SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation

UNSCEAR 2000 Report to the General Assembly, with Scientific Annexes

VOLUME II: EFFECTS

UNITED NATIONS
New York, 2000

NOTE

The report of the Committee without its annexes appears as Official Records of the General Assembly, Fifty-fifth Session, Supplement No. 46 (A/55/46).

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UNITED NATIONS PUBLICATION
Sales No. E.00.IX.4
ISBN 92-1-142239-6

ANNEX H

Combined effects of radiation and other agents

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INTRODUCTION

1. Living organisms are exposed to numerous natural and man-made agents that interact with molecules, cells, and tissues, causing reversible deviations from homeostatic equilibrium or irreversible damage. Many aspects of aging and many diseases are thought to stem from exogenous and endogenous deleterious agents acting on key components of cells within the body. Because of the worldwide proliferation of a number of man-made agents and the increasing release of natural agents due to human activities into the environment, the assessment of toxicity, carcinogenicity, and mutagenicity of a specific chemical, physical, or biological agent is, in fact, a study of combined exposures [G10]. Although this has been recognized for a long time, risk assessment is generally performed with the simplifying assumption that the agent under study acts largely independently of other substances. Studies of interactions have indicated, however, that, at least at high exposures, the action of one agent can be influenced by simultaneous exposures to other agents. The combined effects may be greater or smaller than the sum of the effects from separate exposures to the individual agents. The action at low levels of exposure, which are commonly encountered in occupational and environmental situations, is less clear. Continued, critical review of studies on the effects of combined exposures to radiation and other toxic agents is necessary, particularly at the lowest levels of exposure, to be sure that any modifications of the radiation effects caused by other environmental or occupational agents are recognized and, as far as possible, taken into account in risk assessments.

2. In the UNSCEAR 1982 Report [U6], the Committee discussed the problem of the combined action of radiation with other agents. In reviewing the approaches and the many reports in which synergisms were claimed, the Committee noted that, in general, an adequate conceptual framework was lacking. Despite many reports showing the potential importance of interactions between different agents under specific conditions, mostly occupational, information on the mechanisms of action was largely missing, and the methodologies for data analysis in different branches of the biological sciences were based on different approaches. The UNSCEAR 1982 Report concluded that it was not possible to document clear cases of interaction that could justify substantial modifications to the existing radiation risk estimates. The Committee felt that systematic investigations of combined effects were needed to allow this field to move forward from its early stage of development.

3. The objective of this Annex is to update the Committee's previous review of this subject [U6] and to reconsider whether interactions of radiation and one or more other agents should be taken into account in evaluating radiation risks at low doses. To achieve this objective, the following subjects are considered:

(a) the concepts of doses, targets, and detriments currently used in risk assessments of radiation and chemical agents;
(b) recent developments from research on the possible mechanisms of combined effects from low-level exposures to radiation and other agents;
(c) results and evaluations of data from experimental and epidemiological studies;
(d) mechanistic models applied to experimental and epidemiological results, with generalizations and extrapolations that might be pertinent to low and chronic exposures;
(e) concepts and approaches in other areas of biological science (for example, molecular biology and toxicology) that could suggest ways to develop databases and to identify and assess the effects of interactions important for human populations.

4. Combined effects must be viewed in the light of the considerable insights gained from wider studies of cancer induction (see Annex E, "Mechanisms of radiation oncogenesis", in the UNSCEAR 1993 Report [U3] and Annex G, "*Biological effects at low radiation doses*"), heritable defects (see Annex G, "Hereditary effects of radiation" in the UNSCEAR 1993 Report [U3]), and DNA integrity (see Annex F, "*DNA repair and mutagenesis*"). Where necessary, the following text refers to these and other Annexes.

5. Since at low levels of exposure, the main endpoints from ionizing radiation alone and from its interaction with other agents are stochastic in nature, this Annex will mainly focus on this type of effect and consider cancer induction, mutation and the possibility of prenatal effects. Several specific areas where the combined action of high doses of radiation and chemical agents are known to lead to considerable deviation from additivity will also be considered but only in so far as they help to elucidate the mechanisms of combined exposures. These areas include the interaction of chemotherapeutic compounds and sensitizers to enhance radiation effects in clinical radiotherapy, the effects of protective agents on acute radiation exposure, and stimulatory responses to radiation (reviewed in the UNSCEAR 1994 Report [U2]). The endpoints of interest in these situations of high-dose exposure are deterministic effects.

6. The Annex begins by introducing the problem of combined effects, considering the additivity or non-additivity of biological effects and the possible differences between radiation and chemical carcinogenesis. This is followed by concepts and definitions of physical and biological dosimetry for radiation and other agents. Interactions of other agents in the development of radiation-induced cancer are then considered from a mechanistic point of view. A very important part of the Annex is a review of data on the effects of specific combined exposures on carcinogenesis. This is followed by a chapter on interactions in humans that produce effects other than cancer. Finally, conclusions are drawn and recommendations are offered. A detailed account of the combined effects of radiation and specific physical, chemical, and biological agents is provided in the Appendix.

I. IDENTIFYING INTERACTIONS AND COMBINED EFFECTS

A. SCOPE OF THE PROBLEM

7. When discussing combined effects, it is of utmost importance to provide clear definitions and terminology. Multiple-agent toxicology uses many concepts the nomenclature for which is not unambiguous. Different names are sometimes used for the same phenomenon, and sometimes the same name is used for different mechanisms. The confusion arises in part because the concepts were developed in different disciplines, such as pharmacology, toxicology, biology, statistics, epidemiology, and radiation biology. Starting from different basic assumptions and with different aims in mind, attempts are made to describe the effects of combined exposures to chemical and physical agents. The confusing terminology inhibits clear understanding and thwarts the comparison of different investigations and results. In this Chapter some basic problems concerning combined exposures are discussed.

1. Additivity and deviations from additivity

8. One of the basic questions surrounding the combined effects of two agents is the question of whether the effect of a combined exposure to two or more agents is the same as or different from the sum of the effects of each agent separately. Many terms and synonyms are used to indicate the result (Table 1). They are, in general, based on deviations from the expected outcome (additivity). On a descriptive level, two classes of combined effects can be considered. In the first case, both ionizing radiation and the other agent (or agents) are deleterious on their own and combine to produce an effect not directly predictable from the single exposures. In the second case, only ionizing radiation produces an effect, but its nature or severity may be modified by the other agent, which is non-toxic by itself.

Table 1
Terms and synonyms for combined effects

Effect smaller than anticipated	*Effect as anticipated*	*Effect larger than anticipated*
Antagonism	Additivity	Augmentation
Antergism	Additivism	Enhancement
Depotentiation	Independence	Positive interaction
Desensitation	Indifference	Potentiation
Inhibition	Non-interaction	Sensitation
Infra-additivity	Summation	Superadditivity
Negative interaction	Zero-interaction	Supra-additivism
Negative synergism		Synergism
Subadditivity		Synergy

9. On a mechanistic level, insights gained in more recent years indicate that a much more refined classification may be needed. The main classes of genotoxic and non-genotoxic agents must be considered in relation to specific targets of action. For example, a chemical may act specifically at the site of a radiation-induced lesion, modifying DNA repair fidelity, or it may modify cell growth, strongly influencing the clonal expansion of precancerous cells. The many possibilities for interaction are related to the complexity of the development of the radiation effect and the many steps involved in carcinogenesis. These steps are prone to the influence of many classes of agents, both endogenous and environmental. The multi-step process and the many levels of interaction to be considered are schematically depicted in Figure I. In view of this complexity, it is not surprising that many models, both descriptive and mechanistic, have been developed to describe the combined effects of exposures to different agents [B11, L2, L8, L28, M16, S15, S16, S23, S25, Z1]. In the UNSCEAR 1982 Report [U6], the Committee reviewed these approaches.

10. Although classical epidemiology is important in identifying critical combined effects, it has little potential for dissecting such interactions from the complex interplay possible among the undocumented (and sometimes unknown) exposures that the individuals in these studies incur during their lifetimes. In epidemiological studies, effects that may be associated with exposures to specific agents or circumstances may be the result of interactions among components of a mixture of agents and may have resulted from, or been influenced by, previous exposures. The emerging field of molecular epidemiology may be able to address such questions in the near future.

11. Most knowledge of interaction effects has been provided by experimental studies. These studies have an advantage over epidemiological studies: they retain control of

(a) the population (e.g. selection of systems ranging from DNA to intact animals and of species, strain, age, gender and previous exposure history);
(b) the exposure (e.g. precise knowledge of the type, dose, dose rate and timing of exposure); and
(c) the endpoints (e.g. selection of sampling time and frequency, use of invasive and destructive tests, consistency and completeness of health status evaluations).

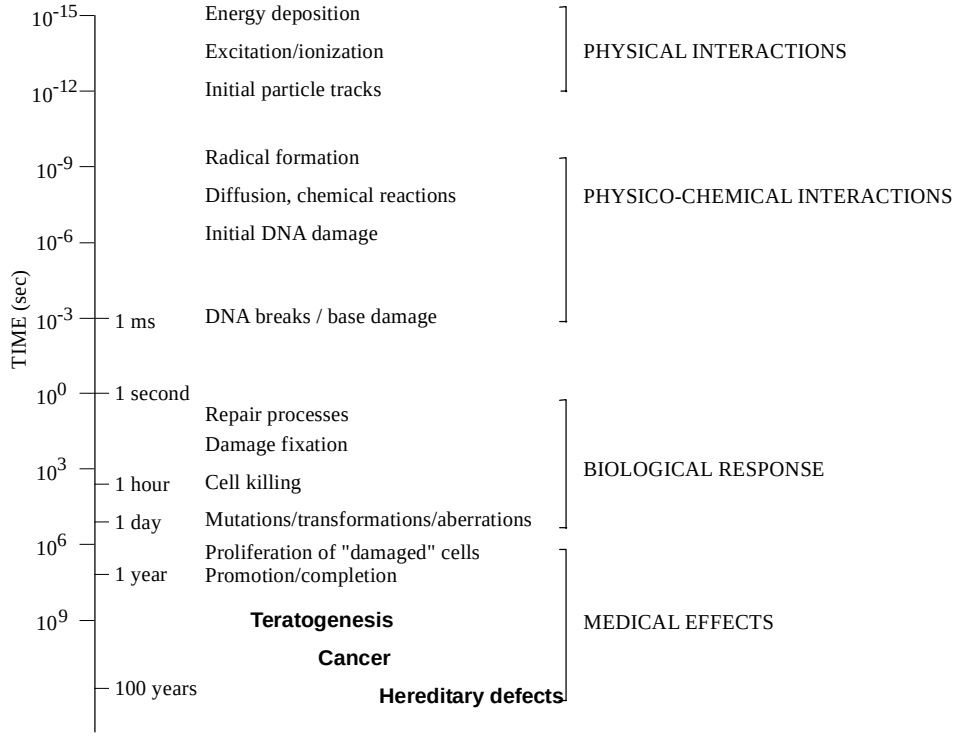

Figure I. Schematic development of the events leading to stochastic radiation effects.

Moreover, in experimental studies one can relate exposures to effects more directly than is typically possible in human studies. This is due, in part, to the fact that both the history of the subject and the exposures under study are known and controlled, making cause-effect linkage easier. In addition, experimental scientists can often determine that an exposure actually results in a dose to the tissue manifesting an effect.

12. Experiments with animals or cells have the disadvantage that the results and conclusions have to be extrapolated to humans. Additionally, conclusions drawn from high-level exposures of animals and cells have to be extrapolated to the low levels of human exposures. The greatest uncertainty is largely a problem of not knowing the shape of the dose-effect relationship at low exposure levels and whether there are effect thresholds. A well balanced conclusion on the combined action of two agents can only be given if the dose-effect relationships of both agents separately and of the combined exposure are known and can be analysed using a (mathematical) model in which the interaction can be consistently and quantitatively defined. The majority of studies on combined effects, including those with radiation, do not meet these conditions.

