study area.

443. Kreuzer et al. [K17] reported on a study of residential radon concentrations and lung cancer in Saxony and Thuringia, which are areas in eastern Germany with naturally elevated radon levels. The study included 1,192 cases and 1,640 controls. Alpha track radon detectors were placed for 1 year in the living room and bedrooms of the subjects' homes. Radon exposure was calculated as the time-weighted average of radon concentrations in each room. The authors also calculated a time-weighted average radon concentration for the whole of the study period, which was 5–35 years prior to the interview date. Mean radon concentrations were 76 Bq/m^3 among cases and 74 Bq/m^3 among controls. Detailed information on demographics and potential confounding factors was obtained using a standardized questionnaire similar to the one used in the study by Kreienbrock et al. [K18]. Approximately 12% of cases and 14% of controls were women. Among the men, only 2% of the cases and 26% of the controls had never smoked. Among the women, 51% of the cases and 77% of the controls had never smoked. ORs and 95% CIs were calculated using conditional logistic regression (for example, smoking and work history with asbestos exposure were quantified). The OR for smokers compared with those who had never smoked was 18 (95% CI: 12, 29) for men and 2.8 (95% CI: 1.70, 4.8) among women. For men, about 30% of cases and 28% of controls had occupational asbestos exposure. Asbestos exposure in women was negligible. ORs adjusted for age, sex, smoking and occupational asbestos exposure, as well as 95% CIs were calculated using conditional logistic regression. Overall, an ERR of 0.08 (95% CI: –0.03, 0.20) per 100 Bq/m^3 and 0.09 (95% CI: –0.06, 0.27) per 100 Bq/m^3 was found for subjects with complete measurements for all 30 years. Smoking acted as a negative confounder, and there was a moderate increase in lung cancer, which for small cell cancers was pronounced.

444. Conrady et al. [C9, C32] conducted a residential radon study among females living in the Schneeberg and Schlema areas of Saxony in eastern Germany. This study looked at all female lung cancer cases in the study area between 1 January 1952 and 31 December 1989. The final group included 72 cases and 288 controls. About 78% of cases and 94% of controls were non-smokers. Radon measurements were made using alpha track detectors. For 24 houses in Schneeberg, the authors compared the radon data with measurements of ^{210}Po in glass samples, which were used for backward extrapolation of the radon concentrations, and concluded that indoor exposure conditions had been "stable" over the study period. They also noted a large variation in indoor radon levels by week and by season, and cautioned that, while 1-year measurements will yield "quite constant" values, shorter-term measurements could be misleading. The authors reported radon levels of 730 Bq/m^3 in case homes in Schneeberg, about 540 Bq/m^3 in "register" controls and about 290 Bq/m^3 in "hospital" controls. Logistic regression was used in the risk analysis. Elevated odds ratios (OR = 4.35; 95% CI: 1.47, 12.90 and OR = 1.94; 95% CI: 0.59, 6.33) were observed in the two highest exposure categories (radon concentrations of 1,000–1,500 Bq/m^3 and >1,500 Bq/m^3) compared with the reference category of 50 Bq/m^3. No elevation in risk was evident in the lower exposure categories, but this might be attributed to the low statistical power. When data were restricted to non-smokers and lung cancer cases with histological confirmation (38 cases, 172 controls), the risk remained elevated in the highest exposure category, with a radon concentration of >1500 Bq/m^3, showing a borderline significant trend only among small cell lung cancers.

445. Pisa et al. [P6] carried out a residential radon study in an alpine valley in Italy where residential radon levels averaged 132 Bq/m^3, compared with the Italian national average of 77 Bq/m^3. "Ecological" evidence suggested the possibility of a weak association between lung cancer and residential radon. Measurements of residential radon levels were carried out over the course of 1 year using alpha track detectors in the bedrooms of the most recent residence for each subject. Between 1 January 1987 and 31 December 1993, 224 residents in the study area died from lung cancer. Of these, interviews were completed for 138 cases (122 men and 16 women) and 291 controls matched for sex and year of birth. Lifetime smoking, dietary variables and occupational history were all considered in the statistical analysis, in which multiple unconditional logistic regression, with radon treated as a continuous variable, was used to estimate the OR and its 95% CI. The OR showed a strong association with smoking in both males and females. No association was observed between lung cancer and exposure to potential occupational carcinogens. An association between radon and lung cancer was seen in men only. Men who lived in homes with radon levels of 40–199 Bq/m^3 (mean 80.4 Bq/m^3) showed an OR, adjusted for age, sex and smoking, that was approximately a factor of 2 greater than that of men who lived in homes with radon levels of below 40 Bq/m^3. The authors reported an OR of 1.4 (95% CI: 0.3, 6.6) per 100 Bq/m^3 for the group as a whole.

446. Oberaigner et al. [O2, S59] carried out a residential radon study in the highly radon-prone district of Imst in Tyrol, Austria. Lung cancer deaths and a sample of deaths with causes other than lung cancer during the years 1970–1992

(causes of death highly related to smoking were excluded) were matched by age, sex and year of death. The next of kin of the cases and the controls were interviewed for residential history, smoking and other risk factors. Radon concentrations were measured in the last residence for approximately 1 year by means of alpha track detectors placed in the bedrooms and the living rooms. The study included 194 cases and 198 controls. The percentage of men was 88% among both cases and controls. Measurements covered 68% (cases) and 75% (controls) for the period 5–35 years before death. The mean radon concentrations were 266 Bq/m^3 among the cases and 123 Bq/m^3 among the controls. ORs were estimated using conditional regression models adjusted for smoking and occupation. The ERR at an increased radon exposure of 100 Bq/m^3 was 0.25 (95% CI: 0.08, 0.43).

447. Wichmann et al. [S61, W19] reported the results of a pooled analysis of the two German radon studies. These two case–control studies were performed during 1990–1997 in eastern and in western Germany [K17, K18], with identical study design. The original data were extended and pooled, and included a total of 2,963 incidences of lung cancer and 4,232 population controls. Radon measurements were carried out over the course of 1 year in houses occupied during the 5–35 years prior to the interview date. Conditional logistic and linear relative risk regression was used for the analysis. Measurements showed an average radon exposure of 61 Bq/m^3. The smoking- and asbestos-adjusted ORs were 0.97 (95% CI: 0.85, 1.11) for radon concentrations of 50–80 Bq/m^3, 1.06 (95% CI: 0.87, 1.30) for radon concentrations of 80–140 Bq/m^3 and 1.40 (95% CI: 1.03, 1.89) for radon concentrations of above 140 Bq/m^3, compared with the reference category with radon concentrations of <50 Bq/m^3. The linear increase in the OR was 0.10 (95% CI: –0.02, 0.30) per 100 Bq/m^3 for all subjects and 0.14 (95% CI: –0.03, 0.55) per 100 Bq/m^3 for less mobile subjects who had lived in only one home in the previous 5–35 years. The risk coefficients generally were higher when measurement error in the radon concentrations was reduced by restricting the population to those for whom good measurements had been made. With respect to histopathology, the risk for small cell carcinoma was much higher than for other subtypes.

448. Bochicchio et al. [B43] reported on a case–control study of lung cancer and residential radon in Lazio, central Italy, characterized by high levels of indoor radon and by Mediterranean climate and diet. All subjects — 384 cases and 404 controls, aged 35–90 years — were recruited in the hospital. Detailed information regarding smoking, diet and other risk factors were collected by direct interview. Residential history during the 30-year period ending 5 years before enrolment was ascertained. In each dwelling, radon detectors were placed in both the main bedroom and the living room for two consecutive 6-month periods. A quality assurance programme was set up for radon measurements [B41]. ORs and 95% CIs for time-weighted radon concentrations were computed using both categorical and continuous unconditional logistic regression analysis and adjusting for smoking, diet and other variables. Approximately 89% and 91% of the cases and the controls, respectively, were concluded to have good radon exposure data. The adjusted ORs were 1.30 (1.03, 1.64), 1.48 (1.08, 2.02), 1.49 (0.82, 2.71) and 2.89 (0.45, 18.6) for radon concentrations in the range 50–99, 100–199, 200–399 and above 400 Bq/m^3, respectively, compared with the reference category with radon concentrations of 0–49 Bq/m^3 (OR = 1; 0.56, 1.79). The adjusted odds ratio risk for 100 Bq/m^3 was 0.14 (–0.11, 0.46) for all subjects, 0.24 (–0.09, 0.70) for subjects with complete radon measurements and 0.30 (–0.08, 0.82) for subjects who had lived in no more than one or two dwellings. There was a tendency towards higher risk among subjects with low to medium consumption of dietary antioxidants (EOR = 0.32; –0.19, 1.16). In conclusion, both categorical and continuous analyses clearly support an association between residential radon concentration and lung cancer. Moreover, subjects with a presumed lower uncertainty in the concentration assessment showed a higher risk. Finally, this is the first study indicating that dietary antioxidants may act as an effect modifier for radon.

449. Baysson et al. reported on indoor radon concentration and lung cancer in France [B33]. The study took place from 1992 to 1998 in French districts with elevated concentrations of radon. The study included 486 lung cancers cases recruited from university hospitals, and 984 controls. Subjects were eligible if they had lived in the study area for at least 25 of the previous 35 years. Full residential histories covering the previous 30 years were obtained, with specific information collected for the dwellings that had been occupied for more than 1 year. Radon concentrations were measured using track etch detectors placed for a 6-month period in the current and the former residences. The authors estimated a time-weighted average radon concentration for each subject for the period of 5–30 years prior to the interviews. Finally, the authors considered the effect of smoking and occupational exposure to carcinogens. The ORs and 95% CIs were calculated using logistic regression with adjustments for sex, age, region, smoking and occupational exposure. Adjusted odds ratio risks for 100 Bq/m^3 were estimated for all subjects as OR = 1.04 (95% CI: 0.99, 1.11) and for subjects with complete measurements (850 subjects) as OR = 1.07 (95% CI: 1.0, 1.14). The French study supports a small excess risk of lung cancer arising from exposure to residential radon.

C. Ecological studies of residential radon

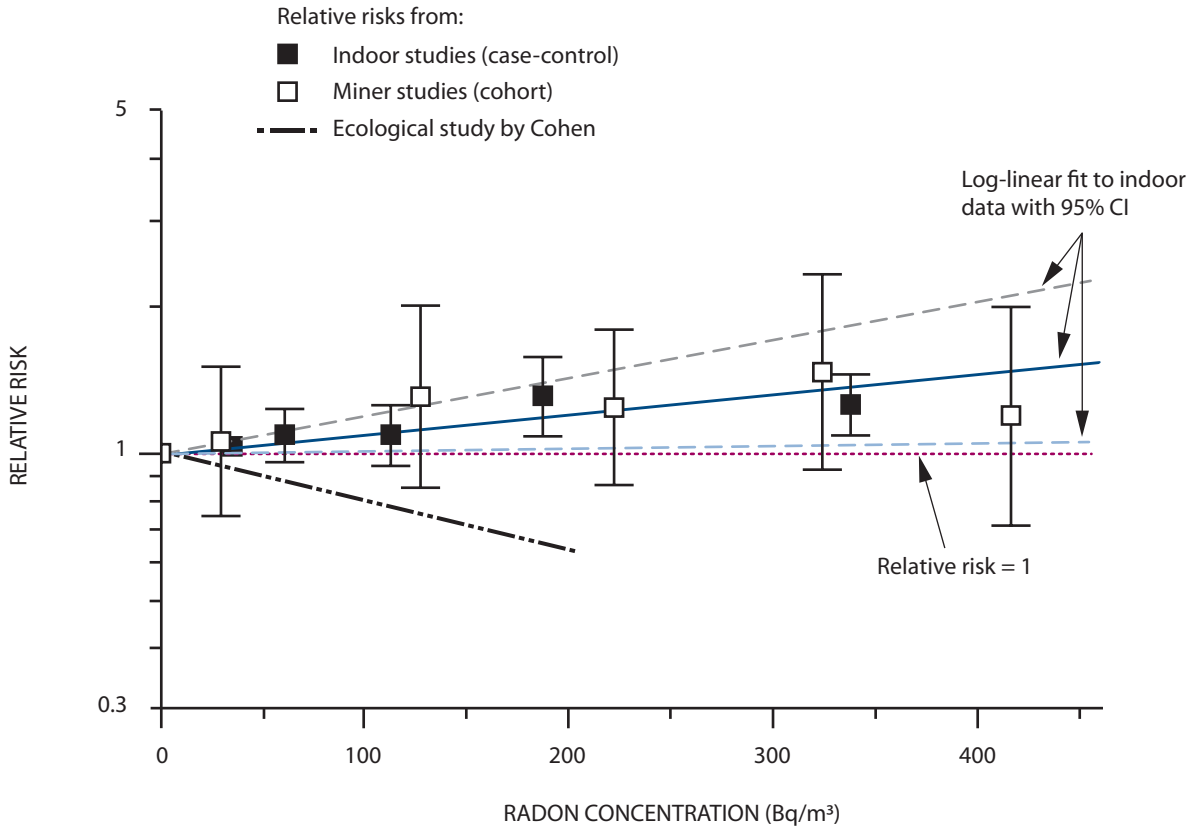
450. The limitations of ecological epidemiological analyses are well discussed in references [C20, L8, L28, U2, U5], as well as in annex A, “Epidemiological studies of radiation and cancer”. Nonetheless, a few short comments on ecological versus case–control studies are appropriate here. Radon studies that rely on averages over geographical areas are especially vulnerable to biases that are not present in results based on individual-level data such as those used in case–control or cohort studies. This is because radon levels are highly variable even within limited geographical areas. In addition, smoking is the major cause of lung cancer, and risk is highly dependent on individual smoking habits. The

distribution of smoking habits across the population in a particular geographical area is an important confounding factor.