13. For the basic case of a single agent acting on a biological system, the resulting effect will be dependent on the dose of the agent and will follow some kind of functional dose-effect relationship. The effect level in the absence of the agent is termed the spontaneous or background effect. The simplest relationship between dose and effect is linear. In the realm of linear dose-effect relationships, the three most commonly considered types of interactions between two agents are additivity, synergism and antagonism, giving a combined effect equal to, greater or less than the effects of independent actions, respectively (reviewed in [M16]).

14. For combined effects of agents with non-linear dose-effect relationships, the analysis is complicated, and more precise definitions of the terms antagonism, additivity, and synergism must be provided [S25, S49]. For example, for an upward-bending dose-effect relationship (Figure II), an additional increment of dose from a single agent will result in a non-linear increase in response, even in the case of additivity. The term synergism has sometimes been erroneously used for such situations [Z3]. Although correct on a descriptive and mathematical level, such a broad definition would render the term synergism practically useless in the study of combined effects. With such a definition, different agents with the same action spectrum, i.e. fully independent agents, would

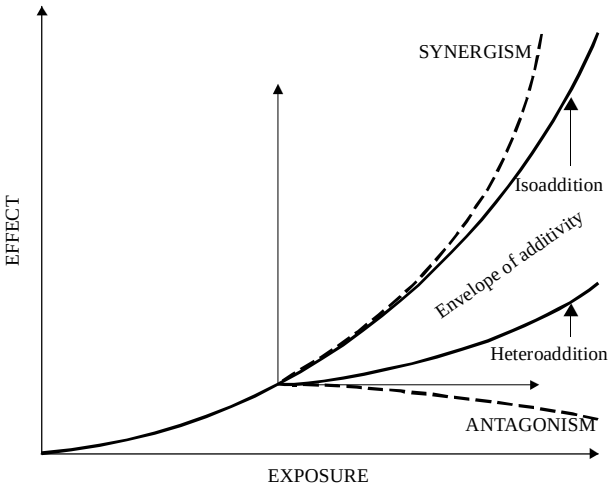

Figure II. Interaction of two agents having non-linear dose-effect relationships. Isoaddition results for mechanically similar agents, heteroaddition for independently acting agents [I3, S49].

produce an apparent synergism in any combination of concentrations as long as the single dose-effect relationships are bent upwards, as is often the case in the dose range of interest. From a mechanistic point of view, synergism can be defined more narrowly to imply that agents combine by acting at different rate-limiting steps of a multi-step process or at different sites of a molecule, thereby enhancing the chance for a negative outcome, such as cancer, by different mechanisms [B23]. Such an assessment is often hindered by insufficient knowledge of the underlying mechanism of action and therefore can rarely be made. Clearly, deviation from additivity is a poor indicator of synergism or antagonism, since non-linear dose-effect relationships and threshold phenomena are the rule rather than the exception for most endpoints in biological systems, and interaction in the statistical-mathematical sense does not define an interaction in a biological-mechanistic sense [B69].

15. In this Annex the term synergism will be used in a narrow sense. The most important question is whether data on combined effects do show some modification of stochastic radiation effects as a result of combined exposure with another agent. If not, no interaction will be assumed, and the resulting effect is additive; if the result of combined exposure is different, some form of interaction has to be assumed, and the resulting effect will be called sub- or supra-additive, depending on whether the effect is lesser or greater, respectively, than the sum of the single-agent effects separately.

2. Radiation effects and effects of other agents

16. As far as carcinogenesis is concerned, the primary effects of ionizing radiation are on DNA, compromising cell survival, cell proliferation, and proper physiological cell functioning. Although the deposition of energy along the track of ionizing radiation can directly affect DNA, most of the damage to DNA from low-LET radiation comes from the formation of radical intermediates stable enough to diffuse several nanometers and interact with critical cellular constituents (for details see Annex F, "*DNA repair and mutagenesis*"). Only a small fraction of the radiation-induced molecular modifications occur in the DNA of the cell nucleus, but practically all experimental and theoretical evidence indicates that DNA, the main carrier of genetic information in living matter, is the critical target. Especially at the doses under consideration in this Annex, damage to structural and functional proteins and lipids has not been shown to contribute noticeably to the detriment from ionizing radiation. To protect the integrity of the genetic information, most cells have highly intricate enzyme systems to repair DNA damage efficiently and effectively based on information contained within the undamaged complementary DNA strand. Despite that, residual fixed damage may result even from low-dose exposures, especially when both DNA strands are damaged. Such damage may lead to reproductive cell death, and therefore possible deterministic effects; to somatic cell mutation, enhancing the risk of cancer; or to mutations in germ cells, with possible deleterious effects in offspring.

17. Longer wavelength radiation, such as ultraviolet (UV) light, although not ionizing itself, still acts mainly by modifying DNA. The UV portion of the electromagnetic spectrum covers the wavelengths between 200 and 400 nm. Conventionally, a distinction is made between UV-C (200–280 nm), UV-B (280–320 nm), and UV-A (320–400 nm). The effects of UV light depend on the wavelength and the absorption properties of the target. Ultraviolet radiation mainly causes the formation of pyrimidine dimers and 6–4 photoproducts, which may also lead to residual DNA damage after repair. Apart from visible light up to 525 nm, which can still interact with photosensitizers to generate reactive species and, subsequently, oxidative damage to DNA, infrared, microwave, and low-frequency electromagnetic radiation have no direct genotoxic effects of their own. Indirect effects might arise from local heating or from charge effects across membranes activating signal transduction pathways and neurons. Such cellular changes may be long-lasting or even be passed from one cell to its progeny. Sugahara and Watanabe [S10] reviewed the epigenetic aspects of radiation carcinogenesis. Studies using cell culture systems show that magnetic fields, depending on their frequency, amplitude, and wave form, interact with biological systems. Such effects have been seen on enzymes related to growth regulation, on intracellular calcium balance, on gene expression, and on peripheral levels of the oncostatic hormone melatonin [H45]. These effects are potentially related to tumour promotion. However, the considerable research conducted thus far has not elucidated critical mechanisms or revealed important health risks from non-thermal exposures. Other than crude effects present only at high exposures, for example strong irritations or protein denaturation, cellular perturbations resulting from non-ionizing radiation cannot be labelled harmful per se.

18. Chemical agents may act as genotoxicants by, for example, forming direct covalent links, by transferring reactive molecular subgroups to DNA, by inducing DNA-DNA or DNA-protein cross-links, or by generating strand breaks. The mode of action may be direct, by the formation of small or bulky DNA adducts as well as strand breaks, or indirect, by the formation of radicals in the vicinity of DNA, leading to strand breaks or small adducts. On the epigenetic or non-genotoxic level, chemicals may interfere with DNA synthesis or repair or may prevent radical scavenging, thereby promoting DNA damage. Non-genotoxic agents may also influence a broad spectrum of other cellular events. Of concern in cancer induction is any interference with cell proliferation, cell differentiation, cell senescence, and apoptosis or with the regulation of these processes.

19. Biological agents may also act at the genetic and epigenetic levels, i.e. they may be genotoxic or non-genotoxic, respectively. Viruses are effective transport vectors for genome fragments and may activate or block the expression of endogenous genetic information. Viral involvement in many animal tumours and also in human malignancies is well established, e.g. the DNA tumour viruses of the papilloma family in cervical carcinoma and the retroviruses HTLV-1 in adult T-cell leukaemia (reviewed in [H13]). In addition,

biological-agent-induced influences on immune responses, inflammation, fever, and endogenous radicals may lead to cytotoxic and/or growth stimulatory responses that are co-carcinogenic, as described later.

B. EXPOSURE ASSESSMENT

20. The most important prerequisite for a comparative assessment of biological effects of different agents, and also of their possible interactions, is the characterization of the exposure or the dosimetry of both agents that may be related to subsequent effects. Some of the main concepts used in toxicology and radiation biology to convert exposures into meaningful measures of dose and health impact are introduced in the following paragraphs.

21. The toxicity of an agent can be defined as its inherent ability to adversely affect living organisms. The spectrum of undesired effects is very wide, ranging from local, reversible effects to irreversible changes leading to the failure of critical organ systems and then to death. The objective of dosimetry is to relate the amount of agent presented to the organism in a way that is relevant to the effects observed and that is measurable in a physical, chemical, or biological manner. Identification of processes occurring at the molecular level, i.e. at a mechanistic level of the effect, would give the most basic indication of a dosimetric measure. The present approaches and possibilities are discussed below. In Section I.B.1, dosimetry based on the measurement of physical or chemical parameters of the agent itself, the physical or chemical dosimetry, is considered. In Section I.B.2, measurement of immediate biological damage caused by the agent (biochemical monitoring) is discussed; this damage may or may not be directly related to the biological effect being considered.

22. Sometimes, when physical, chemical, or biochemical measurements are not possible or cannot be made accurately enough, certain biological effects may be detectable. Such effects may serve as indicators of the exposure to biologically active agents. These "biological markers" reflect damage resulting from toxic interaction, either at the target or at an analogous site that is known or believed to be pathogenically linked to health effects. A wide variety of biological markers fall into this category, including gene mutation; alterations in oncogenes and tumour-suppressor genes; DNA single- and double-strand breaks; and unscheduled DNA synthesis, sister chromatid exchanges, chromosomal aberrations; and micronuclei. None of these markers is highly agent- or exposure-specific, and other factors (lifestyle and environment) that affect these endpoints can act as confounding variables in molecular studies. Some possibilities for assay systems to measure biological markers such as specific gene mutations and cytogenetic damage in exposed humans are presented in Sections I.B.3 and I.B.4, respectively.

1. Dose concepts for physical and chemical agents

23. Ionizing radiation exposure is generally measured in terms of absorbed dose, i.e. the average energy deposited per unit mass. The unit of absorbed dose is the gray (Gy), with 1 Gy equal to 1 J kg^{-1} [I3]. At the level of a cell or cell nucleus, the minimal dose is determined by the ionization density of a single track. Averaged over the volume of a cell nucleus, a single event amounts to between one and several milligray (mGy) for electrons and about 300 mGy for an alpha particle [U3]. Below these dose levels, the probability of a cell being hit varies but the absorbed dose per cell nucleus does not. For internally deposited radionuclides, their location and fate in the organism are used to calculate the absorbed dose in the organs of interest, and usually the average absorbed dose in the organ is taken as the relevant dose that causes the biological effect, assuming a rather homogeneous distribution of energy absorption in the tissue.

24. The definition of exposure or dose for non-ionizing radiation and for most chemical and biological agents is more difficult than for radiation. Ultraviolet radiation can penetrate into tissue at most only for several millimetres, depending on wavelength. The energy absorbed in the tissue of interest, and thus the effectiveness of UV, cannot be easily estimated. Exposure to a toxic agent may be estimated by environmental monitoring (referred to as external dose evaluation in toxicology), internal monitoring (internal dose evaluation), and biochemical effect monitoring (tissue dose or biologically effective dose determination) [E1].

25. For chemical and biological agents, the dose can be based on the time integral of concentration, as for internal exposures with radionuclides. However, in addition to the common important question of defining the critical cellular targets, it is the activation and biodegradation of a chemical agent in the different compartments of the organism that will determine the degree of genetic damage or strength of an epigenetic signal. Although the local concentrations of receptors or reactants could possibly be estimated or determined, these vary considerably in their response to endogenous and environmental factors, which can lead to different sensitivities to the physical or chemical agent. This may restrict the use of biochemical markers somewhat, because their concentrations in body fluids will depend on the mechanisms of uptake, the formation of reactive molecular species, and their breakdown. Somewhat like the dose concept for ionizing radiation, exposure can be related to the number of primary chemical events on DNA leading to the effect under consideration. The above-mentioned quantitative link between DNA alkylation and the product of concentration and time for ethylene oxide may serve as an example [E3] (see also paragraph 34). However, only rarely is the nature of such events known or quantifiable.

26. To give exposure (or dose) its full biological meaning, the concentration-time product at the level of the cellular target structure should be known. Even this is difficult to determine owing to the many membranes and other barriers to be crossed between the intake port and the place of action. Many chemicals also undergo modifications by detoxification in the liver, lung, and other organs, which change both their toxicity and their biokinetics. One of the best known carcino-

gens, benzo(a)pyrene, becomes toxic only after metabolic activation, leading to the ultimate reactive electrophilic carcinogen. Such transformations, called metabolic activation, may differ considerably among species and even between male and female subjects, making inferences from experimental systems still more difficult. The induction of kidney cancer in male rats by a group of chemicals (n-1,4-dichlorobenzene, hexachloroethane, isophorone, tetrachloroethylene, and unleaded gasoline) may serve as an example. It took a great deal of research [B29, E4] to show that the risk for the endpoint under consideration, namely kidney tumours in male rats, is a species- and sex-specific finding that relates to male rats but not to female rats, mice of either sex, or humans. Mechanistic studies showed that male rats have a specific circulating protein, alpha-2u-globulin, that binds the chemicals under consideration and leads to renal accumulation, with subsequent kidney damage and the development of kidney tumours. This protein was shown to be absent in humans. Only such detailed molecular information allows a reasonable risk estimate to be generated for humans [B29]. Unfortunately, the species-specific detection and quantification of toxic agents formed in biochemical pathways are rarely achieved.

2. Biochemical monitoring

27. For chemical agents, internal dose evaluation involves the measurement of the amount of a carcinogen or its metabolites present in cells, tissues, or body fluids. Analysis of internal dose takes into account individual differences in absorption or bioaccumulation of the compound in question. It may be relatively easy to measure the concentrations of the compound in body fluids. However, doing so does not provide data on the interactions of the compound with critical cellular targets. Examples of this type of monitoring include organic compounds or metals (e.g. lead) in the diet, cigarette smoke, or industrial exposures that can be detected in blood or urine [P3]. The binding of chemicals with cell constituents may be measured directly with radioactive labels. Even *in vivo*, correlations between the administered amount of the toxicant, the number of molecules bound to critical targets, and the biological effect can be established [P6].

28. From the energy deposition pattern of ionizing radiation in the tissue constituents and from some critical biochemical parameters, such as oxygen pressure and the local concentrations of radical scavengers, the primary damage, i.e. the number of primary DNA lesions, can be estimated. A few of these parameters are even stable enough to be used as biological indicators such as cytogenetic changes in peripheral blood lymphocytes to assess exposures retrospectively.

29. In toxicology, the tissue dose or biologically effective dose reflects the amount of carcinogen that has directly interacted with cellular macromolecules at a target site. It can be assessed from the amount of DNA and protein damage (strand breaks, DNA adducts, protein adducts) in the target tissue or by extrapolating from damage levels found in surrogate tissues, such as white blood cells. Experiments have shown that, in general, DNA damage levels in target tissues and non-target cells are proportional to the external dose. This class of markers is more mechanistically relevant to carcinogenesis than internal dose, since it takes into account differences in metabolism (activation vs. detoxification) of the compound in question, as well as the extent of repair of carcinogen-altered DNA. Perera and Santella [P3] provided examples of compounds and exposures that might be analysed using this type of biologically effective dosimetry, as well as the populations that have been studied.

30. DNA and protein adducts are measures of exposure to carcinogenic compounds [E1]. They are mechanistically linked to cancer, as they cause DNA damage and mutations in important genes, such as genes coding for growth control or damage repair enzymes. Adducts have been used to estimate cancer risk by comparing their mutagenicity relative to that of x rays. In the same way that the unit cancer risk of x rays is defined, the relative mutagenicity is used to estimate the cancer risk of a chemical exposure that causes adducts (gray-equivalent approach) [E1, E3].

31. In the case of agents binding covalently to different cellular macromolecules, the degree of alkylation of proteins can be used as a surrogate measure for their effects on DNA. Ehrenberg et al. [E3, E10] showed in the mouse that the tissue dose of ethylene oxide, i.e. the concentration of the alkylating agent integrated over time, correlated well with the alkylation pattern. In male mice, the authors were able to show with this method that the tissue dose for ethylene oxide, an agent rapidly distributed to all organs after inhalation, was about 0.5 mM h per ppm h for most organs, including the testes. On the basis of dose-effect curves of ethylene oxide and x rays in barley, the same authors [E3] set a tissue dose of ethylene oxide in humans of 1 mM h equal to 0.8 Gy of low-LET radiation. Such an approach facilitates the comparison and combination of risks of various agents.

32. Despite their relevance as dosimeters of biological effects, the limitations of the current methods should be noted. Most available assays provide information on total or multiple adducts and are rarely capable of pinpointing the critical adducts on DNA. Only for a few target organs, such as the lung or bladder, are epithelial cells available for routine analysis. For other organs, DNA is not readily accessible; many studies therefore use surrogate tissues (e.g. peripheral blood cells and placentas). However, the relationship between adducts in the target and those in surrogate tissues has not been well characterized in humans, although for certain carcinogens it has been characterized in experimental animals [S17]. Again, it must be considered that there are species- and sex-dependent differences in the absorption and metabolism of chemicals in their various forms.

33. By definition, all types of ionizing radiations generate ions. Ionizing radiation can directly induce ionizations in DNA, causing direct damage. However, the majority of damage from low-LET radiation occurs in an indirect manner via the formation of free radicals and H_2O_2, which are precursors of oxidative damage [B8, S34]. When living cells or organisms are irradiated, OH radicals are generated in cells or tissue, which leads to many DNA

lesions, including oxidative DNA base products. Both ionizing radiation and oxidative stress generate free radicals near DNA. Most of these radicals (–R) interact with oxygen, forming peroxyl intermediates (–ROO) and final products (–P). Most of the products are eliminated by nucleotide excision repair and glycosylases [F10], while a small fraction remain in the DNA [S35]. Critical are lesions leading to double-strand breaks or even more complex local damage.

34. Free radicals are difficult to detect, identify, and monitor because of their short half-life, particularly in living organisms. Such detection and monitoring can be achieved only by detecting and measuring the products of their reaction with endogenous bio-components or exogenous components selectively added to a biosystem. Specific products of such reactions or their metabolites may qualify as markers of a particular process or specific free radical. In biosystems, these products are called molecular markers, a subclass of biomarkers [G9]. For a product to qualify as a molecular marker, there must be unequivocal proof of an exclusive origin of the product. First, a comprehensive understanding of the kinetics, energetics, and mechanisms of product generation is required. Then other possible sources of the product must be excluded [S39].

35. Although a molecular marker can be quantified by measurement *in vivo*, quantification of oxidative stress is considerably more complex. The reactivity of all five bases, adenine, cytosine, guanine, thymine and uridine, with OH radicals is extremely high, whereas that of deoxyribose is about five times lower [B34]. The distribution of damage will therefore be governed by the relative abundance and reactivity of DNA and RNA components. Each DNA and RNA base contains more than one site of attack. For example, OH adds to the double bond of thymine at C-5 (56%) and C-6 (35%) and removes hydrogen from the methyl group (9%) [J7]. The 5-hydroxythymidine intermediate leads to formation of thymine glycol. The 6-hydroxythymidine intermediate is an oxidizing radical that gives rise to unstable hydroxyhydrothymine. The radical on the methyl group of thymine, however, is a reducing radical that yields 5-hydroxymethyluracil as the final product (reviewed in [S39]). Addition of OH to the C-8 position of guanine yields a well-known product, 8-hydroxyguanine or 8-oxoguanine, which was discovered by Kasai et al. [K1, K47] and described in detail [J4, S39]. Numerous other products have been identified, and the kinetics and mechanisms of their formation have been described [B8, S12, S34, S36].

36. On the basis of extensive studies in radiation chemistry and radiation biology of the kinetics and mechanisms of OH radical reaction with DNA components, it was suggested that detection of thymine glycol, thymidine glycol, and 5-hydroxymethyluracil indicated endogenous OH generation in rats and humans [C3, H18, W8]. Because thymine glycol can be absorbed through the gastrointestinal tract and 5-hydroxymethyluracil may be generated by enzymatic hydroxylation of thymine, these products may not always qualify as biomarkers for oxidative damage in organisms. Thymidine glycol is less prone to such confounders and qualifies as one of the best endogenous markers of OH [S39]. It was suggested that 8-hydroxyguanine could be another OH marker in biosystems [B12, F3, K1, R9, S30, W12]. Enzymatic hydroxylation of guanine, however, has not been ruled out unequivocally. Hence it is prudent to monitor more than one marker for each specific free radical under investigation. 8-Hydroxyguanosine was analysed in the DNA of peripheral blood leukocytes of patients exposed to therapeutic doses of ionizing radiation [W12]. Radiation-generated oxidative DNA base products were also measured in the DNA of irradiated cells [N2].

37. The chemical reaction products in DNA are excised from damaged DNA over a certain period of time by repair mechanisms and eventually appear in the cell medium or urine. Some oxidative DNA base products have been measured in the urine of irradiated humans and mice. The radiation yields of these markers, i.e. the increments per unit of energy (mass × dose), were obtained from the level one day after irradiation minus the level before irradiation and are shown in Table 2. In contrast to the metabolic levels of these markers, the irradiation yields per unit energy are the same for both mouse and human, as expected, because the same number of OH radicals is generated in both cases [S39]. The metabolic rate plays an important role in the variability of relative rates of oxidative DNA damage. A high metabolic rate, as in rodents, generates a high yield of urinary markers, i.e. higher rates of DNA damage. The rate of DNA damage, however, is not always proportional to the specific metabolic rate because the efficacy of inhibition and scavenging of oxygen radicals and peroxides as well as of DNA repair systems varies in different species.

Table 2
Yield in urine of biological markers of oxidative DNA damage [B12]

Species	*Specific metabolic rate* ($kJ\ kg^{-1}\ d^{-1}$)	*Metabolic yield* ($nmol\ kg^{-1}\ d^{-1}$)		*Increment induced by radiation* ($nmol\ kg^{-1}\ Gy^{-1}$)	
		Thymidine glycol	*8-Hydroxy-guanine*	*Thymidine glycol*	*8-Hydroxy-guanine*
Human	100	0.3±0.1	0.3±0.1	3.1±0.8	6.7±1.5
Mouse	750	7.3±1	11±2	3.0±0.6	6.9±1.3

3. Gene mutation analysis

38. The analysis and quantification of genomic changes are important steps in monitoring and in the elucidation of mechanisms leading to critical health effects. Functional changes, i.e. changes in the phenotype of oncogenes and tumour-suppressor genes, may be of direct relevance to the process of carcinogenesis. The ultimate effects of ionizing radiation and other genotoxic agents are genetic changes, which are heritable, i.e. which can be passed in a clonal fashion from one somatic cell to the following cell generations, or from a germ cell to the offspring. Therefore critical studies of combined effects should include gene mutation analysis as one very important biological endpoint for stochastic health effects. Several methods are available for the study of gene mutations arising in human somatic cells *in vivo*. These methods allow determination of the frequency of mutant lymphocytes or erythrocytes or characterization of mutations at the molecular level in lymphocytes. The study of types, frequencies, and mechanisms of human somatic mutations *in vivo* is valuable in its own right and may also improve the understanding of individual variation in sensitivity to environmental exposures, the influence of DNA repair and metabolism, and the relationship between mutagenesis and carcinogenesis [L7, M21]. Genetic changes are key events in carcinogenesis. Most human tumours contain more or less specific mutations that are directly or indirectly related to the carcinogenic process. A description of mutations in human tumours and the scientific background of many of the concepts and methods addressed in this and the following Chapter are presented in more detail in Annex F, "*DNA repair and mutagenesis*".

39. Early and probably single-step biological end points, such as morphological changes in *in vitro* cell lines, might also serve as indicators of genetic changes. The development of cell culture systems has made it possible to assess the oncogenic potential of a variety of agents at the cellular level. Many assays for oncogenic transformation have been developed, ranging from those in established rodent cell lines, where morphological alteration is scored (e.g. loss of contact inhibition in 10T½ cells), to those in human cells growing in nude mice, where tumour invasiveness is determined. The mutational changes involved are rarely defined. In general, simple *in vitro* systems that deliver reproducible results are the least relevant in terms of human carcinogenesis and human risk estimation. The most important potential of these systems lies in the opportunity they offer to identify and quantify factors and conditions that prevent or enhance cellular transformation by radiation and chemicals [H11].