451. Stidley and Samet [S49] reviewed 15 ecological studies of residential radon exposure from a number of countries. A positive association between radon concentration and lung cancer was observed in 7 of the studies, no association was observed in 6 and statistically significant inverse relationships were observed in 2. The authors discussed methodological issues associated with the ecological studies and suggested that ecological studies should be given little weight in assessing the potential risks of residential radon. In a subsequent paper [S3], the authors showed by simulation that even modest levels of error in exposure or misspecification of the risk model could introduce significant biases into the results of ecological studies. As discussed in section B, the pooled analysis of case–control studies in Europe [D17] and North America [K1] have gone to considerable effort to correct for uncertainty in radon concentrations. Further discussion of this limitation of ecological studies is provided in annex A.

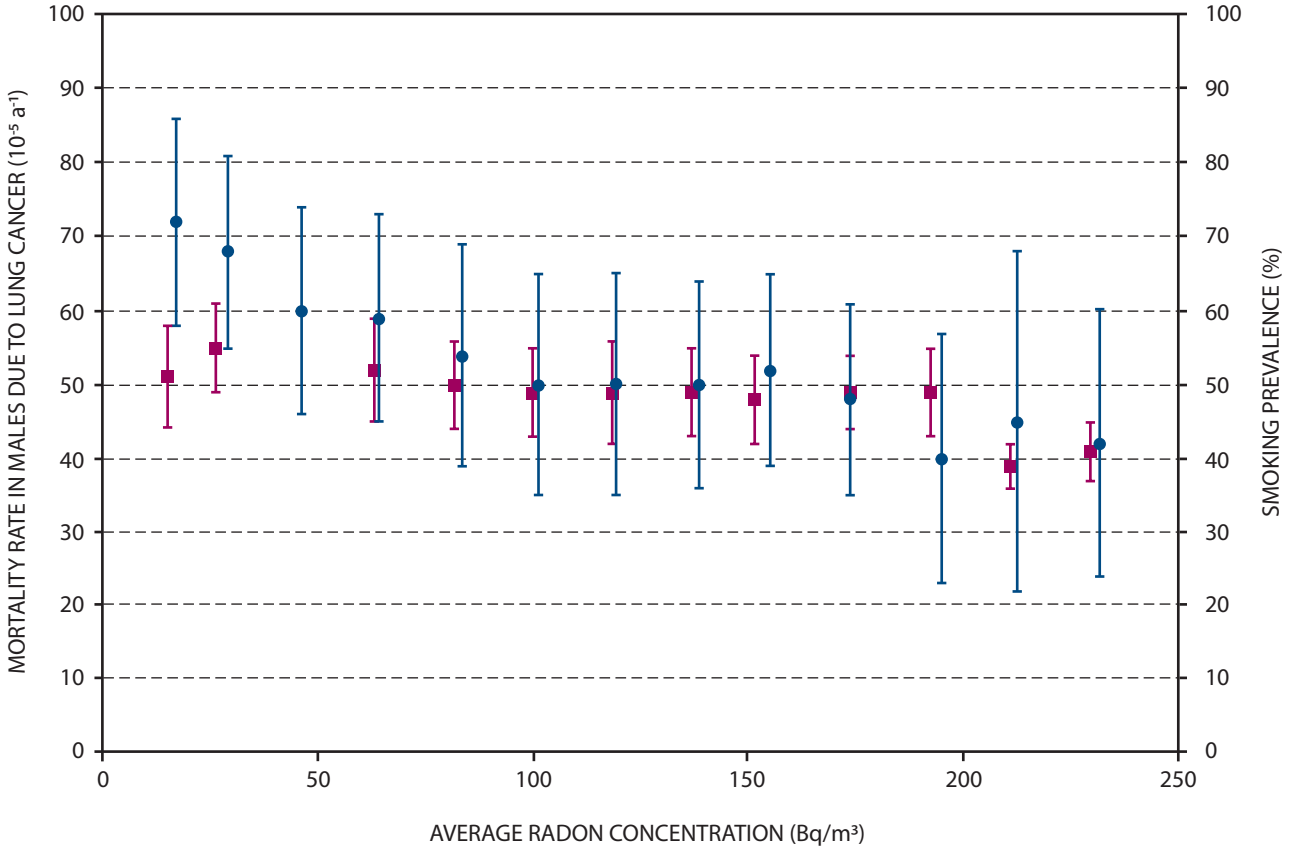
452. Cohen used ecological epidemiological studies to investigate the linear no-threshold theory (LNT) by looking at whether (county average) lung cancer rates in United States counties decreased with increasing (county average) radon concentration. Cohen's work, involving 275,000 measurements in all 50 states [C23, C24, C25, C26, C27, C28], generated a great deal of discussion, including references [A21, F8, F12, G15, G16, L24, L28, L29, L30, S48]. The focus of the discussion was the use of ecological rather than analytical studies to investigate the potential risks from residential radon. Puskin [P10] provided a plausible explanation of Cohen's observation of a negative association (see figure XV) between lung cancer and residential radon concentration. However, Cohen points out that, while an ecological study such as that given in reference [C24] cannot determine a risk versus exposure relationship, it can test the LNT theory. Puskin [P10] found that the inverse association between lung cancer and residential radon was also seen in other smoking-related cancers not related to radon exposure. The result suggested that Cohen's observations could largely be explained by a negative correlation between smoking and radon exposure [P10]. On the other hand, Cohen [C37] argued that Puskin's observation of similar dependence on radon exposure for lung cancer and for other smoking-related cancers is not affected by data on smoking prevalences.

Figure XV. Risk estimates of lung cancer from exposure to radon (adapted from reference [L4]).
Shown are the summary relative risks from meta-analysis of eight indoor radon studies and from the pooled analysis of underground miner studies, restricted to RDP exposures of less than 50 WLM [L19], together with the estimated linear relative risk from the correlation study by Cohen [C24]. Note that references [L4] and [C20] both show Cohen's data extrapolated to beyond where they were actually analysed, about 200 Bq/m^3. The figure has been adjusted to reflect this.



453. Heath et al. [H29] discussed exposure to residential radon and lung cancer risk, as well as providing commentary on Cohen's county-based studies [C24, C25, C35]. To understand Cohen's work, the authors obtained the data sets on which his study was based and carried out an independent analysis of lung cancer mortality on a subset of the data (lung cancer mortality for 1970–1979). Figure XVI (adapted from reference [H29]) shows the results of the analysis of lung cancer mortality in relation to (ecological) radon levels but uncorrected for smoking (solid circles). The same figure shows the pattern of smoking frequencies superimposed (solid squares). The smoking frequencies shown in figure XVI paralleled the pattern of lung cancer except at the lowest radon levels, and this led the authors to suggest that confounding by smoking is particularly important at low levels of radon. Cohen argued that figure XVI is meaningless because the effects of smoking prevalences were fully taken into account in his analyses, and these smoking prevalences were derived from three independent sources, all giving the same results. He also argued that no remotely plausible correlations between radon concentrations and smoking prevalences or intensities of smoking could substantially change his results. Nonetheless, Heath et al. [H29] suggested that Cohen's ecological studies are of limited value in assessing the risk from residential radon exposure because of their reliance on grouped data, coupled with confounding by cigarette smoking.

Figure XVI. Average annual lung cancer mortality per 100,000 males, 1970–1979 (●), and average percentage of cigarette smokers among males (■) within counties, grouped by average county radon level.
Confidence intervals are expressed as the standard deviation of the distribution for each county group (adapted from [H29]).



454. Large ecological studies such as Cohen's [C24, C25], which show an inverse relationship between county averages of radon exposure and lung cancer mortality, continue to generate controversy, despite the results of the numerous analytical (case–control) studies such as those discussed in the preceding section. As discussed in references [S3, S49] and in annex A, ecological studies have a number of methodological challenges, notably their inability to adjust for mobility and smoking habits. The pooled studies involved control of both individual exposures and smoking habits, and provide a methodologically sound basis for estimating the risks from residential radon.

D. Overall evaluation of residential radon studies

455. Baysson and Tirmarche [B29] provided an overview of the case–control studies of residential radon concentration and lung cancer carried out since 1990. They suggested that the results of these studies indicated a positive association

between lung cancer risk and residential radon concentration with, an EOR of 0.06–0.09 per 100 Bq/m^3. Table 22 and figure XVII summarize much of the currently available data on the risks per unit concentration in air from residential case–control studies. In considering the residential case–control studies summarized above, it is important to understand that some studies have greater statistical power than others, owing to factors such as study size and data quality. The key residential case–control studies were included in the pooled analyses of Darby et al. [D17, D21] and Krewski et al. [K1, K26], as previously discussed. Uncertainty in exposure assessment is a factor in residential radon studies, as are the effects of smoking (both active and passive). However, when the results of individual studies are pooled and analysed in a consistent format, there is a coherent trend of increasing risk of lung cancer with increased radon exposure.

456. Bochicchio [B42] reviewed the application of solid-state nuclear detectors in residential radon studies. He argued that exposure uncertainty is generally non-differential and therefore not only adds to the uncertainty in estimated risks but also introduces a bias towards underestimation of risks. Among the factors discussed by Bochicchio are: seasonal variability, which can vary from house to house depending on the source of the radon; the characteristics of the house and of people's living habits; and the difficulties in retrospective dosimetry. Most of the radon studies reviewed by Bochicchio covered periods of up to 35 years, during which there may have been changes to the house, the house ventilation system and residents' habits, all of which can affect both the levels of radon and the levels of RDP exposure. Further discussion of the effects of measurement error in assessing radon exposure is provided in references [H37, H41]. As noted previously, the pooled studies in Europe [D17, D21], North America [K1, K26] and China [L26] have attempted to account for measurement error uncertainty (uncertainty in the measurement of radon concentration and exposure) by restricting the assessment to individuals who had lived in only one or two homes. In addition, as noted earlier, Darby et al. attempted a statistical correction for measurement uncertainty [D21].

457. Bochicchio et al. also provided a convenient summary of residential case–control studies [B44]. The OR per unit concentration in air and 95% CIs for each study are shown in figure XVII. Also shown in figure XVII are the results of several meta-analyses and three pooled analyses in Europe, North America and China. These include the Lubin and Boice meta-analysis of the residential studies [L4], Lubin's analysis of North American and Chinese residential studies [L36], the meta-analysis of 17 case–control studies by Pavia et al. [P17], the Wichmann et al. pooled study of residential exposure in Germany [W19], two pooled studies of 13 case–control studies in Europe by Darby et al. [D17, D21], and the pooled study by Krewski et al. [K1, K26] of 47 case–control studies in North America. All of the studies included in the meta-analyses by Wichmann et al. [W19] and Lubin and Boice [L4] are also included in the pooled analyses [D17, K1].

Table 22 Indoor radon case–control studies (adapted from reference [B29])

Reference		*Region*	*Population*	*Number of cases (controls)*	*Radon measurements*	*OR*[a]	*95% CI*
Year	*Author*						
1990	Schoenberg et al. [S42]	New Jersey, United States	Women	480 (442)	1 year	1.49	0.89, 1.89
1990	Blot et al. [B25]	Shenyang, China	Women	308 (356)	1 year	0.95	Undefined 1.08[g]
1992	Pershagen et al. [P16]	Stockholm, Sweden	Women	201 (378)	1 year	1.16	0.89, 1.92
1994	Pershagen et al. [P11]	Sweden	Both sexes	1 281 (2 576)	3 months	1.10	1.01, 1.22
1994	Letourneau et al. [L25]	Winnipeg, Canada	Both sexes	738 (738)	1 year	0.98	0.87, 1.27
1994	Alavanja et al. [A19]	Missouri, United States	Women, non-smokers	538 (1183)	1 year	1.08	0.95, 1.24
1996	Auvinen et al. [A5]	Finland	Both sexes	517 (517)	1 year	1.11	0.94, 1.31
1996	Ruosteenoja et al. [R3]	Southern Finland	Men	164 (331)	2 months	1.80	0.90, 3.50
1997	Lagarde et al. [L1]	Sweden	Both sexes	1 281 (2 576)		1.17[b]	1.03, 1.37
1998	Darby et al. [D15]	South-west United Kingdom	Both sexes	982 (3 185)	6 months	1.08 1.12[b]	0.97, 1.20 0.95, 1.33
1999	Alavanja et al. [A18]	Missouri, United States	Women	247 (299) 372 (471)	1 year	0.85[c] 1.63[d]	0.73, 1.00 1.07, 2.93

Reference		*Region*	*Population*	*Number of cases (controls)*	*Radon measurements*	*OR*[a]	*95% CI*
Year	*Author*						
2000	Field et al. [F11]	Iowa, United States	Women	413 (614)	1 year	1.24	0.95, 1.92
2001	Kreienbrock et al. [K18]	Western Germany	Both sexes	1 449 (2 297)	1 year	0.97[e] 1.09[f]	0.82, 1.14 0.86, 1.38
2001	Pisa et al. [P6]	Trentino, Italy	–	138 (291)	1 year	1.40	0.3, 6.6
2001	Lagarde et al. [L32]	Sweden	Non-smokers	436 (1649)	3 months	1.10	0.96, 1.38
2001	Tomasek et al. [T10, T29]	Pluton, Czech Republic	–	210	1 year	1.087	1.017, 1.208
2002	Wang et al. [W13]	Gansu, China	Both sexes	768 (1 659)	1 year	1.19	1.05, 1.47
2002	Barros-Dios et al. [B26]	Spain	–	163 (241)	150 days	2.48	1.29, 6.79
2002	Lagarde et al. [L31]	Sweden	Non-smokers	110 (231)	3 months	1.33[c] 1.75[d]	0.88, 3.0 0.96, 5.30
2002	Oberaigner et al. [O2]; Schaffrath et al. [S59]	Tyrol, Austria	Both sexes	194 (198)	1 year	1.25	1.08, 1.43
2003	Kreuzer et al. [K17]	Eastern Germany	Both sexes	1 192 (1 640)	1 year	1.08	0.97, 1.20
2004	Wichmann et al. [S61, W19]	Eastern and western Germany	Both sexes	2 963 (4 232)	1 year	1.1	0.98, 1.3
2004	Baysson et al. [B33]	France	Both sexes	486 (984)	6 months	1.04	0.99, 1.11
2005	Bochiccio et al. [B43]	Lazio, Italy	Both sexes	384 (404)	6 + 6 months	1.14	0.89, 1.46