(a) Mutation frequencies

40. Five systems for biomonitoring humans exposed to carcinogenic agents have been developed in which gene mutation is the endpoint. Two of these use as markers haemoglobin variants (Hb) [S18, T4] and loss of the cell-surface glycoprotein glycophorin A (GPA) in donors heterozygous at the MN locus in erythrocytes [L1, L3, L6]. The other three involve detection of mutations in T lymphocytes in the X-linked locus for the purine salvage pathway enzyme, hypoxanthine phosphoribosyltransferase (*hprt*) [A5, A6, A8, M22, R7, R8, T10], in the autosomal locus for human leukocyte antigen-A (HLA-A) [J3, M18, T7, T8], and in the autosomal T-cell receptor genes (*TCR*) [K23, K24, N3, U15].

41. Mean background mutation frequencies in human cells *in vivo*, as analysed by the five mutation assays, differ by about four orders of magnitude. In summary, the relative order of background mutation frequency values from normal adults for the five markers are Hb ($5\ 10^{-8}$) < *hprt* ($5\ 10^{-6}$) < GPA ($1\ 10^{-5}$) < HLA-A (>$1\ 10^{-5}$) < *TCR* (>$1\ 10^{-4}$) (reviewed in [C23]). For at least three of these mutation systems, sufficient numbers of donors have been tested to show that, as a general rule, the mutant frequency in normal, non-exposed donors is low at birth, increases with age, is often elevated in smokers, and is increased in people who have been exposed to known mutagens and carcinogens. Despite the great variation in mutant frequency among individuals at each of the loci studied, these findings show the potential relevance of mutational analysis in the assessment of combined environmental exposures. More recently, the polymerase chain reaction (PCR) has also been applied in the analysis of mutational spectra. A fairly complete database has been compiled by Cariello et al. [C51, C52].

42. The frequencies of *hprt* mutant cells in healthy adults range from <0.5 to $112\ 10^{-6}$. In most cases the frequency of *hprt* mutant cells is significantly increased after smoking [C17, C19, H2, T4, T11]. There seems to be no effect of sex on the *hprt* mutant frequency. In most studies, an age-related increase in mutant frequency is seen at the *hprt* locus, estimated to be 1%–5% per year in adult donors. Radiochemotherapy for various malignant disorders, including breast cancer, hepatoma, other solid tumours, and lymphoma increased the frequency of *hprt* mutant T cells by a factor of 3–10 [D5, M20, N8]. Cole et al. [C18, C20] examined factory workers exposed to styrene or to nitrogen mustard. In contrast to styrene, nitrogen mustard significantly increased the number of mutant *hprt* cells in these donors. Tates et al. [T9] described a significantly increased mutant frequency in a group of factory workers exposed to ethylene oxide.

(b) Mutational spectrum

43. The spectrum of mutational changes that arise spontaneously or that may be induced by a physical or chemical agent in human cells is broad. At the DNA level it encompasses, at one extreme, single-base events, and at the other, chromosomal rearrangements involving small to large deletions or translocations. In addition, an important category of mutational events in humans involves losses or gains of whole chromosomes. The mutation spectrum in the mammalian genome is reviewed in Annex F, "*DNA repair and mutagenesis*".

44. Many known mutagens form covalent DNA adducts that are released from DNA either spontaneously or by biological repair processes [H16]. Mutations induced by a large number of compounds, e.g. alkylating agents, arylating

agents, and radiation, have been scored and characterized using shuttle vectors. These experiments elucidate the sequence specificity of adduct formation and, subsequently, the mutations and the mutational efficiencies of different adducts [D9, I6, M10].

45. About 15%–20% of *hprt* mutations in normal adults result from gross structural alterations [A8, B31, H8, N7, T10], as detected by Southern blot analysis. These include deletions, insertions, and rearrangements. The break points or alterations are distributed randomly within the gene, with no hot spots having thus far been identified [A8]. The remaining 80% of the background *in vivo hprt* mutations in adults consist of point mutations or small deletions, insertions, and frameshifts beyond the resolution of Southern analysis. Considering only the *in vivo hprt* mutations (46 Lesch-Nyhan germinal, 51 normal adult somatic, 86 exposed adult somatic), several hot spots of point mutations were observed. In particular, four base-pair sites have been observed to be mutated in all groups [C13].

46. Ionizing radiation is known to induce gross structural alterations in *hprt* and other reporter genes in cultured human cells. After exposure to radionuclides for diagnostic purposes, an increase in the frequency of mutants with gross structural alterations on Southern blots was observed to be 33%, compared with 13% before receiving radionuclides [B31]. Mutations from post-radioimmunotherapy patients showed clearly greater frequencies of gross structural alterations than mutations from pre-radioimmunotherapy patients or normal individuals. The latter two frequencies are quite similar, suggesting that cancer per se does not produce this sort of damage at *hprt* [A9]. Taken *in toto*, the data from Albertini et al. [A9] on *in vivo hprt* T-cell mutations indicate that ionizing radiation produces deletions, particularly large deletions.

47. The yield of mutations caused by ionizing radiation may be influenced strongly by adaptive responses to other toxicants or earlier exposures to the same agent. This topic was reviewed in Annex B, "Adaptive responses to radiation in cells and organisms" of the UNSCEAR 1994 Report [U2]. A 70% reduction in *hprt* mutant frequency in radioadapted human lymphoblastoid cells has been reported, as analysed by Southern blot analysis and multiplex polymerase chain reaction assay [R10, R12]. The treatment was 4 Gy from gamma rays alone or in addition to an adaptive dose of 0.02 Gy. The proportion of deletion-type mutations was decreased in adapted cells (42%) compared with that in mutants treated with the high dose alone (77%).

48. Using a shuttle vector system, Kimura et al. [K12] analysed mutational spectra of the human cDNA *hprt* gene, a recombinant DNA copy of the *hprt* RNA, arising spontaneously or induced by the mutagens methylnitrosourea (MNU); 3-amino-1-methyl-5H-pyridol[4,3-b-]-indole (Trp-P2), a tryptophan pyrolysate; and acetylaminofluorene (AAF). Most mutations induced by MNU are G:C to A:T transitions. This can be predicted by the major premutagenic lesion in DNA produced by MNU, namely O^6-methyl-guanine that specifically mispairs with thymine [S41]. Mutations that arise spontaneously or are caused by x rays, Trp-P2, or AAF give rise to a similar mutation spectrum of c-*hprt*. Base substitutions account for about one third of all mutations. Mutations other than base substitutions make up some two thirds of all mutations. The main mutational event in these cases is deletion. A noticeable feature of these deletion mutations is the frequent presence of short, direct repeats at the site of the deletion.

49. Mutational alterations in *p53*, a tumour-suppressor gene, are mostly (more than 85%) missense mutations, while those of *APC*, another tumour-suppressor gene, and *hprt* are largely composed of nonsense, frameshift, deletion, and insertion mutations, resulting in truncated gene products or loss of genes. The mutational spectrum in *p53* is therefore clearly different from that of other genes. Mutations in the *p53* gene detected in tumours seem to be the result of a functional selection process for mutant *p53* protein that gives growth advantages to the cell. On the other hand, large deletions in the *p53* region may not be compatible with cell survival. This suggests that the mutations of *p53* observed in tumours may reflect only those mutations of the initial events that are compatible with cell proliferation and may even reflect those that give the transformed cell a growth advantage over the surrounding cells. Mutational selectivity in tumour-suppressor genes is discussed in detail in Annex F, "*DNA repair and mutagenesis*".

50. With respect to interaction mechanisms leading to combined effects, present knowledge indicates that the mutational spectrum found in tumours often reflects not the agent responsible for the primary DNA damage but rather growth selection based on specific changes in the phenotype or general chromosome instability emerging during carcinogenesis. Analysis of marker cells in peripheral lymphocytes may overcome this problem, albeit at the expense of losing the direct link to human disease.

4. Cytogenetic analysis

51. The main conceptual basis for using cytogenetic assays for biological monitoring is that genetic damage in easily available cells, such as peripheral blood lymphocytes, reflects comparable events in target cells. The fact that chromosomal abnormalities are often a characteristic feature in malignant cells points to the direct relevance of such markers for clastogenic agents to be considered in combined exposures. In addition, long-term follow-up of populations screened for chromosomal aberrations shows a clearly higher cancer risk for the subgroup with an elevated level of chromosome damage [B20, H5]. Microscopically recognizable chromosomal damage includes numerical aberrations and structural chromosomal aberrations, in which a gross change in the morphology of a chromosome has occurred. Chromosome and chromatid breaks, dicentrics, and ring chromosomes are important examples of this class of damage [N11]. The yield of sister chromatid exchanges, which represent apparently symmetrical intrachromosomal exchanges between the two identical sister chromatids and which are already quite

frequent in unexposed cells, is also increased. Micronuclei arising either from acentric chromosome fragments or from a lagged whole chromosome with centromere [W15] are also important markers, although the second production pathway points to a mechanism driven partially by epigenetic factors.

(a) Chromosomal aberrations

52. Induced chromosomal aberrations can be divided into two main classes: chromosome-type aberrations, involving both chromatids of a chromosome, and chromatid-type aberrations, involving only one of the two chromatids. Ionizing radiation induces chromosome-type aberrations in the G_0 or G_1 stage of the cell cycle (e.g. prior to replication), while chromatid-type aberrations are produced during the S or G_2 stage (e.g. during or after replication of the affected chromatid segment). In peripheral lymphocytes, most of which are in the G_0 stage of the cell cycle, ionizing radiation induces mainly chromosome-type aberrations.

53. Most chemical mutagens are S-dependent clastogens and therefore produce mainly chromatid-type aberrations. S-dependent compounds have no direct effect on the chromosomes of peripheral lymphocytes *in vivo*, because they replicate only after stimulation in cell culture. Peripheral lymphocytes can, however, carry unrepaired/misrepaired, long-lived lesions that may lead to aberrations during replication of DNA *in vitro* [S28].

54. The classical chromosome aberration assay for measuring dicentrics is a reasonably good measure of dose down to 100 mGy whole-body exposure [L31] or, with much effort, even lower. However, it is based on a genetic change that considerably impairs the survival of indicator cells and their stem cells, so that the signal fades with time. Reciprocal translocations are considered less disruptive to the proliferative future of affected cells. It is possible to score translocations with G-banding or FISH (fluorescent *in situ* hybridization) techniques, with the latter technique having a higher detection limit, about 500 mGy. In such systems, the preferential loss of affected cells may still be a minor problem; in addition, clonal expansion of cells carrying translocations conferring a growth advantage may lead to an overestimation of the dose with time. Biological dosimetry using cytogenetic parameters will be discussed later. It seems that all agents that apparently induce single-base changes (i.e. base deletions, transversions, or transitions) also induce gross chromosomal changes that are visible under the microscope. However, the number of agents clearly shown to induce cytogenetic changes in humans is still relatively limited [A24, S31]. From known or suspected carcinogenic agents, mixtures, or complex exposures to humans, cytogenetic data are available for 27 compounds in Group 1 of the IARC classification (known carcinogens to humans), for 10 compounds in Group 2A (probable carcinogens to humans), and 15 compounds in Group 2B (possible carcinogens to humans) [I1, I2]. Chromosome damage in humans was found in 19/27, 6/10, and 5/15 cases in these groups, respectively.

55. Most of the informative data on induced chromosomal aberrations in humans arise from high-exposure occupational situations. The comparisons of experimental animal data and human data for the endpoint of chromosomal aberration are generally in good agreement. However, in a few cases there are discrepancies between animal and human data. High occupational exposure to radon induces chromosomal aberrations in humans. Animal experiments with comparable exposures are negative. The most likely explanation is a confounding by other clastogenic exposures in humans, e.g. smoking.

56. Unlike radiation exposure, chemical exposures have been considered in very few cytogenetic follow-up studies. Studies on the induction of chromosomal aberrations after exposure to alkylating agents expressed in peripheral lymphocytes show, like studies after radiation exposure, that damage can be conserved over several months or even years after treatment [G3]. The persistence of chromosome damage, however, varies with the type of exposure and the cytogenetic endpoint examined.