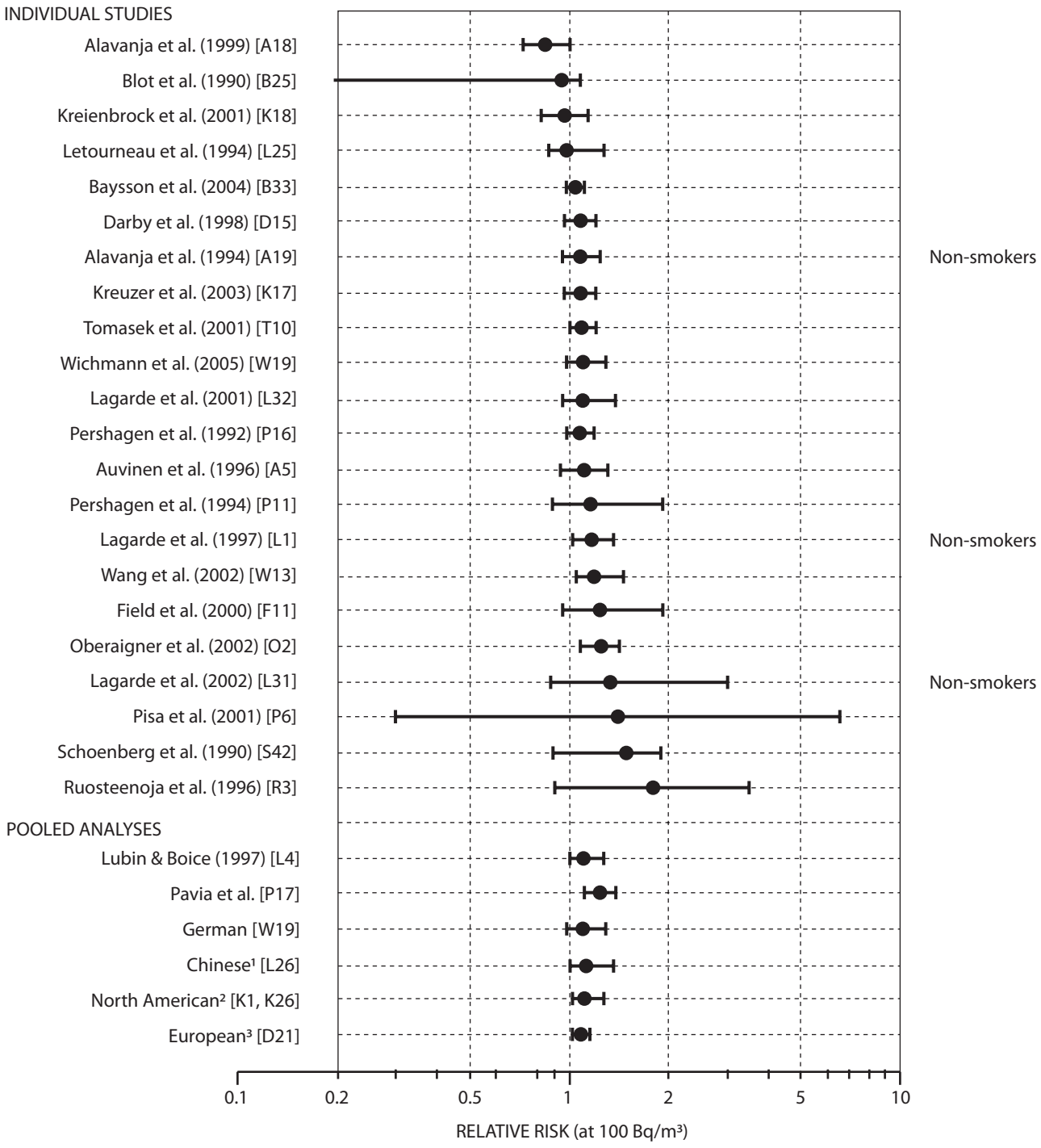
[a] OR at 100 Bq/m^3 (calculated).
[b] Analysis including measurement error.
[c] Analysis based on air monitors.
[d] Analysis based on surface monitors.
[e] Entire study period, 5–15 years.
[f] Radon-prone areas.
[g] As reported in reference [B29], Blot et al. [B25] found no association between lung cancer and radon.

458. Figure XVII is a graphical presentation of the data for the individual studies shown in table 22. In addition, the results of pooled analyses are also shown in the figure along with an estimate of the ERR from the combined pooled studies (see table 22 and previous paragraph). As discussed earlier, corrections for measurement uncertainty increase the predicted ERR somewhat, as shown in table 22.

459. Lubin [L36] discussed several case–control studies of residential radon in North America and two in China. He acknowledged the inherent difficulties in establishing an association between lung cancer risks and residential radon concentration as a consequence of the overall expected risk from radon and the large uncertainties. He noted the latest studies in dosimetry, and suggested that pooling may permit more detailed assessment of the risk. This has been demonstrated, for example, in the pooled residential case–control studies in Europe [D17, D21], North America [K1, K26] and China [L26].

460. Lubin and Boice [L4] undertook a meta-analysis of eight case–control residential radon studies carried out in China, Finland, Sweden, the United Kingdom and the United States. The meta-analysis included those studies with 200 or more cases and long-term measurements of indoor radon concentrations. The pooled study contained in total 4,263 lung cancer cases and 6,612 controls. Using published data from each of the eight studies, the authors carried out regression analyses. The risks (OR) and 95% CIs for each of the studies are also shown in figure XVII. As was shown also in figure XIV, such results are generally consistent with extrapolations from miner studies based on the results of BEIR IV and BEIR VI [C19, C20]. As noted by Lubin and Boice, the CIs for the individual studies are large and include a relative risk of unity, which is consistent with the possibility that there is no effect from exposure to residential radon. Overall, however, the authors found that the excess risk from the combined data was significantly different from zero. For residential exposure to 150 Bq/m^3, the authors estimated a combined OR of 1.1 (95% CI: 1.0, 1.3). Overall, Lubin and Boice concluded that the risk from residential radon is unlikely to be larger than that predicted on the basis of the miner data, that the negative exposure response seen in some studies is likely to have been due to exposure misclassification or uncontrolled confounding factors, and that their results are consistent with a small effect on lung cancer from residential radon [L4].

Figure XVII. Risk estimates from residential radon studies [B29].
Shown are the summary relative risks for exposure at a radon concentration of 100 Bq/m^3 (except for references [L4, P17] at 150 Bq/m^3) and the corresponding 95% confidence intervals.



¹Includes [B25, W13]
²Includes [A18, A19, F11, L25, S42, S76]
³Includes [A5, B26, B33, B43, L32, O2, P11, P16, R3, T29, W19]

461. Pavia et al. [P17] reported the results of a meta-analysis of residential exposures to radon gas and lung cancer from 17 case–control studies. Their analysis suggested an association between residential radon concentration and lung cancer. A weighted log-linear regression analysis was used to develop estimates of pooled ORs for exposure to a radon concentration of 100 Bq/m^3, reported as OR = 1.15 (95% CI: 1.07, 1.24). The authors indicated that not all studies were adjusted for smoking and that their results cannot exclude the possibility that their inability to fully adjust for smoking (or other confounders) could account for the increased risk seen in their study.

462. Darby et al. [D17] reported a pooled analysis of 13 European case–control studies of the risk of lung cancer from residential radon. This analysis is of great interest, especially since heterogeneity among the results of the various individual studies disappeared once the data from the 13 studies were put into a common format and analysed in a consistent manner. The study included 7,148 lung cancer cases and 14,208 controls. The mean radon level measured using long-term alpha track-etch detection in the houses of the control group was 97 Bq/m^3, and in the houses of lung cancer cases was 104 Bq/m^3. Radon exposures during the previous 5–34 years were considered in the analysis. The authors investigated the association between radon concentration and lung cancer using two models. In one model the risk of lung cancer was proportional to $(1 + \beta\chi)$, where χ is the measured radon level and β is the proportional risk factor per unit increase in radon. The second model subdivided cases and controls by categories of radon exposure. The authors noted that the dose–response relationship appeared to be linear with no threshold and did not depend on smoking status. Before correcting for random uncertainties in measuring radon concentrations, the authors reported an increased excess odds ratio (EOR) of about 0.08 (95% CI: 0.03, 0.16) per 100 Bq/m^3. Figure XVIII shows the relative risk of lung cancer according to the time-weighted average observed residential radon concentration, after stratification by study, age, region of residence and smoking habits. The relative risks and 95% CIs for categories of radon concentration are shown in table 23. When the analysis was repeated with only those exposed below a radon concentration of 200 Bq/m^3, the dose–response relationship remained statistically significant ($p = 0.04$).

Figure XVIII. Relative risk of lung cancer versus observed residential radon concentration.

The estimated linear relationship RR = 1 + 0.00084χ (solid line), with 95% confidence limits (dashed lines). The relative risk is equal to 1 at 0 Bq/m^3 (adapted from figure 2 of reference [D21]).

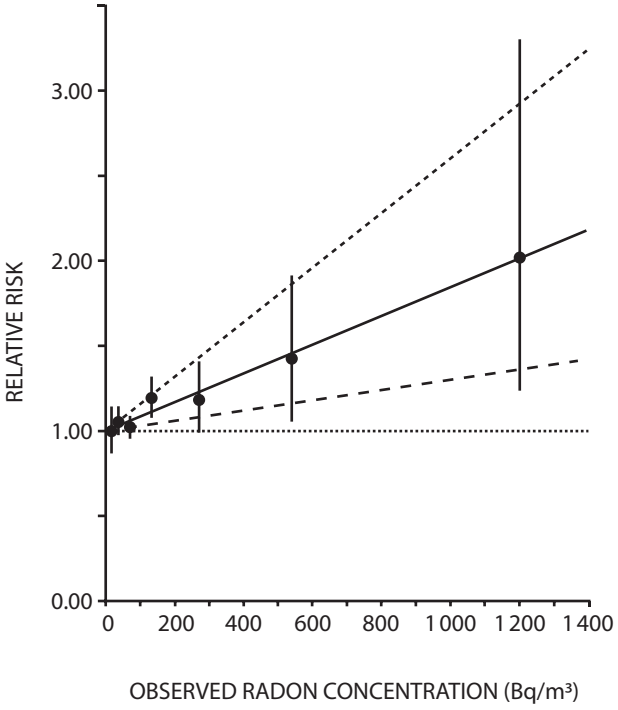


Table 23 Relative risk of lung cancer according to time-weighted average observed residential radon concentration
(adapted from table 16 of reference [D21])

Observed radon concetration[a] (Bq/m³)	*Number of cases*	*Number of controls*	*Mean observed radon concentration*	*Relative risk (95% CI)*
<25	566	1 474	17	1.00 (0.87, 1.15)
25–49	1 999	3 905	39	1.06 (0.98, 1.15)
50–99	2 618	5 033	71	1.03 (0.96, 1.10)
100–199	1 296	2 247	136	1.20 (1.08, 1.32)
200–299	434	936	273	1.18 (0.99, 1.42)
400–799	169	498	542	1.43 (1.06, 1.92)
≥800	66	115	1 204	2.02 (1.24, 3.31)
Total	7 148	14 208		

[a] Observed radon concentration for each address in the 30-year period ending 5 years prior to the index date weighted according to the length of time that the person lived at that address.

463. When the analysis was restricted to people who had lived in at most two residences during the previous 30 years, the excess (EOR) increased to 0.094 (95% CI: 0.034, 0.175) per 100 Bq/m^3. Finally, after correction for random uncertainties in the assessment of radon concentrations, the dose–response relationship remained linear, but the relative risk doubled to 0.16 (95% CI: 0.05, 0.31) at 100 Bq/m^3. Darby et al. also discussed the combined effect of smoking and residential radon exposure on the absolute risk of lung cancer, and indicated, using the same relative risk factor of 0.16 for lifetime exposure (taken as 75 years) to a radon concentration of 100 Bq/m^3, that the risks of lung cancer in lifelong non-smokers and cigarette smokers would be about 0.47% and 11.6%, respectively. Expressed differently, almost all of the risk accrues to the population of smokers.

464. Krewski et al. [K1, K26] reported a pooled analysis of residential radon exposure and lung cancer risk in seven case–control studies in North America. The combined study included some 3,662 cases and 4,966 controls. Residential radon levels were determined using long-term alpha track detection spanning 12 months. The analysis focused on exposures 5–30 years prior to the interview date. Data were analysed using conditional likelihood regression and the linear model OR (χ) = 1 + $\beta\chi$, where χ is the cumulative radon exposure in the previous 5–30 years. The authors stated that the EORs for individual studies ranged from 0.01 (95% CI: <0.00, 0.42) per 100 Bq/m^3 in a Missouri study [A18] to 0.56 (95% CI: 0.22, 2.97) per 100 Bq/m^3 in a New Jersey study [S42]. The authors also investigated potential modifying effects of smoking and demographic factors, and noted that, while there was no apparent heterogeneity in EOR by sex or education level, there was some suggestion of a decreasing radon-associated risk with increasing age. The authors also indicated that they had found no significant differences in EOR with measures of smoking status. Overall, an EOR = 0.11 (95% CI: 0.00, 0.28) per 100 Bq/m^3 was estimated. The authors also indicated that analyses restricted to subsets of the data with "presumed more accurate radon dosimetry" resulted in increased estimates of the EOR, of 0.18 (95% CI: 0.02, 0.43) per 100 Bq/m^3.

465. Smoking is a potential confounding factor in both the residential and the miner studies. However, there is far more information about smoking histories available in the residential studies. Furthermore, several analyses show that the information collected on smoking habits gives estimates of the risks of lung cancer that are very much in line with those of other studies of lung cancer and smoking [C20, C39, P20].

466. Becker [B34] reviewed the radon experience of miners, residential radon exposure and "the therapeutic use" of radon. Becker noted that BEIR VI [C20] indicated that smoking has a much greater risk of lung cancer than does exposure to radon. He then argued that the correction of the miner data for smoking is complicated by the under-reporting of actual smoking, and hence that the uncertainties associated with retrospective analysis of smoking greatly confound the analysis of radon risk in miners. Similar arguments were made about the residential radon studies, noting that uncertainties in smoking "by far dominate" the uncertainties associated with the retrospective analysis of exposure to radon.

467. ***Conclusion***. The main studies of residential radon are the pooled analyses of European [D17, D21], North American [K1, K26] and Chinese [L26] residential case–control studies. These studies indicate a significant association between the risk of lung cancer and exposure to residential radon. The studies also examined the effect of restricting analyses to those who had lived in at most two residences. In addition, Darby et al. [D17, D21] also carried out analyses that adjusted for exposure uncertainty. Both the European and the North American studies have looked at the estimated relative risk from radon for individuals with different smoking habits and demonstrated not only that there is no significant heterogeneity, but that the risk estimates on the relative scale are very similar for individuals in different smoking categories.

468. Table 24 shows the ERR per unit residential radon concentration from three pooled analyses of case–control studies. The pooled analyses also reported the ERR for analyses restricted to individuals who had lived in only one or two residences and hence, whose radon exposures are presumed to be more precisely known than those of individuals who changed residences many times. The ERR estimates from the restricted analyses were higher than the ERR estimates from the primary analyses. The analysis by Darby et al. used a regression model correction for exposure uncertainty, which approximately doubled the ERR obtained from analysis of the primary data [D21].

Table 24 ERR per unit radon concentration in air (per 100 Bq/m^3) and 95% confidence intervals from combined residential radon studies

Study	*Primary analysis*	*Restricted analysis*	*Exposures adjusted for uncertainty*
European [D17, D21]	0.084 (0.03, 0.158)	0.094[a] (0.034, 0.175)	0.16[c] (0.05, 0.31)
North American [K1, K26]	0.11 (0.00, 0.28)	0.18[a] (0.02, 0.43)	
Chinese [L26]	0.133 (0.01, 0.36)	0.319[b] (0.07, 0.91)	
Combined	0.093 (0.04, 0.15)	0.11 (0.05, 0.19)	

[a] Only one or two residences and at least 20 years of coverage.
[b] Only one residence with complete coverage.
[c] Correction for measurement uncertainty.

469. An analysis was conducted to (approximately) combine the risk estimates from these three pooled analyses using weighting by the inverse of the variance in the ERR estimate. The ERR estimate for the combined primary analyses was 0.093 per 100 Bq/m^3, and for the restricted analyses, the combined ERR estimate was 0.11 per 100 Bq/m^3. The European study [D21] provided 72% of the weight (of information) from the primary analyses and 82% of the weight (of information) from the restricted analyses.

470. The European study provided an ERR estimate of 0.16 per 100 Bq/m^3 of residential radon, based on a correction for measurement uncertainty that was about twice as high as the ERR from the primary analyses [D21]. The measurement error correction of Darby et al. [D21] requires a number of assumptions concerning the distribution describing the long-term average residential radon levels within a geographical area and the variability associated with repeated measurements of radon concentrations in the same dwelling. However, as discussed by Darby et al. [D21], there is also information to support many of the assumptions concerning, for example, the magnitude of year-to-year variability. Nonetheless, at this time it seems reasonable to adopt the estimate corrected for random uncertainties in the assessment of radon concentrations from Darby et al. [D21], namely an ERR of 0.16 (95% CI: 0.05, 0.31) per 100 Bq/m^3, as an appropriate, if possibly conservative, estimate of the (lifetime) risk from residential radon.

VI. EFFECTS OF RADON ON ORGANS AND TISSUES OTHER THAN THE LUNG

A. Dosimetric considerations

471. It is generally recognized that the main hazard from inhaled RDPs is from irradiation of the lung. However, in some circumstances, irradiation of the stomach from ingestion of water containing dissolved radon gas may need to be considered. One important factor is the length of time that ingested radon remains in the stomach [I14]. Various estimates of dose to the stomach are within a factor of about 10 [N9], which is quite a small variation considering the uncertainties associated with such estimates. Calculations also suggest that decay products deposited on the skin may be capable of irradiating the sensitive basal cells [S4].

472. In addition to the dose to the lung, Jacobi and Eisfeld [J2] and Harley and Robbins [H38] calculated the dose to organs other than the lung, such as kidney, bone marrow and skin. Kendall and Smith [K21] applied ICRP dose models to estimate effective doses to organs and tissues from radon and its decay products, including doses arising via inhalation, external exposure of the skin and ingestion. The aim was to provide a self-consistent summary that would allow the various hazards to be compared and put into context. The largest dose overall from inhaled radon and its decay products was to the respiratory tract; doses to other organs were usually at least an order of magnitude smaller (see table 25, adapted from reference [K21, table 2]). In particular, doses to tissues with a relatively high fat content (such as red bone marrow), while somewhat higher than those to most other tissues, did not appear to be high enough to present a particular problem. The conclusion was that the conventional focus on risk of lung cancer from inhaled RDPs was appropriate

473. Kendall and Smith [K21] also considered the dose to the foetus. For RDPs, they adopted the foetal discrimination factors of the ICRP. The ICRP makes no recommendation for radon gas. Kendall and Smith noted that, for many radionuclides, the dose to the foetus is similar to that to maternal muscle. Arguing that the fat content of the foetus is low, they assumed that maternal muscle provides a reasonable surrogate. An alternative approach is discussed below.

474. According to Kendall and Smith [K19], the general pattern of doses to different tissues for inhalation and ingestion of radon and its decay products by children is similar to that in adults. Both for inhalation and for ingestion, the organ of intake receives much higher doses than any other organ. In the case of inhalation, the largest doses are to the lung and the extrathoracic part of the respiratory tract (the nose, pharynx and larynx). In the case of ingestion, the stomach receives a much higher dose than any other organ. Of the other organs and tissues, those with a high fat content receive somewhat higher doses from radon gas. Red bone marrow, thought to be the tissue in which childhood leukaemias originate, does not receive doses that are large compared with those to other tissues. Nevertheless, the calculated doses are high enough to suggest that radon may be responsible for a small proportion of childhood leukaemias [H38]. It is possible that alpha particles from RDPs irradiate the cells in which skin cancers originate and thus induce skin cancer. However, the location of these sensitive cells is not known with certainty, and it is possible that they are too deep to receive a significant dose. If they are irradiated, it is likely that the doses would be larger in the case of children than in adults. However, the evidence so far available is inconclusive.

475. Robbins and Harley [R5] suggested that maternal ingestion of radon in water can deliver a dose to the foetus. Radon is transported by the blood and diffuses throughout the body, including the placenta. Radon and its short-lived decay products can thus reach an embryo/foetus. There is an interval during pregnancy when the foetus is at the highest risk of severe effects from radiation exposure. For the early embryo, there are two important factors to consider: the very small size of the embryo yields a small target for alpha particle hits, but conversely, alpha particle damage to DNA may have major consequences. The dosimetric calculations for the developing embryo and foetus used the maternal and the foetal placental blood supply at different points in time [R5]. These calculations relied on available published data for these, and for foetal weights. The accumulation of radon in various compartments following the ingestion of 100 Bq of radon dissolved in water was estimated, and the dose determined as a function of time, on the basis of the pharmacokinetic model developed. The ratio of the weight of blood in the embryo/newborn infant to its total weight was assumed to be a constant, as was blood flow to the placenta at 115 mL kg^{-1} min^{-1}. These parameters need verification, as they are critical not only for such calculations but for other basic toxicological calculations. The clearance half-times of radon for the various tissues in the mother were based on published human data [H24, H25]. For an average intake of 0.6 L of raw tap water per day, containing a radon concentration of 100 Bq/L, the calculated total equivalent dose to the foetus over the term of pregnancy was 250 μSv. The highest calculated equivalent dose of 3 μSv/week occurred between weeks 6 and 16 [R5]. The foetal doses estimated in reference [R5] are considerably higher than those reported by Kendall and Smith [K19], who have noted that the foetus has little fat until late in gestation. The difference between these estimates originated from the assumptions made in the models.

Table 25 Summary of dose coefficients from inhaled radon decay products together with annual doses from decay products and radon at 200 Bq/m³ (see reference [K21] for discussion)

	Type F					*Type M*					^{222}Rn
	Dose per unit intake (Sv/Bq)					*Dose per unit intake (Sv/Bq)*					
	^{218}Po	^{214}Pb	^{214}Bi	*Mixture*	*Annual dose (mSv) at 200 Bq/m³*	^{218}Po	^{214}Pb	^{214}Bi	*Mixture*	*Annual dose (mSv) at 200 Bq/m³*	*Annual dose (mSv) at 200 Bq/m³*
Lung	$1.1\ 10^{-8}$	$1.4\ 10^{-8}$	$3.9\ 10^{-8}$	$2.5\ 10^{-8}$	35.8	$2.7\ 10^{-8}$	$1.3\ 10^{-7}$	$1.1\ 10^{-7}$	$1.1\ 10^{-7}$	159	1.2
Ext.thor.	$4.4\ 10^{-9}$	$2.7\ 10^{-8}$	$6.3\ 10^{-8}$	$3.0\ 10^{-8}$	44.5	$6.7\ 10^{-9}$	$4.8\ 10^{-8}$	$9.3\ 10^{-8}$	$4.9\ 10^{-8}$	70.9	
Stomach	$3.3\ 10^{-11}$	$1.7\ 10^{-10}$	$9.4\ 10^{-11}$	$1.3\ 10^{-10}$	0.19	$1.2\ 10^{-11}$	$7.7\ 10^{-11}$	$4.9\ 10^{-11}$	$5.6\ 10^{-11}$	0.08	0.06
Small intestine	$3.0\ 10^{-11}$	$1.7\ 10^{-10}$	$7.5\ 10^{-11}$	$1.2\ 10^{-10}$	0.17	$6.9\ 10^{-12}$	$4.9\ 10^{-11}$	$1.8\ 10^{-11}$	$3.2\ 10^{-11}$	0.05	0.06
Colon	$2.8\ 10^{-11}$	$1.5\ 10^{-10}$	$8.2\ 10^{-11}$	$1.1\ 10^{-10}$	0.16	$3.9\ 10^{-12}$	$2.4\ 10^{-11}$	$9.9\ 10^{-12}$	$1.7\ 10^{-11}$	0.02	0.05
RBM	$3.9\ 10^{-11}$	$3.2\ 10^{-10}$	$6.8\ 10^{-11}$	$1.9\ 10^{-10}$	0.28	$4.2\ 10^{-12}$	$3.7\ 10^{-11}$	$7.4\ 10^{-12}$	$2.2\ 10^{-11}$	0.03	0.65
Bone surface	$3.4\ 10^{-11}$	$2.1\ 10^{-9}$	$6.8\ 10^{-11}$	$1.0\ 10^{-9}$	1.48	$3.6\ 10^{-12}$	$2.5\ 10^{-10}$	$7.3\ 10^{-12}$	$1.2\ 10^{-10}$	0.17	0.03
Liver	$5.8\ 10^{-11}$	$5.0\ 10^{-10}$	$6.8\ 10^{-11}$	$2.9\ 10^{-10}$	0.43	$6.3\ 10^{-12}$	$5.6\ 10^{-11}$	$7.6\ 10^{-12}$	$3.2\ 10^{-11}$	0.05	0.09
Breast	$2.8\ 10^{-11}$	$1.4\ 10^{-10}$	$6.8\ 10^{-11}$	$1.0\ 10^{-10}$	0.15	$3.0\ 10^{-12}$	$1.6\ 10^{-11}$	$7.8\ 10^{-12}$	$1.2\ 10^{-11}$	0.02	0.42
Kidney	$8.7\ 10^{-11}$	$4.8\ 10^{-9}$	$5.8\ 10^{-9}$	$3.6\ 10^{-9}$	5.20	$9.5\ 10^{-12}$	$5.0\ 10^{-10}$	$5.9\ 10^{-10}$	$3.7\ 10^{-10}$	0.54	0.05
Gonads	$2.8\ 10^{-11}$	$1.4\ 10^{-10}$	$6.8\ 10^{-11}$	$1.0\ 10^{-10}$	0.15	$2.9\ 10^{-12}$	$1.5\ 10^{-11}$	$7.1\ 10^{-12}$	$1.1\ 10^{-11}$	0.02	0.05
Brain	$2.7\ 10^{-11}$	$1.5\ 10^{-10}$	$6.8\ 10^{-11}$	$1.1\ 10^{-10}$	0.15	$2.9\ 10^{-12}$	$1.5\ 10^{-11}$	$7.1\ 10^{-12}$	$1.1\ 10^{-12}$	0.02	0.06
Bladder	$2.8\ 10^{-11}$	$2.1\ 10^{-10}$	$1.0\ 10^{-10}$	$1.4\ 10^{-10}$	0.21	$2.9\ 10^{-12}$	$2.2\ 10^{-11}$	$1.0\ 10^{-11}$	$1.5\ 10^{-11}$	0.02	0.05
Muscle	$2.8\ 10^{-11}$	$1.4\ 10^{-10}$	$6.8\ 10^{-11}$	$1.0\ 10^{-10}$	0.15	$3.0\ 10^{-12}$	$1.6\ 10^{-11}$	$7.4\ 10^{-12}$	$1.1\ 10^{-11}$	0.02	0.05
CED	$1.4\ 10^{-9}$	$2.5\ 10^{-9}$	$5.6\ 10^{-9}$	$3.6\ 10^{-9}$	5.30	$3.3\ 10^{-9}$	$1.6\ 10^{-8}$	$1.4\ 10^{-8}$	$1.3\ 10^{-8}$	19.7	0.28
Foetus	$1.3\ 10^{-11}$	$5.3\ 10^{-11}$	$2.6\ 10^{-11}$	$4.1\ 10^{-11}$	0.06	$1.6\ 10^{-12}$	$6.2\ 10^{-12}$	$2.9\ 10^{-12}$	$4.9\ 10^{-12}$	0.01	0.04
Skin					25					25	

Note: The activity of decay products in comparison with radon is taken to be ^{218}Po:^{214}Pb:^{214}Bi in the ratio 0.9:0.45:0.225; F = 0.41. 90% of decay products are taken to be attached to aerosols, 10% unattached. Annual volume breathed taken to be 7,300 m³. Ext.thor. is the extrathoracic part of the respiratory tract. RBM is red bone marrow. CED is committed effective dose. Kidney is shown as the organ receiving the next highest dose after the respiratory tract. The dose to the foetus from radon gas taken to be that to muscle over 9 months. Dose to skin is taken from reference [N9]. It does not depend on type.