(b) Sister chromatid exchange

57. The induction of sister chromatid exchange can be observed in cells that have undergone two rounds of DNA replication in the presence of bromodeoxyuridine (BrUdR), which results in chromosomes having sister chromatids that are chemically different from one another: one is unifilarly labeled with BrUdR and the other bifilarly labeled. Such sister chromatids stain differently from one another, and any exchanges that occur between the sister chromatids can be clearly seen and counted [W7]. A number of studies confirmed the ability of low-LET radiation to induce sister chromatid exchanges in rodent cells [G4, L22, R5, U14] and human lymphocytes [G14]. However, in other studies, when normal human lymphocytes in G_0 were assessed for their ability to express sister chromatid exchanges following low-LET radiation exposure, they failed to do so, in contrast to the quantifiable induction of chromosomal aberrations [L21, M28, P2]. This difference could possibly be attributed to the presence of BrUdR, a known radiosensitizer, at the time of irradiation in the rodent cell studies [L25]. Nevertheless, low-LET ionizing radiation and radiomimetic chemicals are not very effective at inducing sister chromatid exchanges, contrary to S-dependent agents such as UV light [W11], alkylating agents [T1, Y4], and cross-linking agents [S4]. High-LET radiation (neutrons and alpha particles), however, induces sister chromatid exchanges in normal human peripheral lymphocytes exposed in G_0. This suggests that the relative biological effectiveness for sister chromatid exchange induction is very large, since there is little low-LET response [A2, S11]. The induction of sister chromatid exchange as a function of charged-particle LET in Chinese hamster cells was recently described [G7]. At each LET examined there was a dose-dependent increase in the frequency of sister chromatid exchanges. In contrast to the majority of biological endpoints, however, where relative biological effectiveness increases as LET increases up to a maximum and then declines, it was found that sister chromatid exchange

induction already declined as LET changed from 10 to 120 keV mm^{-1} [G7]. These observations can be explained on the basis of repair differences for DNA damage induced by radiations of different LET, i.e. the faster the repair, the less likelihood there will be of unrepaired DNA damage at the time of replication when sister chromatid exchanges are formed.

(c) Micronuclei induction

58. Micronuclei can be formed from entire chromosomes or chromosome fragments [M36]. They result from chromosome breakage and/or damage to the mitotic spindle and are used as a measure of genotoxicity [H15]. Techniques to block cytokinesis in mitogen-stimulated lymphocytes [F4, F5, M24, P19] allow these micronuclei to be observed in binucleated cells found after the abortive attempt of the cell to divide. There is, however, a large and variable background frequency of some 5–12 micronuclei per 10^3 binucleated cells [F5, Y2]. The background frequency increases with age from about 4 per 10^3 among those $\geq$20 years, to 8 per 10^3 for those $\geq$30 years, and nearly 12 per 10^3 for those $\geq$40 years [Y2]. The increase is about 4% per year [F5]. The range of variability increases with age as well. Farooqi and Kesavan [F18] also found that the yield of radiation-induced micronuclei in mouse polychromatic erythrocytes was strongly influenced by small conditioning doses (25 mGy). Micronuclei assays are faster and have a greater potential for automation than the scoring of chromosome aberrations [M36].

59. Caffeinated and alcoholic beverages have no significant effects on *in vivo* mean micronuclei frequency in binucleated lymphocytes. Even the intraperitoneal (ip) injection of large amounts of caffeine (15 mg kg^{-1} body weight) did not induce chromosomal aberrations in mice [F19]. However, the estimated number of diagnostic x-ray examinations to an individual in the year prior to measurement was significantly correlated to micronuclei frequency [Y2, Y5]. The effect of age and x rays on lymphocyte micronuclei has been shown repeatedly [A11, E2, F5, I1, I2]. Tobacco smoke and tobacco-related exposures are listed in the IARC Monograph series [I1, I2] as micronuclei-inducing agents.

60. In an analysis of micronuclei frequency in survivors of the atomic bombings, Ban et al. [B4] confirmed the age dependency of background micronuclei levels in peripheral lymphocytes. Females showed a somewhat higher frequency of binucleated cells. Age and sex were independently acting factors. There is no evidence for an effect of radiation dose on present-day background micronuclei frequency in the survivors.

5. Summary

61. The primary molecular and cellular effects of the many agents potentially involved in combined effects are extremely diverse. No unifying concept of dose can therefore be applied. However, comparisons of toxicity may be based on relevant experimental and clinical endpoints with sometimes only loose and enigmatic links to primary lesions and interactions. A large number of quantitative and semi-quantitative indicators of exposure are presently available. On the level of genotoxicity, DNA damage can be measured up to the functional level of single genes, thus allowing a comparison of the biological activity of different agents and an assessment of possible interactions on a directly relevant level. The accessibility of critical cells and tissues to standard analysis remains a problem. Qualitative and quantitative monitoring of biological effects at the different levels of organization, from molecules to organisms, not only might allow an assessment of the exposure to the different agents involved but could also form the basis for a better understanding of the mechanisms of combined effects and for the elucidation of dose-effect functions for cellular and clinical endpoints.

II. MECHANISTIC CONSIDERATIONS

62. In view of the many different agents that may be involved in combined exposures with radiation and the complexity of the possible interactions, it is necessary to gain some insight from the mechanistic point of view. This Chapter will give a qualitative insight into the interaction processes by describing important steps in the development of the radiation effect and by suggesting how the radiation effect might be influenced by other agents. For a quantitative insight, various models have been developed to describe the biological effects. Examples of such models will be briefly discussed, in so far as they serve to improve understanding of the mechanisms involved in combined effects. However, it should be kept in mind that models have limited applicability, and agents do not always have only a single mode of interaction.

63. Since cancer is the most important health effect for radiation at low doses, the review presented in this Chapter deals mainly with mechanisms that are central to the emergence of malignant growth. An in-depth review of the scientific background of some of the concepts discussed here was presented in Annex E, "Mechanisms of radiation oncogenesis", of the UNSCEAR 1993 Report [U3], Annex F, "*DNA repair and mutagenesis*" and Annex G, "*Biological effects at low radiation doses*".

64. The timescale of events for the various stages of radiation-induced cancer ranges from less than a second to tens of years. Schematically, three crude time-scale-based phases can be defined on the molecular, the cellular, and the tissue/organ level. The molecular phase ranges from the early interaction of the radiation track until initial damage in biologically important molecules has occurred (of the order of seconds). The cellular phase follows and lasts until the biological reactions of the cells involved have occurred and biological cellular effects are induced (of the order of a few

days). Ultimately, on the tissue/organ level, cellular damage may progress in due time, with or without cooperation from other damage, to clinically detectable cancer, which can occur up to 40 or more years after the initial irradiation. These phases are described below. A schematic representation of the processes is given in Figure I. The separation into these phases is arbitrary; it is time-scale-motivated and serves here only to describe the possible interactions of the radiation effect with other agents. In reality, the processes are not separated that rigorously, and interactions with another agent may occur on more than one level or phase.

65. Radiation-induced effects other than cancer, such as deterministic and teratogenic effects, involve similar phases in the development of the radiation damage. For conciseness, these effects are not explicitly mentioned and considered here, but the data in humans are reviewed briefly in Chapter V.

A. EFFECTS ON THE MOLECULAR LEVEL

66. Following the primary interaction of a radiation track with biological matter, an avalanche of events occurs, and various reactive species are left after passage of an ionizing particle or photon: molecules are excited and ionized, radicals are formed, and secondary electrons progress through the material. Most of these species are chemically very reactive and produce other molecular species. These initial processes develop in a very short time (of the order of microseconds) and at short distances from the radiation track. The processes are dependent on the physical and chemical characteristics of the material, the type of radiation, and the conditions in the immediate environment of the target molecule, such as the availability of oxygen, the presence of sensitizing or protecting agents, the ambient temperature, and the ionization density of the radiation. The processes involved in the interaction of radiation at the molecular level are extensively studied in radiation biochemistry and microdosimetry, the concepts of which have been described by the International Commission on Radiation Units and Measurements (ICRU) [I7].

67. The biological effects of radiation arise mainly from damage induced in DNA molecules. Important types of DNA damage are DNA single- and double-strand breaks, base damage, intra- and intermolecular cross-links, and multiply damaged sites (mds) (see Annex F, "*DNA repair and mutagenesis*"). A review of special models with emphasis on the importance of the DNA damage is given by Goodhead et al. [G17]. As far as epigenetic damage or modifications of other cell constituents are concerned, cytoplasmic changes and mitochondrial or membrane damage may also play a role in certain types of radiation effects, but the importance of these for radiation-induced cancer is disputed. Indirect effect modifiers such as growth stimulation as a result of stem cell killing may become important at higher doses.

68. The possibility of another agent interacting with the radiation effect in this early phase is dependent on changes in the DNA environment. The direct environment of the DNA defines the fate of radiation-induced reactive species, such as water radicals, and the possibility for direct or indirect damage to the DNA. Interaction leads to changes in the dose-effect relationship for DNA damage and consequently to changes in the dose-effect relationship for cellular effects (see Section II.B). A well known modification of the radiation effect is caused by a change in the oxygen content. Anoxic cells, in general, are more resistant to radiation than well oxygenated cells. Typical agents interacting with the radiation effect at this level are electrophilic compounds, such as N_2O, NO_2, NO, CO_2, SO_2, and SO_3, and nucleophilic agents, such as cysteamine and cysteine [G17, O11]. For interaction with the radiation effect, the agents should, in general, be present in the DNA environment during irradiation. They may modify radiation effects by a factor of up to 3. More indirect effects may result from vasodilators and constrictors modulating oxygen pressure in irradiated tissue.

69. An important class of agents are hypoxic cell radiosensitizers, also called oxygen-mimetic agents, which have potential use in radiotherapy to enhance the effectiveness of the radiation treatment in anoxic or poorly oxygenated parts of the tumour. These sensitizers must be present at the instant of irradiation. The mechanisms are free-radical-based: the compounds, in general, have increased electron affinity and are believed to involve fast electron transfer processes in DNA [A1]. Well-known agents include nitroheterocyclic compounds, such as metronidazole, misonidazole, and related compounds, metal-based compounds containing Pt, Rh, Fe, Co, and other metals, and nitro-compounds, such as nitrosoureas [S2].

70. Other chemicals protect healthy cells against the radiation effect. They may also be used in radiotherapy. These radioprotectors are mainly sulphur-containing compounds. They act, in part, as radical scavengers and have to be present at the time of irradiation to produce their protective effect. The radioprotective effect is a factor of 3 or less. Typical compounds of this type are cysteine, cysteamine, aminoethylisothiourea (AET), mercaptoethylamine (MEA), and other sulfhydryl-group-containing agents [M4].

B. EFFECTS ON THE CELLULAR LEVEL

71. When the radiation has induced molecular damage, the cell reacts by attempting to remove the damage and restore normal cellular function. The reaction depends on the type of damage. For simplicity, only damage to the DNA is considered here, which may be characterized as single-strand or double-strand damage. Single-strand damage, such as breaks or base damage, may be readily and effectively repaired. Complex localized damage, such as a double-strand break, is more difficult to repair and may lead to a biologically different behaviour of the cell. Repair depends on the cell's genotype. It takes place within a few hours after the irradiation. Some of the damage may be persistent and lead to a radiation effect at the cellular level. The most important cellular effects are chromosomal aberrations, mutations and cell inactivation, killing, and apoptosis. Changes leading to

malignant transformation, which can be considered a specific class of somatic mutations or chromosomal aberrations, are particularly important for radiation carcinogenesis.

72. Attempts to characterize the initial biological effect of a radiation exposure and its dose-effect relationship have led to the development of mechanistic biophysical models of radiation action. The aim of these models is to present a mathematical description of radiation action based on realistic assumptions related to basic mechanisms [G12]. Broadly, a common characteristic of these models is that they describe the cellular radiation effect E(D) by a linear quadratic dose-effect relationship:

$$E(D) = E_0 + \alpha D + \beta D^2 \quad (1)$$

where E(D) is the cellular effect from a dose D, E_0 is the effect without radiation (D = 0), α is the contribution to the effect per unit dose and β is the contribution to the effect per unit dose squared.