476. Kendall and Smith [K19] also investigated differences between doses to adults and those to children aged 1 and 10 years. They calculated both dose coefficients, i.e. the equivalent dose resulting from an intake of unit activity of the material in question, and also annual equivalent doses. The former tend to be higher in children than in adults. The differences between the annual doses to children and adults are smaller than the differences in the dose coefficients, because children breathe less air and ingest less water than adults.

B. Epidemiology of cancers other than lung

477. Annex A, "Epidemiological studies of radiation and cancer", provides a discussion of the epidemiological methods as well as information complementary to that provided in this annex.

478. The currently available epidemiological evidence indicates that risks other than lung cancer from exposure to radon and its decay products are likely to be small. A number of studies of residential radon and non-lung cancer are available. Laurier et al. [L18] reviewed 19 ecological studies, 8 residential case–control studies and 6 miner cohort studies published between 1997 and 2000 in order to examine a possible association between radon exposure and leukaemia. While the ecological studies suggested a possible positive association, the case–control studies and the miner cohort studies did not. Overall, the authors concluded that the available data (i.e. to 2000) did not provide any evidence of an association between radon exposure and leukaemia [L18].

479. A series of papers [E6, L38, T28] followed the review by Laurier et al. [L18]. Eatough [E6] noted that potential leukaemia risk to miners was investigated by looking for trends in relative risks with increasing exposure to RDPs. However, studies of the dosimetry for radon gas and RDPs suggest that the doses to the red bone marrow are not appreciable [H38]. Tomasek [T28] reported a study of Czech miners in which an excess of leukaemia related to duration of exposure was observed, and went on to suggest that duration of exposure in the mine environment is likely to be a surrogate for exposure to uranium dust, which he considered to be the predominant dose contributor. Laurier et al. [L38] commented that results published since their review [L18] did not modify the conclusions that available epidemiological data do not demonstrate an association between leukaemia and exposure to radon. They also noted that if such a relationship exists, the association would be slight and of little significance for residential exposure.

480. An ecological study of cancer incidence and radon levels in the south-west of the United Kingdom looked at 14 major cancer sites, using data for the South-Western Cancer registry [E5]. Average radon levels for residences were sorted into 10 categories, from low (<40 Bq/m^3) to very high (>230 Bq/m^3), and age standardized cancer rates were calculated for each category. Incidence rates for lung cancer were similar across all radon categories. Except for non-melanoma skin cancer, the authors found no significant positive correlation with radon. Overall, the authors found no significant difference in the corrected survival rates for any cancer site between the low- and high-radon areas.

481. A research letter by Law et al. [L20] commented on residential radon exposure and leukaemia, referring to an earlier study [K24] of acute leukaemia in the south-west, north and north-west of England of residents aged 16–69 years. Radon measurements (of about 6 months' duration) were made in the living rooms and bedrooms of 1,881 houses (about 78% of the homes of the subjects, 76% for the cases and 80% for the controls). No association was found between acute leukaemia and radon concentration; the authors concluded that their study did not support exposure to residential radon as a causal factor in leukaemia in the United Kingdom.

482. Thorne et al. [T24] investigated possible associations between residential radon exposure and paediatric cancers in Devon and Cornwall, United Kingdom. This study compared the incidence of childhood cancers in postal sectors with low radon concentrations (<100 Bq/m^3, average 57 Bq/m^3), and high radon concentrations (>100 Bq/m^3, average 183 Bq/m^3) in a total of 238 postal sectors. The authors found no significant difference in cancer incidence rate between low- and high-exposure sectors.

483. Steinbuch et al. [S46] reported an investigation of residential radon exposure and risk of childhood acute myeloid leukaemia (AML). Alpha track detectors were placed in the houses of 173 cases and 254 controls for 1 year. Overall, there was no association between residential radon concentration and the risk of AML. Lubin et al. [L12] reported an age-matched study of childhood acute lymphocytic leukaemia (ALL) and residential radon exposures of children in the United States. Radon levels for the 505 cases and 443 controls were estimated for 97% of the exposure period. The mean radon level was lower for cases (65.4 Bq/m^3) than for controls (79.1 Bq/m^3). No association between ALL and radon exposure was found.

484. Evrard et al. evaluated the ecological association between indoor radon concentrations and acute leukaemia incidence among children under 15 years of age in France [E7]. The study considered the whole country, divided into 348 geographical units. Incidence data included 4,015 cases of acute leukaemia registered by the French National Registry of Childhood Leukaemia and Lymphoma between 1990 and 1998. Exposure was based on a national campaign of 13,240 indoor radon measurements. A positive ecological association was observed between indoor radon concentration and childhood leukaemia incidence, on the borderline of statistical significance (p = 0.053). A significant association was observed for AML (p = 0.004) but not for ALL (p = 0.49). Consideration of exposure to terrestrial and cosmic radiation did not modify the observed association between radon exposure and incidence of AML [E8].

485. In a 1993 paper, Tomasek et al. [T51] reported on a study of RDP exposure and cancers other than lung cancer in a cohort of uranium miners in western Bohemia. These authors investigated site-specific cancer mortality in 4,320 miners who had been followed-up for an average of 25 years. An average exposure of 219 WLM was reported. Data on smoking habits and alcohol consumption were not available. An analysis of rates of observed to expected numbers of deaths (O/E) showed that overall, the risk of death from non-lung cancers was slightly greater than the natural rates but not significantly so. A significant excess of non-lung cancer mortality in men who started to mine when they were younger than 25 years of age was found, but the increase was not related to cumulative RDP exposure. Overall, the authors concluded that there is no significant risk of any cancer other than lung cancer, although further investigation is needed of the effect of RDP exposure on cancers of the gall bladder and extrahepatic bile duct and on multiple myeloma.

486. Rericha et al. [R13] reported an investigation of the incidence of leukaemia, lymphoma and multiple myeloma, a total of 177 cases, in Czech uranium miners, using a retrospective case–cohort design in a study of 23,043 uranium miners. The authors found no apparent association between RDP exposure and either non-Hodgkin's lymphoma or multiple myeloma, but did find an association between RDP exposure and an increased risk of leukaemia (chronic lymphocytic leukaemia, CLL), which was not previously thought to be radiogenic. The association is based on a comparison of the relative risk (RR) of CLL in miners who had an RDP exposure of 110 WLM with those who had an exposure of 3 WLM. An RR of 1.98 (95% CI: 1.10, 3.59; $p = 0.016$) was reported.

487. Darby et al. [D22] reported on RDP exposure and non-lung cancer in a group of Swedish iron ore miners. These authors observed that, when the mortality from all cancers other than lung in miners was compared with that expected for the northernmost county of Sweden, the O/E ratios were higher in men with exposures of >100 WLM than in men with lower exposures, but the trend was not significant. As in the western Bohemian study [T51], excesses were seen for cancer of the gall bladder and extrahepatic bile duct and for multiple myeloma; however, the increases were not statistically significant.

488. Darby et al. [D12] carried out a collaborative analysis of 11 miner studies to look for risks of cancer other than lung cancer. The miner populations included 10 of the 11 miner cohorts covered by the joint analysis by Lubin et al. [L10] (the Radium Hill study [W15] was excluded since follow-up was incomplete). The 11th miner cohort added in the analyses by Darby et al. was a cohort of Cornish tin miners [H29]. Overall, the study [D12] included some 64,209 men who had worked for an average of 6.4 years, accumulated an average exposure of 155 WLM and been followed for an average of 16.9 years. External mortality data were available for 10 of the studies (but not for the Chinese study of Xuan et al. [X1]). In total, some 1,179 non-lung cancer deaths were observed, which was close to the expected number (O/E = 1.01; 95% CI: 0.95, 1.07). Among the 28 individual cancer types studied, the only sites of statistically significant excess cancers were the stomach and the liver; however, mortality from these cancers was not related to cumulative RDP exposure in WLM and was thus unlikely to have been caused by radon. Among the leukaemias, the O/E ratio for acute myeloid leukaemia was larger than for other leukaemias, but was not statistically significant. Overall, the authors concluded that exposure to high concentrations of radon in the air is unlikely to result in an increased risk of cancer mortality other than from lung cancer [D12].

489. Möhner et al. [M44] reported on a study of leukaemia in German miners. This case–control study included 377 cases and 980 individually matched controls. Exposures were based on a job–exposure matrix that included exposure to radon and its decay products as well as exposure to external gamma radiation and to long-lived radioactive dust. Using logistic regression methods and taking study power into account, the authors concluded that a "casual relationship between radon progeny and risk of leukaemia can largely be excluded". The study did, however, suggest an elevated risk of leukaemia for cumulative exposures of $\geq$400 WLM.

490. Kreuzer et al. [K29] reported on a study of the risk of lung cancer and other cancers in the German uranium miner cohort. The study, based on external comparisons, showed a statistically significant excess of lung cancer risk and a trend of increasing risk with increasing cumulative RDP exposure. The study also found an excess, although not statistically significant, mortality from liver and lung cancer. The authors indicated that the excess is unrelated to cumulative RDP exposure and noted that, in the early years of mining, the Wismut mining company provided miners with alcohol and cigarettes free of charge. Tirmarche et al. [T9] reported an excess of larynx cancer in French miners; however, Villeneuve et al. [V4] found no significant relationship between RDP exposure and other cancer sites.

491. The update of data for the Eldorado miners [H35] involved 17,660 workers. The update extended the mortality analysis by almost 20 years and added 30 years of new information on cancer incidence. A total of 5,332 deaths occurred between 1950 and 1999, and 2,355 workers developed at least one cancer between 1969 and 1999. Mortality and cancer incidence were compared with those of the general Canadian population. Lung cancer was the only cancer site with an excess for both mortality and cancer incidence. In the internal analysis, there was no meaningful evidence of any causal relationship between RDP exposure and increased risk of any cancer other than lung cancer.

C. Effects other than cancer

492. A full discussion of this subject is provided in annex B, "Epidemiological evaluation of cardiovascular disease and

other non-cancer diseases following radiation exposure". This section provides a few additional observations from epidemiological studies of miners.

493. Villeneuve and Morrison [V2] investigated the mortality from coronary heart disease (CHD). In this study, the cohort consisted of 1,743 underground miners and 321 millers or surface workers. Men in this cohort had a mean exposure to RDPs of 378.6 WLM over an average of 5.7 years. As for other analyses of these miners, exposures were based on the analysis of Corkill and Dory [C12]. Smoking data from a 1993 survey were used to update data from previous surveys in 1960, 1966, 1970 and 1978. Smoking status (current, former or never smokers) was determined for 59% of the cohort. Finally, data on the mortality experience of the cohort were available up to 1990. Multivariate Poisson regression analysis was used to estimate the relative risk of CHD from RDP exposure, with adjustments for attained age, duration of exposure and smoking status. Unlike previous studies of this cohort, the analyses [V2] used internal comparisons to control for bias potentially introduced through the healthy worker effect. This study found that workers with high cumulative exposures to RDPs (over 1,000 WLM) had an elevated risk of CHD (RR = 1.5; 95% CI: 0.77, 2.75) compared with those with no exposure. However, no statistically significant trend of increasing risk with increasing exposure was found, nor was there a statistically significant interaction between cumulative RDP exposure and smoking status. The authors found that smoking status was a significant predictor of CHD.

494. Villeneuve et al. [V6] further explored the relationship between mortality from CHD and RDP exposure using both external and internal cohort comparisons in their most recent update of this cohort. There was no association between cumulative exposure in WLM and CHD mortality. A reduced CHD mortality rate was observed relative to the population of Newfoundland males (SMR = 0.86; 95% CI: 0.74, 0.98); the authors attributed this reduction to a "healthy worker effect".

495. Kreuzer et al. [K30] reported an investigation of the mortality from cardiovascular diseases in a cohort of German uranium miners. The cohort included 590,001 male subjects who were employed for at least 6 months between 1946 and 1989 at the former Wismut uranium company in eastern Germany. As for other studies of Wismut miners, exposures to RDPs, long-lived radionuclides and external gamma radiation were estimated using a detailed job–exposure matrix. As of 31 December 1998, the cohort included (about) 16,598 deceased miners, with 5,417 deaths from cardiovascular diseases. Linear Poisson regression models were used to estimate the ERR per unit of cumulative radiation exposure after adjusting for attained age and calendar period. The study found no trend in risk of circulatory diseases with increasing cumulative exposure to RDPs (ERR was 0.0006 (95% CI: -0.004, 0.006) per 100 WLM), external gamma radiation (ERR was -0.26 (95% CI: -0.6, 0.05) per Sv) or long-lived radionuclides (ERR was -0.2 (95% CI: -0.5, 0.06) per 100 kBq h m^{-3}. The authors concluded that their results did not support an association between cardiovascular disease mortality and exposure to radiation among uranium miners.

496. More recent data are also available from Canada. Howe [H35], in the update of the Eldorado cohort study, reported that for most of the causes of death, the cohort as a whole as well as various subcohorts had reduced risks relative to the population. In particular, the authors stated that, although an increase of hypertensive disease was observed (significant in only one of the subcohorts examined), the study indicated a statistically significant deficit in males of cardiovascular diseases such as stroke and ischaemic heart disease. The authors go on to suggest that this is probably a consequence of the healthy worker effect, since heart disease would be likely to prevent people working in a strenuous physical occupation such as mining. Villeneuve et al. [V6] found that there was no apparent trend between cumulative exposure to radon and the relative risk of death from CHD (p = 0.63). Furthermore, this finding was unchanged after adjusting for lifetime smoking status, which was available for approximately 54% of the cohort. Additionally, cumulative exposure to radon was found to be unrelated to diseases of the circulatory system, acute myocardial infarction and cerebrovascular disease. All of these findings are consistent with those of Kreuzer et al. [K30].

VII. IMPLICATIONS FOR RISK ASSESSMENT

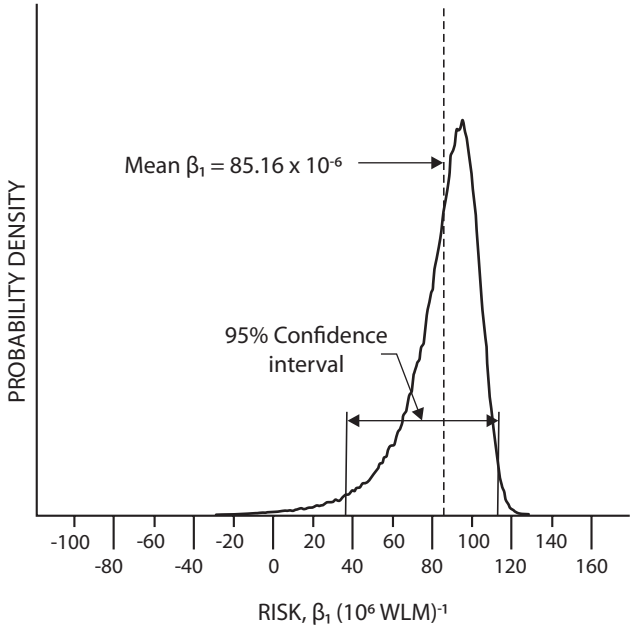
497. Epidemiological studies of underground miners provide the current basis for estimating the risk of exposure to radon and its decay products. Studies of smoking and non-smoking miners show that exposure to RDPs carries an enhanced risk of lung cancer. However, miners were not exposed to RDPs alone but rather to RDPs in association with many other agents, among them silica (quartz), various metals, including arsenic, and diesel exhaust. Traditionally, the risks from domestic radon exposure have been developed by extrapolation from the data for miners; however, the risks from residential radon can now be estimated directly using the results of pooled residential case–control studies (see section V).

A. General studies of exposure uncertainty

498. There is a great deal of interest in dosimetry-based estimates of risk from exposure to RDPs (see section II). There is a discrepancy between the dosimetry-based and the miner-epidemiology-based approaches, with differences in estimates of a factor of 2–3. The miner epidemiology data provide the basis for investigating biological mechanisms and modifiers such as time since exposure and age at exposure. As discussed in section IV, there is considerable uncertainty in the exposures of miners, with uncertainty increasing the further we go back in time. In view of the ongoing importance of miner studies in understanding the risk from exposure to radon and RDPs at work and in the home, it is useful to consider the effects of uncertainty in miner exposures on the epidemiological analyses performed using miner data.

499. A 1989 report considered the effects of uncertainty in exposure estimates for the studies then available of miners in Ontario, Beaverlodge and Port Radium (Canada), Czechoslovakia, Sweden, and the Colorado Plateau (United States) [S10]. Several subgroups of the Colorado Plateau cohort were also studied. The focus of the study was on adjusting the exposure–response parameter in the simple linear model for excess relative and excess absolute risk. The authors used a Bayesian error-in-variable relative risk approach to analytically assess the effect of uncertainty on the exposure–response relationship. Figure XIX shows the posterior probability density factor for the absolute risk coefficient for Colorado miners with no mining exposure prior to 1950. At that time (i.e. follow-up to 31 December 1982), the cohort with no exposure before 1950 included 168 miners with lung cancer. The mean lifetime risk was estimated at about 85 cases per 10^6 person-years per working level month, with a range of about 60–150 cases per 10^6 person-years per working level month [S10]. The estimate may be compared with the BEIR IV [C19] estimate of 140 per 10^6 person-years per working level month for male non-smokers. The approach in reference [S10] was necessarily limited because the authors did not have access to data on individual miners and could only utilize published relative risks within categories of cumulative exposure (J h m^{-3} or WLM); similarly, no attempt could be made to evaluate patterns of error for different mining periods within a study. For the ERR model, the authors estimated that the most likely range for the ERR per unit exposure parameter for this group of studies was 0.009–0.00005 (J h m^{-3})$^{-1}$ (0.5–1.5% WLM^{-1}).

Figure XIX. Distribution of absolute risk for Colorado miners with no exposure prior to 1950 [S10].



500. A subsequent paper by Chambers et al. [C15] reviewed the factors affecting exposure estimation for seven groups of uranium miners. These included the temporal and spatial variability of radon and RDP levels in the workplace, changing mining methods and ventilation practices, and uncertainties about the miners' work histories. Bayesian methods were used to develop posterior probability density functions of absolute and excess relative risk coefficients for each of the cohorts. Figure XX shows the posterior probability density function for the ERR. The authors noted that the estimated RDP exposures in WLM are uncertain in all the miner studies. For the reasons given in reference [S10], the studies of United States miners [H17, P14, S10], Ontario miners [M19, M23], and

Czechoslovak miners [S25] were considered at the time to provide the strongest basis for risk estimation. The strengths of these groups are that they are all large, well-traced cohorts for which considerable information existed on which to base risk estimates for the period of interest. To the best of the authors' knowledge, there was no systematic bias with regard to the magnitude of the group exposures estimated in these studies. However, the uncertainties in exposures of individual miners could be very large, particularly for exposures associated with the early years of mining. Of these three groups, the United States miners were exposed to the highest RDP concentrations, the Czechoslovak miners were exposed to intermediate levels, and the Ontario miners were exposed to the lowest levels. While figure XX showed the posterior probability density function for all cohorts, figure XXI illustrates the posterior probability density function developed by combining the three studies considered "best", together with that from combining all the studies (with and without a notional correction for bias in the Swedish and the Beaverlodge studies). Overall, Chambers et al. [C15] estimated the ERR to have a 95% uncertainty range of 0.5–1.5 per 100 WLM, comparable to the estimates of Lubin et al. [L10].

501. The pooled analysis by Lubin et al. [L10] considered the impact of uncertainties in the estimates of RDP exposure only in the most general way and only within the context of modification of the exposure–response relationship by exposure rate and exposure duration. Exposure uncertainty was considered greatest in the earliest years of mining, i.e. those years in which exposure rates were highest. According to Lubin et al. [L10], exposure uncertainty would therefore have tended to attenuate the effects of high exposures and potentially induce an inverse exposure-rate pattern [L22]. They estimated an ERR and associated 95% CIs as depicted graphically in figure XXII. Lubin et al. [L22] report a combined estimate of ERR of 0.49 (95% CI: 0.2, 1.0) per 100 WLM, where the joint 95% CI is based on a random-effects model.

Figure XX. Excess relative risk (posterior) probability density functions with unadjusted variances [C15].

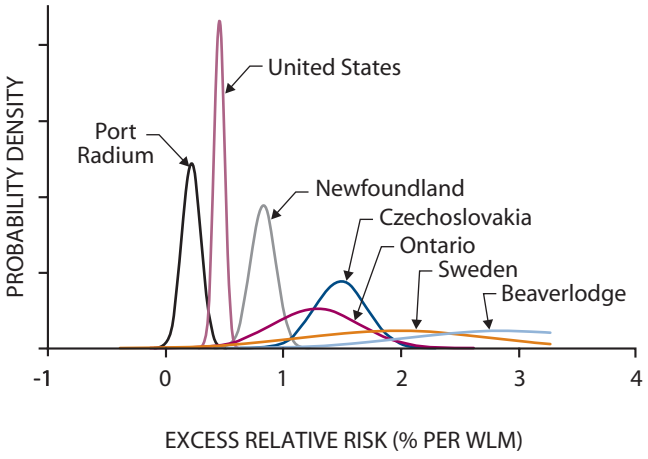
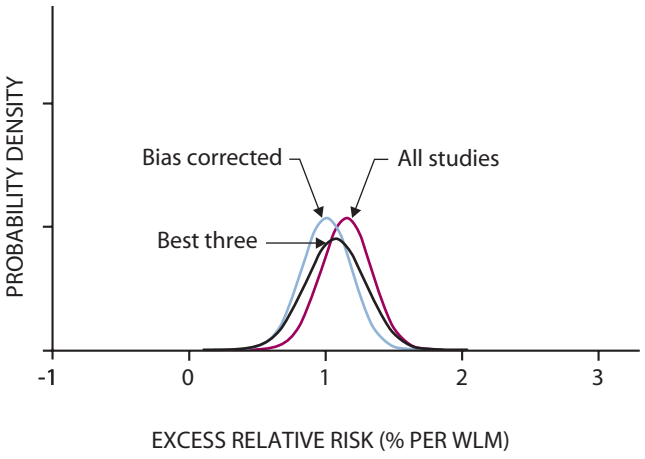


Figure XXI. Combined excess relative risk (posterior) probability density functions [C15].

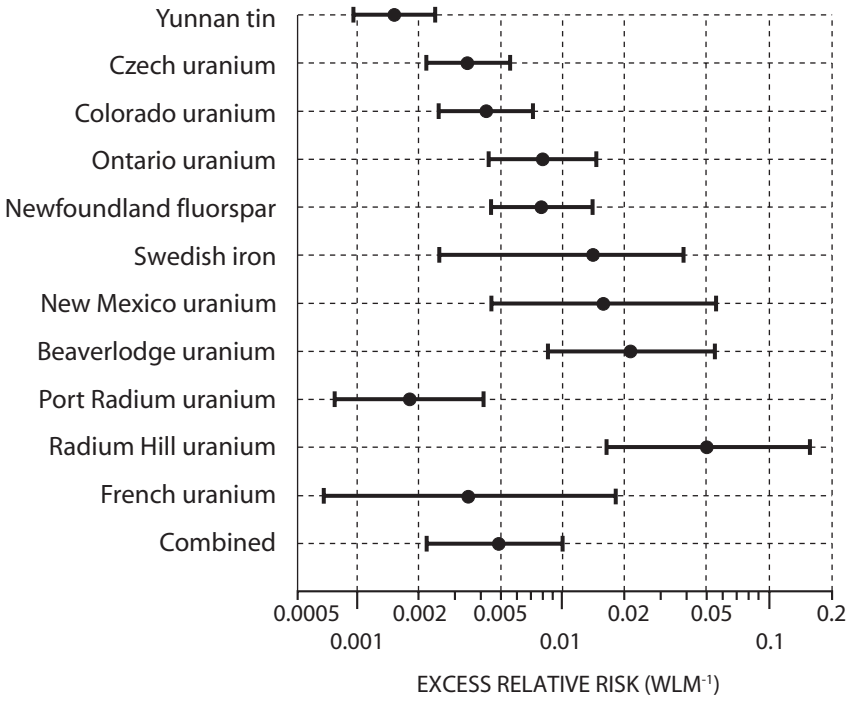


502. BEIR VI [C20] also investigated the role of uncertainty in estimating lung cancer risk, and developed an extensive table summarizing the sources of uncertainty in estimating lifetime risk from residential exposure to radon. The BEIR VI estimates of lung cancer risk were based on analyses of the data from miner epidemiological studies. The BEIR VI Committee acknowledged that there were uncertainties in exposures to RDPs and other potential factors, such as exposure to cigarette smoke and arsenic. No systematic bias in the estimates of miner exposure to RDPs was identified. BEIR VI also suggested that random errors might result in an underestimate of the slope (risk) of the exposure–response relationship. In addition to extensive qualitative discussion, the BEIR VI Committee applied quantitative methods for uncertainty analysis, acknowledging that their analysis should be considered illustrative, "not to replace the Committee's new comprehensive qualitative analysis", since not all sources of uncertainty could be identified and characterized. The BEIR VI Committee's "preferred" uncertainty limit was derived using a simple constant relative risk model fitted to the miner data at cumulative exposures of below 50 WLM. On this basis, BEIR VI estimated an ERR of 1.17 per 100 WLM, with a 95% uncertainty interval from 0.2 to 22.5 per 100 WLM.

503. Notwithstanding the different data and methods that have been used to develop combined estimates of ERR from the studies of miners, there is a remarkable consistency in the ranges of ERR per 100 WLM that have been estimated. As discussed in the preceding paragraphs, the 95% CI for the ERR was estimated by Chambers et al. [C15] as 0.5–1.5 per 100 WLM; by Lubin et al. [L10] as 0.2–1.0 per 100 WLM; and by BEIR VI [C20] as 0.2–22.5 per 100 WLM, with central estimates of ERR ranging from 0.49 to 1.17 per 100 WLM. On the basis of the most recent results of miner studies as described earlier in this annex (see para. 427), an estimate of a combined ERR of 0.59 (95% CI: 0.35, 1.0)

per 100 WLM is consistent with previous estimates but with somewhat narrow 95% CIs. By multiplying a notional lifetime risk of lung cancer of, say, 8%, and an average ERR of 0.59 (95% CI: 0.25, 1.0) per 100 WLM, a nominal absolute lifetime risk of about 4.7×10^{-4} (95% CI: 2.8×10^{-4}, 8×10^{-4}) for 100 WLM can be calculated.

Figure XXII. Estimates of excess relative risk of lung cancer per WLM (adapted from [L10]).