73. The interpretation of the linear and quadratic dose terms depends on the underlying assumptions of the model. The linear term has a single-track nature, sometimes called intratrack damage. The quadratic term has a dual or multitrack nature, involving the accumulation of sublethal damage or sublesions [C16, K5, K8]. Some models do not account for repair; in other models, repair is considered essential for development of the radiation effect. Most models do not specify the initial type of damage [C16, K4], while others are more specific [C45]. In general, double-strand breaks in DNA play an essential role in the radiation effect.

74. Equation (1) broadly describes the dose-effect relationships for exposures within one cell cycle and is generally used to analyse cellular experimental data, such as chromosomal aberrations, mutations, cellular transformation, and cell killing [I14]. The dose coefficients α and β depend on the effect considered, the cell type, the type of radiation, and the development of the radiation damage during the molecular phase [L11]. For instance, α is particularly dependent on the type of radiation and, in general, is larger for densely ionizing radiation than for sparsely ionizing radiation. The coefficient β, in general, tends to decrease with higher-LET radiation. As far as irradiation time is concerned, α hardly changes and is mostly invariable, but β changes markedly: it reaches a maximum for acute irradiation, decreases for lower dose rates, and for irradiation times of more than a few hours is negligible or zero. This implies that for chronic irradiation a linear dose-effect relationship for cellular effects is anticipated.

75. The mechanism of interaction of another agent with the radiation effect at the cellular level is broadly based on three types of action: (a) the accumulation of sublesions and lesions; (b) interference with cellular repair; and (c) changes in cell-cycle kinetics. All types of interaction are most effective when the potentially interacting agent is present in the cell at the time of irradiation or within a few hours later, roughly as long as the radiation effect is not fixed and repair is still possible.

1. Accumulation of (sub)lesions

76. An important category of combined exposures involving accumulation of sublesions is that of combined exposures to different types of ionizing radiation. For cellular effects such as cell killing, mutations, and chromosomal aberrations, it is well known that the combined exposure to two types of radiation can lead to a larger than additive effect. Understanding how cellular damage produced by densely ionizing radiation (high-LET radiation) interacts with that produced by low-LET radiation is important both in radiation therapy and in evaluating risk.

77. With similarity in the underlying radiation mechanism, interaction between different types of ionizing radiation can be shown to be, in general, of the so-called isoadditive type. Modellers of cellular radiation effects tend to describe the larger effect of combined radiation exposures in terms of accumulation of and interaction between sublethal damaged sites, which may lead to an extra contribution to the radiation effect (increase of the quadratic term of the linear-quadratic dose-effect relationship) [B35, C15, L10, Z14].

78. In general, if the (additional) radiation effect E_i of radiation type i is linear-quadratic with dose D_i,

$$E_i(D_i) = \alpha_i D_i + \beta_i D_i^2 \quad (2)$$

then the combined exposure to radiation types 1 and 2 will lead to effect E_c, given by

$$E_c(D_1, D_2) = \alpha_1 D_1 + \alpha_2 D_2 + (\sqrt{\beta_1}\, D_1 + \sqrt{\beta_2}\, D_2)^2 \quad (3)$$

In the absence of interaction, the effect would be given by

$$E_a(D_1, D_2) = \alpha_1 D_1 + \alpha_2 D_2 + \beta_1 D_1^2 + \beta_2 D_2^2 \quad (4)$$

The extra effect is expressed in the difference between equations (3) and (4) and can be calculated to be

$$E_c(D_1, D_2) - E_a(D_1, D_2) = 2\sqrt{\beta_1 \beta_2}\, D_1 D_2 \quad (5)$$

Equation (5) indicates that the extra effect is dependent on β_1 and β_2. Experimental evidence [B5, C6] shows that β is practically independent of radiation type (i.e. low- or high-LET radiation), so that interaction of sublethal damage can be expected. Using this assumption, the radiation effect of combined exposures of acute high- and low-LET radiation could well be described by the equations given here [L10].

79. Considering this interaction process, one has to keep in mind the following restrictions:

(a) sublethal damage can be repaired by the cell, so that when there is time between the two exposures, the extra effect will decrease;

(b) the quadratic term for each radiation type separately is dependent on dose rate, i.e. irradiation time, which implies that the extra term for combined exposures

also vanishes for dose rates below a certain value (i.e. less than 10 mGy min^{-1});

(c) the interaction process described here is, strictly speaking, proven only for exposures occurring within one cell cycle. Deviations may be expected when exposures occur over more than one cell cycle; and

(d) for practical applications in risk analysis, deviations from additivity are generally not very large, with the most significant deviations being expected for acute irradiation exposures such as are used in radiation therapy; additivity is virtually expected for combined chronic exposures.

80. As Lam [L47] has shown, the interaction of two types of radiation can also be described using the linear isobolic relationship, which is usually used for the combined action of two toxic agents. The reverse also applies: the interaction with radiation of a toxic chemical that has a supralinear or quadratic exposure-effect relationship for cellular effects can be similarly described as the interaction of two types of radiation. As described above, if the radiation effect after a dose D is given by equation (1) and the effect after an exposure X of a second agent is given by

$$E_s(X) = \sigma X + \varepsilon X^2 \qquad (6)$$

then the effect of a combined exposure will be

$$E_c(D, X) = \alpha D + \beta D^2 + \sigma X + \varepsilon X^2 + \eta DX \qquad (7)$$

This means that the effect of the combined exposure to radiation and the second agent is given by the sum of the effects of the two agents separately and an extra effect (ηDX), which is proportional to the dose D of radiation and exposure X of the second agent. This extra term is the result of the interaction of sublethal damage of radiation with sublethal damage of the second agent.

81. This description of the effect of combined exposures can be used for a number of compounds with radiation [L51]. In this analysis it is assumed that the cellular effect of physical and chemical agents can be described as a linear-quadratic function of exposure X. Examples of such agents are ultraviolet radiation (UV) [L52]; alkylating agents such as the nitrosouric compounds ethylnitrosourea (ENU), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) [L48]; benzo[a]pyrene (BP); ethylmethane sulphonate (EMS) [C7] and many more. The conditions mentioned in paragraph 84 concerning the interaction of two types of radiation should be kept in mind for this interaction of radiation with another physical or chemical agent as well. Repair of the sublesions from the first agent before the other agent becomes effective can lessen the enhancement effect of the combined exposure and lead to an effect more nearly like additivity. In general, the analysis can be applied to different cellular endpoints, such as cell killing [L48, L50, L51], chromosomal aberrations, and mutations [C7].

2. Cellular repair

82. The speed and fidelity of DNA repair is one of the main determinants of the yield of fixed damage. Most molecular damage to DNA is subject to a sequential series of enzymatic reactions that constitutes the repair process. This topic has been the subject of much recent study, and a spectrum of analytical procedures, operative at both the molecular and cellular level, has been developed to monitor DNA repair [F10]. DNA damage may include altered bases, the covalent binding of bulky adducts, intrastrand or interstrand cross-links and the generation of strand breaks. Altered bases may be generated by spontaneous reactions, most importantly deamination of cytosine to form uracil, of adenine to form hypoxanthine, and of 5-methylcytosine to form thymine. A range of alkylated products is formed in DNA as a consequence of exposure to nitroso compounds and other alkylating agents. Bulky adducts are formed as a consequence of the covalent binding, to purines in particular, of polycyclic hydrocarbons, aromatic amines, aflatoxins, and similar substances. Two types of pyrimidine dimer are induced by exposure to UV radiation: cyclobutyl pyrimidine dimers are most common, and the so-called 6–4 photoproducts are also produced. Cross-linking of DNA strands may occur following exposure to bifunctional alkylating agents and chemicals such as cis-diaminedichloroplatinum. Strand breakage may be caused by ionizing radiation, heavy metals, chemicals such as bleomycin, and endogenously generated active oxygen species (reviewed in [S9]).

83. Efficient repair of DNA damage is necessary to retain genomic stability and to prevent somatic and genetic disease in humans and other organisms as well. There are several modes of repair, and these may also be affected themselves by mutagenic agents. Failure of repair may thus be as much a cause of disease as the initial DNA damage. To safeguard the genome, cells are able to block cell-cycle progression in response to DNA damage at specific transition points to allow DNA repair. Most prominent are the so-called checkpoint control mechanisms at the G_1/S phase and G_2/M phase transition. The subject of DNA repair is reviewed in Annex F, "*DNA repair and mutagenesis*".

84. Programmed cell death, known as apoptosis, obviates the risks from error-prone repair in heavily damaged cells and is, accordingly, another important defence mechanism of the cell, preventing the survival of aberrant cells and, hence, tumour development [D13]. Apoptosis can become activated under physiological conditions and also after damage to DNA [H44, T17]. *p53* plays an important role in DNA damage-induced apoptosis [L32], so the clonal selection of cells with non-functional *p53* by hypoxia [G19] or by UV radiation [Z5] is potentially an important mechanism to increase tumour yield. This was also shown for radiation teratogenesis in mice. Norimura et al. [N18] found that p53-mediated apoptosis strongly reduced fetal malformations after *in utero* exposure to ionizing radiation (2 Gy), whereas $p53^{-/-}$ strains displayed a 70% incidence of anomalies. Such effects may lead to an apparent threshold in the dose-effect relationship for malformations after *in utero* irradiation [N19]. Several other

types of cell loss or irreversible growth arrest occur in mammalian systems in addition to apoptosis; these include terminal differentiation, senescence, and necrosis. Necrosis, in contrast to apoptosis, is not an orderly cellular process but rather the disorganized death of a cell. Several recent reviews of this topic have been published [S5, T17, W6]. Apoptosis is discussed further in Annex F, "*DNA repair and mutagenesis*".

85. A second class of agents that can interact with radiation and cause changes in the radiation effect at the cellular level are agents that modify the repair capacity of cells. Repair inhibitors often influence the DNA structure and may be immunosuppressive [S1]. These agents might have toxic effects themselves. Examples are the intercalating agents actinomycin D, adriamycin, and quinacrine. The xanthine derivatives (caffeine, theobromine, and theophylline) also belong to this type of agent. The different effects reported for these agents may be due to the different kinetics of repair in the cell cycle and the presence of the drugs during different phases of the cell cycle [B3, T16]. Depending on the drugs, the repair of sublethal damage or of potentially lethal damage might be involved in the interaction process.

3. Cytokinetics

86. Another important class of agents are chemicals that change the behaviour of the cells in the cell cycle. These agents are indirectly related to those that interfere with repair, because cells that are irradiated tend to move more slowly through the cell cycle in order to have more time for repair. Some cytokinetic agents inhibit changes in the cell cycle. Caffeine is an agent known to remove or alter the cell's capacity to induce a G_2 block or shorten the S phase after irradiation [S1]. The result is that caffeine enhances radiation-induced cell killing and chromosomal aberrations. Effects of cytokinetic agents are investigated for different purposes, among which is to study the mechanism of radiation-induced cellular response and to answer questions such as, in which phase of the cell cycle is the radiation damage fixed? These effects are also investigated for their possible application in radiotherapy. Cytokinetic agents are not normally considered important for environmental risks of stochastic radiation effects. However, some chemicals, for example those with hormonal side effects such as environmental estrogens, have been shown to be effective even at environmental concentrations [S81].

4. Toxicological analysis

87. The cellular effects of combined exposures to radiation and other agents are part of the broad, classical field of toxicology, in which the effects of exposures to two agents are analysed using the isobolic method. The method is primarily useful for agents with isoadditive effects, but it is used for other agents as well. It has been applied to radiation effects [L47, S1].