B. Biologically based models

504. Several investigators reported analyses of experimental animal data and epidemiological data with multistage models to study mechanisms of carcinogenesis and assess risks from the inhalation of RDPs (e.g. [B20, B28, B29, C20, C29, C30, C31, C33, H26, K22, L16, L23, L33, L34]). The main difference between the approaches was in the choice of exposure–response model to be used for each of the cellular processes in the biologically based model. Age-dependent cancer incidence data do not yet provide the basis for determining the most appropriate model, as evidenced by results of a model comparison using lung cancer data for radon-exposed rats [H26]. The BEIR VI Committee [C20], as noted earlier, acknowledged the importance of biologically based models and indicated that, when biological mechanisms are better understood, such models might become the preferred approach to assessing the risks. Similar views are expressed by Krewski et al. [K22], who applied the two-stage clonal expansion (TSCE) model to two cohorts important to assessing the risks from RDP exposure, namely the Colorado Plateau miners and the Chinese tin miners.

505. An important project, within the framework of the European Union project FIGH-CT1999-00013, looked at a multistage model analysis of the data from the French and Czech uranium miner cohorts [T30]. One of the issues being investigated was how risks can be transferred between populations with different baseline lung cancer incidence rates. The combined cohort includes 5,098 miners and 125 lung cancer deaths in the French cohort and 5,002 miners and 449 lung cancer deaths in the Czech cohort. The corresponding mean total cumulative exposure levels were 37 and 57 WLM, respectively, for the two cohorts. A two-mutation carcinogenesis model with clonal expansion of cells in the intermediate stage was fitted to the individual miner data. Linear exposure-effect relationships were used for the two mutational steps. The authors found that the fitted linear effect of radon on the first mutational step was an order of magnitude larger than for the second mutational step. Although the baseline lung cancer risk in the Czech miner cohort was considerably higher than that for the French miners, both data sets could be described with the same parameter values for the relative effect of exposure to RDPs on the mutation rates. The authors argued that the uniform description of the effect of RDP exposure for two miner cohorts with distinctly different baseline lung cancer risks (0.09 for the French and 0.23 for the Czech miners) demonstrated the possibility of using the model for risk transfer across populations. In addition, the biologically based model implicitly describes age and exposure-rate effects, and thereby allows for extrapolation to lifetime exposures to low radon concentrations. Lifetime risks were calculated for a 75-year continuous exposure to 1 WLM/a (~256 Bq/m^3). The lifetime ERR calculated from the model solution in references [B27, B28] was 1.1 for the combined cohorts.

506. Heidenreich et al. [H32] provided further analysis of the French and Czech cohorts using the biologically based TSCE model together with an analysis of the Chinese and Colorado cohorts. The model allows an action of radiation on initiation, promotion and transformation in cancer induction. While all four studies indicate a highly significant action of radiation on promotion, the action on initiation is not significant in the French cohort and is barely significant in the Colorado miner cohort. No action on transformation is found in the Colorado miners, while the other data sets indicate a borderline significance. The doubling exposure rate for initiation is about 3.5 WLM/a in the new data sets, while it is higher in the historic data sets. For transformation, the doubling rate is about 20 WLM/a for the new data sets, while again the historic data give higher estimates. The action of radiation on promotion is different in the four data sets. The larger power of the French and Czech cohorts requires less extrapolation when the risk at very low exposures is estimated.

507. A 1990 study [M11] reanalysed the data for the Colorado Plateau uranium miners in order to investigate RDP exposure, cigarette smoking and lung cancer using a two-mutation model as the biological basis for their assessment. The authors concluded that exposures to both RDPs and cigarette smoke affect the first mutational step and the rate of cell division but that the second mutational step was independent of RDP and cigarette smoke exposures. The authors also concluded that the age-specific risks arising from joint exposure to RDPs and cigarette smoke are more than additive but less than multiplicative. An inverse dose-rate effect was observed, in that fractionally lower RDP exposure resulted in higher lung cancer risks. The authors also obtained the same estimates of lung cancer risk from exposure to residential radon for both smokers and non-smokers. This observation is consistent with observations from the European [D17] and North American [K1] pooled residential radon studies of no significant heterogeneity across categories of smoking. These studies showed an ERR of 0.2 per 100 WLM for non-smokers at age 70 exposed at a rate of 0.2 WLM/a.

508. Luebeck et al. [L16] provided further discussion based on an analysis of the data for the Colorado Plateau uranium miner cohort using a TSCE model. Exposure to RDPs was suggested to affect both the rate of initiation of intermediate cells in the pathway to cancer and their rate of proliferation. However, the effect of radon on the rate of initiation was not statistically significant. The results of reference [L16] showed that, depending on total radon exposure, the lifetime ERR per unit exposure first increased with duration of exposure, reached a maximum and then declined. Non-smoking miners who were exposed to RDPs for 10 years were found to have approximately the same risk (as measured by lifetime ERR per unit exposure) as a non-smoking individual who spent 10–20 years in a residence with very low levels of radon. These authors stated that if the inverse dose-rate effect observed in "miners at much higher total doses were extrapolated naively to durations (and exposure rates) more typical for homes, the risk (lifetime ERR per unit exposure) would be grossly overestimated."

509. More recent studies such as those reported in references [B27, B28, H32, H36, K22, M11, T30] suggest that mechanistic or biologically based models, which allow the opportunity to investigate mechanisms of carcinogenesis, are likely to find increasing application. Tirmarche et al. [T30], for example, reported estimates of final and second mutation rates based on an analysis of the French and Czech miner cohorts. One observation from their analysis is that an inverse dose-rate effect cannot be excluded for exposure rates of >30 WLM/a.

510. Harley et al. [H4, H36] applied a biologically based model derived from an evaluation of the number of nuclei traversed by an alpha particle that will cycle as a function of time following unit exposure to RDPs. The model was developed in two steps. First, the number of basal cell nuclei traversed by an alpha particle following an exposure of 1 WLM was determined from biological data. Secondly, the number of nuclei traversed and the measured cycling rates in normal bronchial epithelium were used to calculate the dividing population as a function of time after exposure. The cycling rates decreased with a half-time of about 15 years. The authors compared their model fit with the combined excess risk data from the joint analysis of 11 underground mining cohorts [L10], and suggested that their model may explain why the tumour risk decreases with time since exposure and with attained age, in that both could reflect the reduction in cycling frequency of cell nuclei repopulating the basal stem cells.

511. Little [L21] fitted multistage cancer models with clonal expansion to the Colorado miner data, allowing for up to three mutational steps. Both radiation and smoking were allowed to affect the mutation rates as well as cell proliferation in the intermediate stages. The best fit of the data was obtained for a three-mutation model in which the first and second mutation rates increased with RDP exposure, and the first mutation rate increased with smoking rate, in a strongly non-linear way. This three-mutation model was slightly superior to a two-stage model in which the first mutation rate depended in a non-linear fashion on radon and smoking, in combination with a reduction of intermediate cell death/differentiation rate with radon exposure.

512. The preceding examples show that current multistage cancer models with clonal expansion are not specific enough to determine the dose–response relationships for the cellular processes in the model from a fit to cancer incidence data. This was shown in model intercomparison on a large data set of radon-exposed rats [H21]. An alternative approach to this problem of specificity was applied in the two-mutation model fit of Leenhouts to the Colorado uranium miner data [L23]. He used a stepwise fitting approach in combination with biologically motivated mutation equations. The radiation effect in this model was limited to the mutation rates, and no effect of radiation on proliferation rates was assumed. Using a stepwise approach, Leenhouts fitted the background parameters to the lung cancer incidence of non-smokers, the smoking coefficients to the lung cancer incidence of smokers, and the radiation parameters to the miner data. From

the model solution, the following risks were calculated at age 75 for a lifelong exposure to 0.1 WLM/a: EAR of 0.008 for non-smokers, increasing to 0.0014 for smokers, which corresponds to ERR values of 0.19 and 0.009, respectively.

C. Risk projection

513. The ICRP [I3] recommends that radon risk assessment be based on epidemiological studies of miners. The BEIR VI Committee also base their risk estimates on an analysis of pooled miner data [C20]. Both reports predate the pooled analyses of residential case–control radon studies. As discussed below, the pressent report suggests that the risk of lung cancer from domestic exposure to radon can be estimated using the results of the pooled residential case–control radon studies [D17, D21, K1, K26, L26].

514. It is important to develop reliable risk estimates of lung cancer due to radon exposure in the workplace or in the home. Many published evaluations (e.g. [C20, U2, U17, W18]) are available, as described in previous sections. Whether there is a risk from residential radon exposure has been widely debated in the literature (e.g. [B34, C24, C25, C34, F8, F12, H29, P10]). However, notwithstanding the wide range of results from residential case–control studies and the important effects of confounding by smoking and other factors, overall the pooled European [D17, D21], North American [K1, K26] and Chinese [L26] case–control residential radon studies clearly demonstrate an association between risk of lung cancer and residential radon exposure. There is a remarkable coherence both among the pooled residential studies and the downward extrapolation of radon risk estimates from miner studies.

515. Uncertainties associated with downward extrapolation from miner studies include, but are not limited to: uncertainties in the reconstruction of miner exposures; possible exposure to other carcinogens; the high but uncertain level of smoking among miners; and the fact that exposures in mines historically were at relatively high levels compared with levels in homes and in present-day mines. Uncertainties associated with residential radon studies include, among other factors: lack of contemporary radon measurements in residences (e.g. while it may be possible to measure radon levels today in homes previously occupied by subjects in residential studies, there will be large uncertainties associated with assumptions about changes to home ventilation and in the habits of subjects over time); uncertainties about smoking; and low statistical power.

516. Many studies of lung cancer risks in miners exposed to RDPs (see section IV) and several joint analyses of 11 miner cohorts have been published [C20, L4, L10]. Tirmarche et al. [T30] discussed risk estimates among the general population and reported a comparison of different models used to estimate lifetime risk. These authors estimated a lifetime ERR in the range 0.08–2.31, according to the exposure scenario. These results are in the envelope of the results in BEIR VI. However, there are wide variations in these studies in size, duration of follow-up, reliability of exposure estimation, availability of smoking data and other factors. For example, it is questionable whether the following cohorts should be used for risk assessment: *(a)* Radium Hill cohort, because of limited follow-up and the low quality of the exposure data; *(b)* Port Radium, Newfoundland fluorspar and (without further re-evaluation using the revised dosimetry) Swedish iron miner cohorts, because of the low quality of the exposure data; and *(c)* Chinese tin mining cohort, because of extreme confounding from arsenic exposure. In addition, the French cohort data [C20, L4, L10] are being updated, and the new Wismut cohort described in section V, is being developed. The current European project in which a joint analysis is being performed of the extended French cohort and the Czech cohort is an important step, as these two cohorts are both of high quality. In addition, the Eldorado cohort, consisting of miners at the Port Radium and Beaverlodge uranium mines and the Port Hope uranium processing facility, has been updated [H35]. One important aspect of this update is the extensive review of work histories and the use of updated exposure algorithms, both of which significantly improve the quality of the epidemiological studies carried out on this cohort.

517. The BEIR VI model was developed from a pooled analysis of 11 underground mining cohorts and takes account of the reduction of relative risk with increasing time since exposure, adjusting for attained age and exposure rate. Estimates of lifetime risks developed with the model also incorporated sex and smoking status. BEIR VI developed two models, an exposure-age concentration model and an exposure-age duration model. The general structure of the BEIR VI model is illustrated below:

$$\mathrm{ERR} = \beta \left[\theta_{5\text{-}14}\, w_{5\text{-}14} + \theta_{5\text{-}14}\, w_{5\text{-}14} + \theta_{25+}\, w_{25+} \right] \theta_{\mathrm{age}}\, \gamma_z$$

where

- β = slope of the exposure–risk relationship for the assumed reference categories of the modifying factors.
- $\theta_{5\text{-}14}\ \theta_{15\text{-}24,}\ \theta_{25+}$ = weighting factor for time periods 5–14, 15–24 and >25 years post-exposure (values of $\theta_{5\text{-}14}$, etc., are provided in reference [C20] and range from 0.31 to 0.81).
- $w_{5\text{-}14,}\ w_{15\text{-}24,}\ w_{25+}$ = radon exposures in the time windows 5–14, 15–24 and >25 years.
- θ_{age} = parameter to describe the decline in ERR with increasing age. BEIR VI [C20] reported values of 0.13–1.00.
- γ_z = parameter to describe the exposure-rate effect. BEIR VI [C20] reported values of 1.0–10.2.

518. For exposure conditions in modern Saskatchewan uranium mines, the power to detect any excess risk arising from workplace exposure to radon is likely to be very small. A study carried out for the Canadian Nuclear Safety Commission [S55] investigated the feasibility of conducting epidemiological studies of underground miners working under today's exposure conditions. To carry out this assessment, two hypothetical cohorts were considered: a retrospective cohort of miners employed in Saskatchewan uranium mines between 1975 and 2000, and a prospective cohort that included miners employed from 2000 to 2030. The cohorts were developed using demographic data provided by the mining companies, dose data from the National Dose Registry, and reference baseline cancer and mortality rates from all of Canada and Saskatchewan. More than 50% of modern Saskatchewan uranium miners smoke. Adjustments were made for different smoking prevalences in miners and the reference population. In 2000, the average underground miner in northern Saskatchewan was exposed at work to about 0.11 WLM from RDPs (with an upper 95% CI of about 0.43 WLM). At the same time, annual exposure to RDPs at home was estimated to be about 0.4 WLM/a (ranging upward to about 10 WLM/a). The feasibility analysis simulated incremental risks of lung cancer from radon exposure using a relative risk model and an ERR of 0.89% WLM^{-1} based on the Ontario cohort, since it has provided the largest and best exposure data of the Canadian uranium miner studies. Sensitivity analyses were carried out with respect to both reference risk and exposures. Two statistical measures were estimated using probabilistic simulation, standardized mortality ratio (SMR) and regression analysis, which produced an estimate of excess lung cancer risks for a working level month. Both cohorts were modelled to 2030. SMRs were distributed on an average of 1.01, with most (80%) falling in the range 0.93–1.08. Similarly, the slope estimated from the regression analysis had a mean of 1.01, with 80% of the slope estimated to be in the range 0.083–0.105 WLM^{-1}. This quite large range reflected the statistical uncertainty estimated in the cohorts. The results of the regression analyses showed little probability (power) of detecting the predicted excess risks in the cohorts, because the probability of the lower confidence level on the slopes exceeding zero is only about 3% (i.e. much less than the objective of 80%) for most combinations of scenario, cohort and follow-up period. Moreover, exposure at home accounted for about 98% of the total for modern Saskatchewan miners.