88. The use of an isobolic diagram to describe the combined effect of two agents is shown in Figure III. The

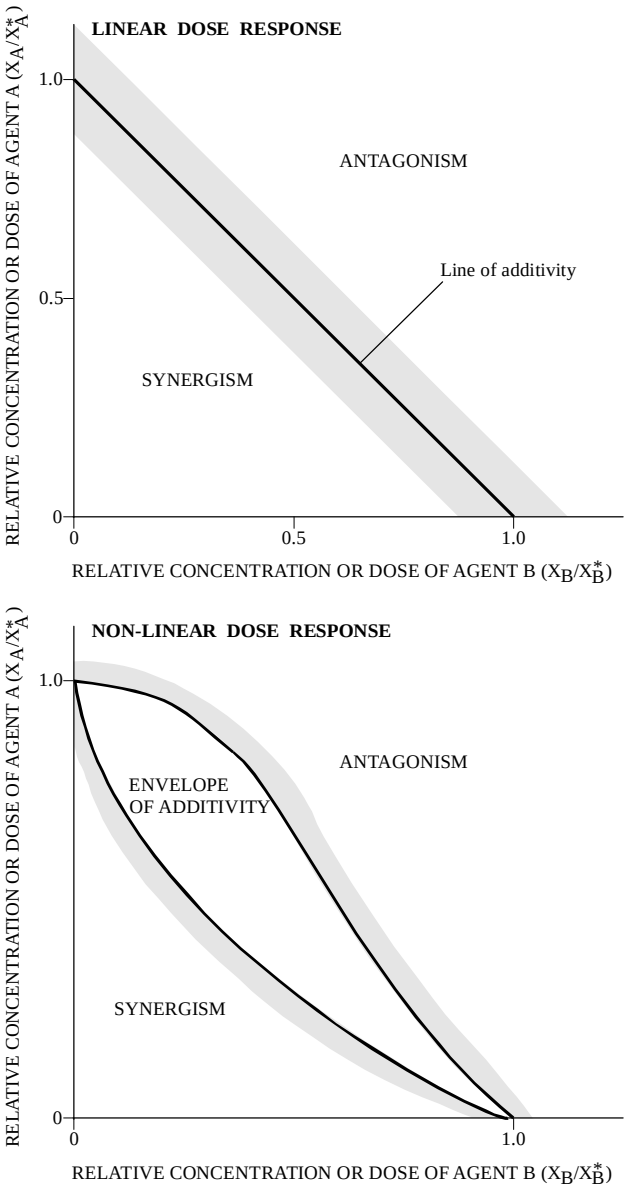

Figure III. Isobolic diagrams for a given level of response in two agents, both acting with linear (upper diagram) and non-linear (lower diagram) exposure response [U6].
The axes are normalized to values of 1.0 for each agent acting separately, i.e. X^*_A and X^*_B.

exposures are indicated on the two ordinates, usually with the single-agent exposures yielding the same effect normalized to one. The case of additivity is described by a straight isoeffect line for any combination of two agents with linear dose-response relationships for separate action (Figure III, upper diagram). If the points deviate significantly to the left of the isobolic line, the interaction is synergistic. An antagonistic interaction is postulated when the experimental points lie to the right of the isobolic line. Even in such a simple theoretical case, to assess the combined action of two agents, several combinations of the two agents leading to the given effect, E_{AB}, have to be tested. Although there are some important biological endpoints, such as frequency of point mutations, that show a linear or nearly linear increase after separate

exposures to genotoxic chemicals or radiation, the dose-effect relationships for health impairments caused by complex multi-stage changes in biological systems are often better described by exponential or sigmoid functions of dose. For these more realistic circumstances, the line of additivity in the isobolic diagram becomes curved and transforms into an envelope of additivity (Figure III, lower diagram). In general, the order of exposure to agents with differing dose-response relationships then becomes important as well [R4].

89. Such isobolic analyses are important tools, for example in optimizing combination therapy [L16, R4], but are of less value in evaluating the effects of chronic exposures in the workplace and in non-occupational settings [B69]. An extended review of this approach and its mathematical background was presented in the UNSCEAR 1982 Report [U6]. This approach is based on producing equal effects with different combinations of the two agents under restricted conditions of time. Owing to the general lack of such ranges of exposures in human populations, this method is not applicable in epidemiology.

90. The interaction at the cellular level is restricted in time, so that damage from one agent seldom interacts with damage from a second one. For low dose rates of radiation and long-term exposures of other agents, the supralinear or quadratic dose terms of the dose-effect relationships tend to diminish, and only a linear dose-effect relationship remains. In these cases, the possible interaction in combined exposure also decreases, and additivity results. This implies that since interaction at the cellular level during low-dose, long-term exposures to radiation and other agents can be expected to have a low probability of occurrence, it is therefore of limited importance for carcinogenesis.

C. EFFECTS ON THE TISSUE/ORGAN LEVEL

91. After fixation of the radiation effect at the cellular level, which occurs within a few days, a much longer time is needed before an effect at the organ level occurs, i.e. before a stochastic radiation effect is evident. The period of occurrence of a stochastic effect is dependent on the type of effect. For example, hereditary defects may occur when a germ cell, after having been irradiated, forms the origin of an organism of the next generation. For radiation-induced cancer, it is the time between the initiation event, or possibly one of the following steps of the carcinogenesis process, and the detection of a tumour. Full consideration of the mechanistic aspects of cancer development is given in Annex G, "*Biological effects at low radiation doses*". The events occurring after the initiation event in the development of tumorigenesis are considered to take place on the tissue and organ level and may occur years or decades later.

92. It is generally accepted that carcinogenesis is a multi-step process. The usual chain of events is considered to be initiation of damage, tumour promotion, possibly with activated proto-oncogenes or deactivated tumour-suppressor genes, and malignant progression. Each of these processes can be related to effects at the cellular level. The basic aspects of these processes were reviewed in Annex E, "*Mechanisms of radiation oncogenesis*", of the UNSCEAR 1993 Report [U3]. The concepts of multi-stage carcinogenesis have evolved over many years of cancer research [A17, B10, B14, B15, C8, F7, M27, R6]. Several lines of evidence that support the multi-stage model of cancer derive from studies of pathology, epidemiology, chemical and radiation carcinogenesis in animals, cell biology, molecular biology, and human genetics [K28, M5]. Germ-line mutations, somatic genetic events, and epigenetic stimulation by the host organism may all play important roles in neoplastic development. The definition of two broad classes of genes, proto-oncogenes with growth-enhancing functions and tumour- suppressor genes with growth-inhibiting functions, brought a biological basis and a unifying concept to the multi-stage theory of cancer [V2]. Owing to the functional diversity of the products of these genes involving cell surface receptors, protein kinases, phosphatases, and DNA-binding proteins, to mention only a few, this concept does not lend itself directly to a better understanding quantitatively. However, in this area the modifications of the cancer process after exposure to external agents may be investigated.

93. The number of genetic changes involved in the evolution of a specific malignant neoplasm is not known with certainty. In some cancers that occur early in life, soon after exposure, or in genetically susceptible individuals, there may be only one rate-limiting change needed for malignant disease. Certain forms of leukaemia, e.g. those resulting from reciprocal translocations [B64] or cancer induction in retinoblastoma heterozygotes, seem to follow this course. Multi-hit models developed on the basis of specific incidence rates of solid cancers from epidemiological data often show an exponential increase in the incidence of specific cancers with the fifth to seventh power of age [K11]. Most colorectal cancers have three or more altered genes, [F6, V1, V2], and estimates of as many as 10 or more mutational changes have been proposed to occur in adult human cancers [B17]. Basically, all these genetic changes might be induced by ionizing radiation, other genotoxic agents, and the inherent instability of DNA alone.

94. The distinction between proto-oncogenes and tumour-suppressor genes has important repercussions for dose-effect models, because the former class would generally express its function dominantly, whereas the latter could fulfill its protective function as long as one allele is functionally intact, i.e. the tumour-suppressor function would be a recessive trait. However, the probability of developing cancer is in many cases higher in heterozygotes than a pure recessive trait would predict, indicating the importance of penetrance in the genetics of the different tumour-suppressor genes. Moreover, mutations in some tumour-suppressor genes like *p53* and WT1 may be of the dominant negative type, in which the mutated protein overrides the action of the suppressor wild-type allele [H4, M25].

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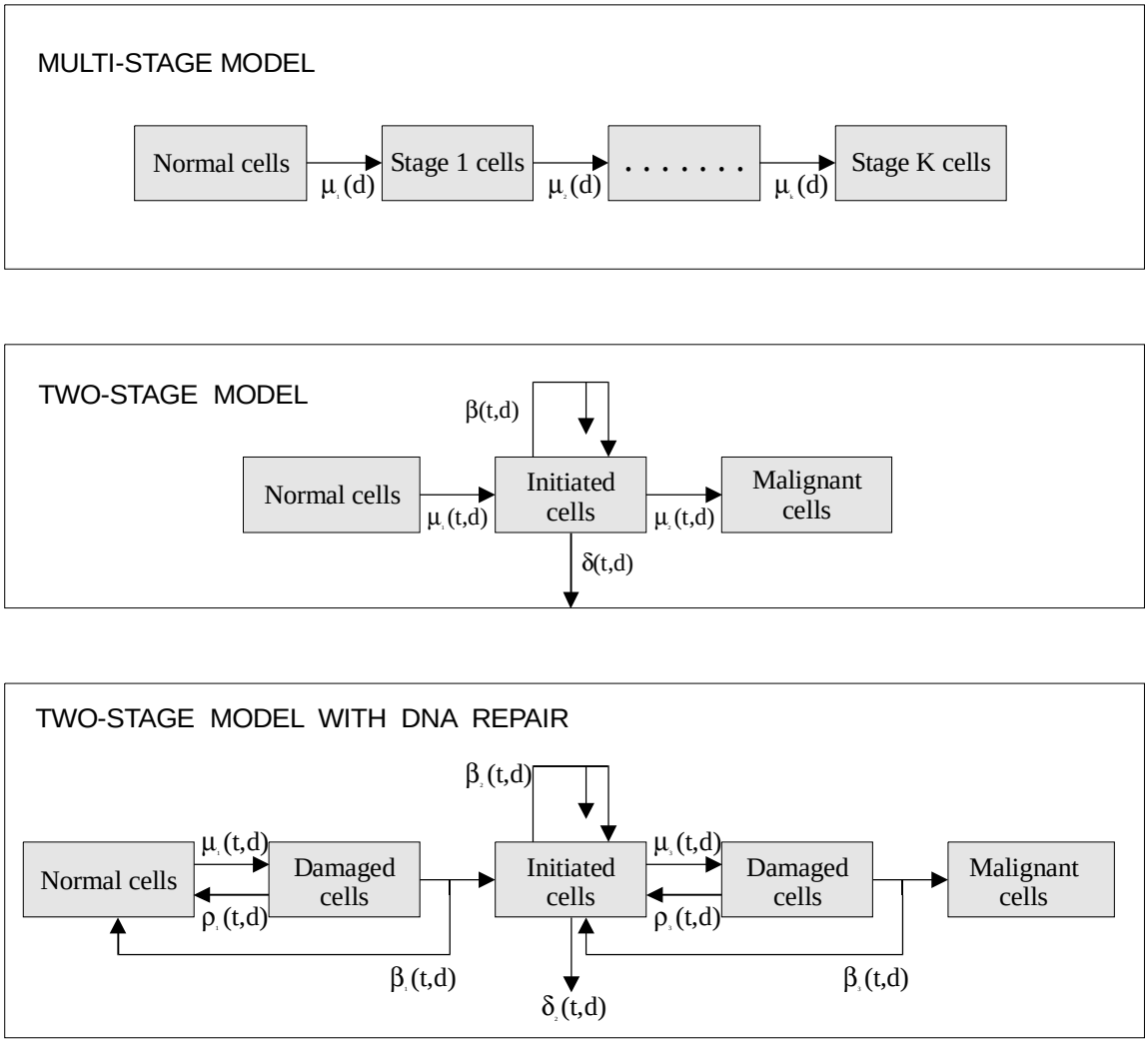

Figure IV. Models of carcinogenesis.
Parameters: μ is the probability of transformation, β_i is the birth or replication rate, δ_i is the death rate, ρ_i is the repair rate, i is the stage, d the dose, and t the time.