519. There is now a great deal of information available concerning the risks from exposure to radon and its decay products. The studies of underground miners exposed to high levels of radon in the past have formed the principal source of information on the risks, and serve as the basis for developing exposure–response models and evaluating modifiers of effects such as time since exposure and age at exposure. A great deal of study has also been given to dosimetric evaluations, both as a means for transferring risk estimates from a miner population to other circumstances and also in their role as a source of ab initio risk estimates. While dosimetric evaluations remain important for understanding the biological and physical mechanisms of carcinogenesis, given the new information that has emerged from the pooling of residential radon case–control studies, the reconciliation of the dosimetric and epidemiological evaluations is now much less important for estimating risks from residential radon. Experimental studies will continue to play an important role in understanding the mechanisms and risks arising from exposure to radon at work and at home, and biologically based models will play an increasingly important role in the future; however, at this time, the epidemiological studies of miners and the residential case–control studies provide the strongest basis for risk assessment.

520. In the past, radon risk estimates for residential exposures were based on downward extrapolation of evidence from studies of miners who were exposed at higher exposures for shorter times. Pooled analyses of European, North American and Chinese residential case–control studies provide strong evidence supporting the evidence from miner studies that exposure to high levels of radon and its decay products in homes leads to an increased incidence of lung cancer.

521. Both the miner and the residential studies have advantages and disadvantages, some of which are briefly summarized below. The advantages of miner studies discussed in section IV include:

- Relatively high (relative to domestic) cumulative exposures and exposure rates, which allowed the development of dose (exposure)–response relationships, at least at high cumulative exposures.
- The ability to examine factors that modify the simple linear dose–effect relationship (time since exposure, age at exposure, exposure rate).
- Information on risks over a lifetime, not just a window of 30 years or so. Miner studies will require continued follow-up to realize this potential to the full.
- Exposure estimates based on contemporary measurements (albeit incomplete, most often area measurements rather than individual measurements and subject to uncertainty). While some evaluations of the effects of the uncertainties in these measurements on the resulting risk estimates were carried out, more remains to be done in this area.
- Information on all causes of death and cancer incidence other than lung cancer.

522. Many residential case–control studies (section V), have been published. Individually, they have limited statistical power, and meta-analysis has suggested that the results of the studies are inconsistent. However, more recent pooled analyses, especially those of European [D17, D21] and North American [K1, K26] studies, combined the data on all the individuals in a number of residential studies and have greater power than the individual component studies. The pooled residential radon studies provide strong, direct

evidence of risk from residential radon. The pooled residential studies have certain advantages over the miner studies:

- Exposures received under aerosol conditions similar to those of interest.
- Exposures received at concentrations similar to those of interest (thus reducing the need to extrapolate from the relatively high rates of exposure in mines).
- Reduced confounding from possible exposure to occupational carcinogens such as arsenic.
- Detailed individual smoking histories for study participants.
- Detailed individual exposure data based on measurements in the homes where the individuals had lived. Nonetheless, exposure uncertainties are also part of residential studies. For example, the measurements were usually made some time after the period over which the risk was to be assessed, and in some instances, there were alterations to the home between the time when the resident lived there and the time when the measurement was made. Data are available regarding the uncertainties in the assessment of residential radon exposures, and the European pooled analysis, in particular, carried out calculations quantifying the effect of this uncertainty on the risk estimates.
- Data for both men and women for a wide variety of ages.

523. Notwithstanding the strengths and weaknesses of risk estimates from studies of miners and of residential radon, there is now a remarkable coherence between the risk estimates developed from epidemiological studies of miners and pooled residential case–control radon studies. While both the miner studies and the residential case–control radon studies are subject to various limitations arising from exposure uncertainty and confounding by smoking, for example, both types of study are suitable for risk estimation. The miner studies provide a strong basis for evaluating risks from RDP exposure to people at work and at home, and for investigating the effects of modifiers to the exposure–response relationship [S2]. However, the results of the pooled residential studies now provide a direct method of estimating risks to people at home without the need for (downward) extrapolation from miner studies. The measurement-adjusted risk coefficients reported, for example from the European pooling study, provide an appropriate basis for estimating risks to people at home.

VIII. OVERALL CONCLUSIONS

524. This annex, "Sources-to-effects assessment for radon in homes and workplaces", discusses: potential sources of exposure of workers and the public to radon; issues of current interest in the dosimetry of radon and its decay products; information from animal experiments and experiments at the cellular and subcellular levels that are important in understanding the mechanisms of carcinogenesis; epidemiological studies of miner and residential exposure to radon; and approaches to risk projection.

525. During daily life, everyone is exposed to radon, an inert radioactive gas that occurs naturally and is present everywhere in the atmosphere. The levels of radon indoors vary widely both within countries and between countries, with (nominal) geometric mean concentrations of radon in air indoors ranging from less than 10 Bq/m^3 in the Middle East to up to around 100 Bq/m^3 in a number of European countries.

526. The annual per caput dose from inhalation of radon gas and its decay products represents typically about one-half of the effective dose received by members of the public from all natural sources of ionizing radiation. For certain occupations, radon gas is the predominant source of occupational radiation exposure.

527. Radon decay products are well established as lung carcinogens. However, the doses to other organs and tissues arising from the inhalation of radon and its decay products are quite small, usually at least an order of magnitude smaller than those to the lung. Moreover, epidemiological data provide little support for increased risks of mortality other than from lung cancer.

528. A factor for calculating the dose from a given exposure to radon and its decay products is needed for risk management, including regulatory purposes, and to allow comparison with other sources of radiation exposure. There are two approaches for deriving a dose conversion factor. A "dosimetric approach" derives the dose from a given exposure based on the deposition characteristics of radon decay products in the respiratory tract. An "epidemiological approach" was used by the International Commission on Radiological Protection (ICRP) to derive the dose conversion factor from epidemiological studies using the ratio of the risk of lung cancer in miners to the overall risk of cancer in the survivors of the atomic bombings in Japan. In the UNSCEAR 2000 Report, there was a difference of about a factor of 2 between the two approaches. However, the most recent data that have been published on the risks to underground miners (derived from updated studies of cohorts of uranium miners) suggest that the two approaches are less different than initially thought. The Committee therefore continues to recommend a radon dose conversion factor of 9 nSv $(Bq\ h\ m^{-3})^{-1}$ to evaluate the effective dose from radon inhalation. The dose conversion convention recommended in ICRP Publication 65 [I2] is approximately 30% lower than this factor but the difference is not considered significant.

529. Studies of miners exposed to radon and its decay products provide a direct basis for assessing lung cancer risk. The United States National Research Council's 6th Committee on the Biological Effects of Ionizing Radiation (BEIR VI) [C20] reported an excess relative risk from exposure to radon that was equivalent to 1.8% (95% CI: 0.3%, 35%) $(MBq\ h\ m^{-3})^{-1}$ for miners with cumulative exposures of below 30 MBq h m^{-3}. There are various sources of error in the exposure assessment of miners, especially in the earliest years of mining, when exposures were at their highest. Other factors that complicate the analysis of data on miners include: the high percentage of miners who smoke; workplace exposure to dust contaminants, such as arsenic, diesel exhaust in the dust and other pollutants; and periods spent working in non-uranium mines. The power to detect any excess risks due to the exposures that miners nowadays receive is likely to be small, in part because the exposures are much smaller than those in the early years of mining. Because of the high exposures in the early days of mining, it is possible to detect trends in lung cancer incidence and to investigate factors that affect the exposure–response relationship, such as the age at exposure, the effect of exposure rate and the reduction of risk with increasing time since exposure, as well as the effect of confounding factors such as smoking.

530. The BEIR VI model developed from the pooled analysis of 11 cohorts of underground miners provides a well-established basis for estimating risks from occupational exposures to radon, and accounts for factors such as the reduced risk with increasing time since exposure. Since the BEIR VI report was published, studies of various miner cohorts have been updated, and confirm the general patterns of risk with dose and with time since exposure that were reported by BEIR VI. They also provide updated coefficients to take account of the effects of time since exposure on ageing populations. Miner studies therefore provide a strong basis for evaluating risks from exposure to radon and for investigating the effects of modifiers to the dose–response relationship.

531. Biological and cellular models of the multistage process of carcinogenesis are used to analyse the data from studies on miners, and offer the possibility to assess uncertainties in our understanding of the mechanisms for the development of cancer and their modelling for the purposes of risk estimation.

532. The extrapolation of radon concentrations in the air in mines to those in homes provides an indirect basis for assessing the risks from residential exposure to radon. However, there are now over 20 analytical studies of residential radon exposure and lung cancer. These studies typically assess the relative risk from exposure to radon on the basis of estimates of residential exposure over a period of 25–30 years prior to the diagnosis of lung cancer. More recent pooled analyses of residential case–control studies support a small but detectable lung cancer risk from residential exposure, and this risk increases with increasing radon concentrations. The excess relative risk of lung cancer from long-term residential exposure to a radon concentration of 100 Bq/m^3 is established with reasonably good precision and is considered to be about 16% for both smokers and non-smokers (after correction for uncertainties in the exposure assessment), with an uncertainty of about a factor of 3 higher or lower than this value. Because the baseline lung cancer rate for smokers is much higher than that for non-smokers, smokers account for nearly 90% of the population risk of lung cancer.

533. Although there are major uncertainties in extrapolating the risks of exposure to radon from the miner studies in order to assess the risks in the home, there is nevertheless remarkably good agreement between the risk factors derived from the miner studies and from the pooled residential case–control studies. The ERR per unit radon concentration in air estimated in this annex from miner studies is 0.12 (95% CI: 0.04, 0.2) per 100 Bq/m^3 (see para. 424); that from the pooled residential case–control studies (based on the restricted analysis) for Europe is 0.094 (95% CI: 0.034, 0.175) per 100 Bq/m^3 [D17, D21] and for North America is 0.18 (95% CI: 0.02, 0.43) [K1, K26] per 100 Bq/m^3 (see table 24). The studies of uranium miners also provide important information on the effects of modifiers to the exposure–response relationship, and further follow-up is encouraged. The pooling of residential case–control studies in Europe, North America and China now provides an appropriate basis for estimating the risks from long-term residential exposure to radon. On the basis of current information, the Committee considers the use of measurement-adjusted risk coefficients from pooled studies as an appropriate basis for estimating the risks to people at home.

Appendix

Quantities, units and conversion factors relevant to radon and its decay products

Term	*Definition*	*Unit and Selected Conversions*
Absorbed dose	The energy absorbed through exposure to radiation divided by the mass of the body or by the mass of the part of the body that absorbs the radiation.	Gy 1 Gy = 1 J/kg = 100 rad
Effective dose	The sum over all tissues and organs of the equivalent doses weighted by the tissue weighting factor, which represents the contribution of that organ or tissue to the total detriment resulting from uniform irradiation of the whole body.	Sv 1 Sv = 100 rem
Equilibrium equivalent concentration	The concentration of radon in air, in equilibrium with its short-lived decay products, which would have the same potential alpha energy concentration as the existing non-equilibrium mixture.	Bq/m^3 $1\ Bq/m^3 = 5.56 \times 10^{-9}\ J/m^3$
Equilibrium equivalent exposure	Time integral of the corresponding equilibrium equivalent concentration of radon to which the individual is exposed over a given time period.	$Bq\ h\ m^{-3}$ $1\ Bq\ h\ m^{-3} = 5.56 \times 10^{-9}\ J\ h\ m^{-3}$ $1\ Bq\ h\ m^{-3} = 1.57 \times 10^{-6}$ WLM
Mache unit	A measure of radon concentration (historically radium emanation), where 1000 Mache units equals the amount in equilibrium with 1/2000 mg of radium.	Mache unit 1 Mache unit = 275 pCi/L
Potential alpha energy concentration	The concentration of short-lived radon decay products in air in terms of the alpha energy released during complete decay through polonium-214.	J/m^3 $1\ J/m^3 = 1.80 \times 10^{8}\ Bq/m^3$
Potential alpha energy exposure	Time integral of the potential alpha energy concentration in air to which the individual is exposed over a given time period.	$J\ h\ m^{-3}$ $1\ J\ h\ m^{-3} = 1.80 \times 10^{8}\ Bq\ h\ m^{-3}$ $1\ J\ h\ m^{-3} = 282$ WLM
Radon levels	Radon concentration in air.	pCi/L 1 pCi/L = 0.037 Bq/L $1\ pCi/L = 37\ Bq/m^3$
Relative risk coefficient	The ratio of the risk in an exposed population to that in a similar unexposed population per unit exposure.	—
Working level	Any combination of the short-lived decay products of radon in one litre of air that will result in the emission of 1.3×10^5 MeV of potential alpha energy.	WL 1 WL = 100 pCi/L (assuming 100% equilibrium, i.e. F = 1) 1 WL = 250 pCi/L (assuming 40% equilibrium, i.e. F = 0.4)
Working level month	The cumulative exposure from breathing an atmosphere at a concentration of 1 WL for a working month of 170 hours.	WLM $1\ WLM = 3.54 \times 10^{-3}\ J\ h\ m^{-3}$ $1\ WLM = 6.38 \times 10^{5}\ Bq\ h\ m^{-3}$ 1 WLM = WL × exposure time (h/a)/(170 h/WLM)

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