95. Multi-stage cancer models are described in Annex G, "*Biological effects at low radiation doses*". The multi-stage model proposed by Armitage and Doll [A16, A17, A18] represents one of the first attempts to develop a biological model of carcinogenesis. They postulated that cancer develops from a single cell that must pass sequentially through a particular series of transformations to become a malignant cell. The multi-stage Armitage-Doll model is illustrated in the upper portion of Figure IV. This model assumes that a normal cell must pass through k sequential stages before becoming fully malignant. This model has k + 1 types of cells: normal cells, stage 1 cells, stage 2 cells, ..., and stage k (malignant) cells. The model supposes that at age t an individual has a population $N_0(t)$ of completely normal cells and that these cells acquire a first mutation at a rate $\lambda_1(t)$. The cells with one mutation acquire a second mutation at a rate $\lambda_2(t)$, and so on until at the (k − 1) stage the cells with (k − 1) mutations proceed at a rate $\lambda_k(t)$ to become fully malignant.

96. The instantaneous tumour incidence rate h(t) at time t in the multi-stage model is therefore approximately of the form

$$dN_k(t)/dt = \lambda_k(t) \int_0^t \int_0^{x_{k-1}} \cdots \int_0^{x_2} N_0(x_1)\lambda_1(x_1) \ldots \lambda_{k-1}(x_{k-1})\, dx_1 \ldots dx_{k-1} \quad (8)$$

where N is the number of cells in the target tissue and $\lambda_i(t)$ is the instantaneous rate of the i-th cellular change (i = 1, ..., k). For simplicity, it is often assumed that the transition rates are linearly related to the dose $d_i(t)$ of the carcinogen at time t for the i-th stage. Therefore the transition rate from one stage to the next is given by $\lambda_i(t) = a_i + b_i d_i(t)$. Here, a_i denotes the transition rate in the absence of exposure and b_i reflects the effects of the carcinogen on the transition rate into stage i = 1, ..., k. With similar values for spontaneous transition rates (a_i to a_k), this model predicts that the age-specific tumour incidence rate will be proportional to the (k − 1)st power of time and provides a good description of human cancer incidence data with $2 < k < 6$ stages [A20, A22].

97. To encompass the growing biological evidence that the process of carcinogenesis involves intermediate cells having

a growth advantage over normal cells, Armitage and Doll [A17] modified their initial model. The initial model is generally viewed as not biologically plausible, because it does not account for cell kinetics, more specifically the birth and death of cells. The modified model that includes cell kinetics must sometimes assume very small and, in the opinion of Armitage and Doll [A17], unlikely values for the growth rate of intermediate cells to fit the data.

98. Moolgavkar and Venzon [M29] and Moolgavkar and Knudson [M23] proposed a two-stage birth-death-mutation model to describe the process of carcinogenesis in adults. By incorporating both cell kinetics and tissue growth, this model can be used to describe a broader class of tumour incidence data than the classical multi-stage model. This model has three cell types: normal cells, intermediate or initiated cells, and malignant cells. The middle portion of Figure IV displays the general two-stage model of carcinogenesis in which for a normal cell to become malignant, it must pass from the normal state through the intermediate state and into the malignant state. The simplicity of this model allows classifying external agents in three categories of carcinogen: initiators, which stimulate the first transition of a normal stem cell into the intermediate stage; completers, which transform an intermediate cell into a malignant cell by the second transition; and promoters, which enhance cell division and the net increase of intermediate cells with time [K46]. Ionizing radiation and other genotoxic agents may be both initiators and completers.

99. The Moolgavkar-Venzon-Knudson model was later extended to account for more than two mutational stages [L23, L24, M14]. For the generalized Moolgavkar-Venzon-Knudson model it may be supposed that at age t there are N(t) susceptible stem cells, each subject to mutation to a type of cell carrying an irreversible mutation at a rate of $\mu_0(t)$. The cells with one mutation divide into two such cells at a rate $\gamma_1(t)$. At a rate $\delta_1(t)$ they die or differentiate. Each cell with one mutation can also divide into an equivalent daughter cell and another cell with a second irreversible mutation at a rate $\mu_1(t)$. For the cells with two mutations there are also assumed to be competing processes of cell growth, death and differentiation, and mutation taking place at rates $\gamma_2(t)$, $\delta_2(t)$, and $\mu_2(t)$, respectively. This continues until at the (k-1) stage the cell will have accumulated (k-1) mutations. It will eventually acquire another mutation and become fully malignant.

100. With the advent of more sophisticated experimental techniques and a growing understanding of the process of carcinogenesis, more refined mathematical models have been developed and continue to be developed to embody the current scientific knowledge and mechanisms of cancer. Mutation is the result of DNA damage and the subsequent fixation and propagation of the damage by DNA replication. This process is included as a single rate constant in the models described in the previous paragraphs. However, agents can affect DNA damage rates, cellular replication rates, and/or the processes of DNA repair. Kopp-Schneider and Portier [K19] expanded the modelling of the mutation process to account explicitly for the process of cellular damage to DNA, DNA repair, and DNA replication. The two-stage damage-fixation model has five types of cells: normal cells, damaged normal cells and damaged initiated cells both of which are subject to DNA repair, initiated cells, and malignant cells (in which damage has been fixed by replication). This model is shown in the lower portion of Figure IV.

101. It is clear that research on quantitative multi-stage models is still in progress and that the complexity of the carcinogenic process inhibits a choice of a universally accepted and applicable model. However, it is also clear that multi-stage models have a biological basis and could describe tumour incidence quantitatively and as such have a future in improving radiation risk estimates and estimates of combined effects. Always important is the question of complexity vs. simplicity. The biology of cancer formation is so complicated that an ever-increasing number of parameters are needed to cover all possibilities of tumour formation mathematically. On the other hand the available data are limited, so the number of parameters that can be fixed is limited as well. As far as the mathematics and statistics are concerned, it is preferable that the number of unknown parameters be as low as possible.

102. Most multi-stage models are used to describe the age dependence of tumour incidence and the influence of chemical carcinogens in animal experiments. In a few cases, they have been used to describe radiation-induced tumours. As far as human data are concerned, the induction of lung tumours by radon in miners [L9, M39] and the lifespan studies of the Japanese atomic bomb survivors [H1, K45, L5] were used to test multi-stage models. In general, ionizing radiation acts mainly as an initiator, although it has some influence on other coefficients. An important conclusion of the use of multi-stage models for radiation carcinogenesis is that radiation generally seems to affect only one step in the carcinogenesis process; in other words, it is a co-factor of background tumour incidence. This implies that the radiation effect is dependent on background tumour incidence as well as on other agents or factors that produce tumours or cancer.

103. The timescale for effects at the tissue or organ level is long and can last for years. The implication is that interaction with another agent is possible even when the exposures of the two agents are separated in time for up to several years. A comprehensive treatment of the carcinogenic effect of combined exposures and the implications for dose-effect relationships using a two-mutation carcinogenesis model is given in Krewski et al. [K46]. They classify carcinogenic agents as initiators, completers, and promoters and conclude that the joint effect of two compounds that both affect the same stage in the carcinogenic process will be described well by the additive risk model; however, the effect of combined exposure to two carcinogens that influence different stages will not necessarily result in a multiplicative model. Short exposures that occur close together in time and do not occur at either very young or very old ages can produce a nearly additive relative relationship. Synergism would, however, arise when the contribution to different transitions by different carcinogenic agents is large compared with the spontaneous rate and when the time course of

exposure is penalizing if the sequence of steps matters. Brown and Chu [B9] concluded that the observation of a multiplicative relative risk relationship in studies of joint exposure to two carcinogens is evidence of action at two different stages of the carcinogenesis process. These examples illustrate the importance of a full understanding of the timescale of exposure for both agents.

104. An overview of interactions for simple binary exposures to agents with specific effects is given in Table 3. According to the terminology used by Krewski et al. [K46], many interactions leading to considerable deviations from additivity are possible although hardly predictable. The effectiveness of a carcinogenic agent depends not only on the exposure but also on the time of exposure, age at exposure, time since exposure, and duration of exposure. This time dependence is completely different from and should not be confused with the time involved in the cellular dose-rate effect. It is therefore not possible to quantitatively predict the dose-effect relationship for tumour induction after combined exposure.

105. This assessment is based on the evaluation of carcinogenesis data using a two-mutation model. Deviations of the carcinogenesis process in other ways, e.g. by disturbing organ functions in a crude way, are ignored. The assumption of otherwise undisturbed functioning of the organ or organism is probably reasonable for low exposures but may complicate analysis for high doses and exposures. The long-term development of tumours implies a long period of time over which the process can be influenced. For interaction in the genesis of radiation-induced tumours, it implies that exposure to a different agent at a time that is completely separated from the time of irradiation may influence the radiation effect in often poorly predictable ways.

Table 3
Anticipated interaction response of two single-agent carcinogens [a]
[K29]

Carcinogen A	*Carcinogen B*	*Interaction response*
Initiator	Initiator	Additive
Completer	Completer	Additive
Initiator	Completer	Multiplicative
Initiator	Promoter	Multiplicative to supra-multiplicative
Initiator	Promoter and completer	Multiplicative to supra-multiplicative
Initiator	Initiator and completer	Supra-additive to sub-multiplicative
Initiator	Initiator and promoter	Supra-additive to supra-multiplicative
Promoter	Promoter and completer	Supra-multiplicative

a See glossary for definition of terms.

D. DOSE MODIFIERS AND OTHER INDIRECT INTERACTIONS

106. Apart from the direct interference with the development of the radiation effect, further indirect interaction mechanisms are possible when an agent changes the retention of the radioactive substance following inhalation or ingestion and consequently changes the organ dose. A well-known case is blockage of ^{131}I uptake to the thyroid by stable iodine, which is used in nuclear medicine and envisaged in future radiological emergencies to greatly reduce the dose to the thyroid gland. Other drugs, such as ethylenediaminetetracetic acid (EDTA), are used for therapeutic reasons, to stimulate the metabolic transfer of inhaled or ingested radionuclides and, consequently, to reduce the relevant organ dose. Natural chelators such as citrate may also modulate the biological half-lives of metal and actinide ions. Synergistic effects on this level are known from the inhibition of mucocilliary clearance by a second agent [F28]. Several examples of dose-modifying agents are given in the Appendix, which covers specific interactions. Mechanistic considerations indicate that the irritants and cytotoxicants implicated here generally display an effect threshold and are therefore of little concern for combined effects at low exposure levels.

107. Other modifications of the radiation effect are possible when the physiological condition of the organism is changed, either intentionally or by chance. Examples are changes in hormone levels or in the immunological system. Such changes may also be induced by radiation (e.g. UV radiation). Also, novel mechanisms of genetic change such as radiation- or chemical-induced genetic instability, which leads to new genetic damage many cell generations after exposure (see Annex F, "*DNA repair and mutagenesis*"), may be prone to more-than-additive effects. The results of this type of interaction are dependent on the conditions of the change and are, in general, poorly predictable.

E. SUMMARY

108. Carcinogenesis and, consequently, also the development of radiation-induced tumours is a long-term process. Mechanistically, three levels can be distinguished in the development of the radiation effect on cancer: the molecular, cellular, and tissue/organ levels. On each level, ionizing radiation induces changes and processes, and these may be influenced by combined exposures to other agents. A summary of the levels, processes involved in cancer development, and examples of the many classes of substances and agent with a potential to interfere at different

levels of radiation-induced carcinogenesis is presented in Figure V. The biological effect of combined exposures to radiation and other agents at low doses is, in general, expected to be additive, especially in the case of chronic exposures. Deviations from additivity may primarily be expected from interactions on the tissue/organ level. In this phase, exposure to other agents may take a long time, up to tens of years, and for interaction to occur, the exposures to radiation and the other agent need not be simultaneous. Thus, interaction can last a relatively long time, and the radiation effect can be influenced to a significant extent by the interactions that take place during this phase. Studies using multi-stage models show that classifying the agents involved in terms of their action as initiators, completers, and/or promoters may help to predict the result of their interaction with radiation and other agents. These studies also indicate that the radiation effect depends on the background tumour incidence.

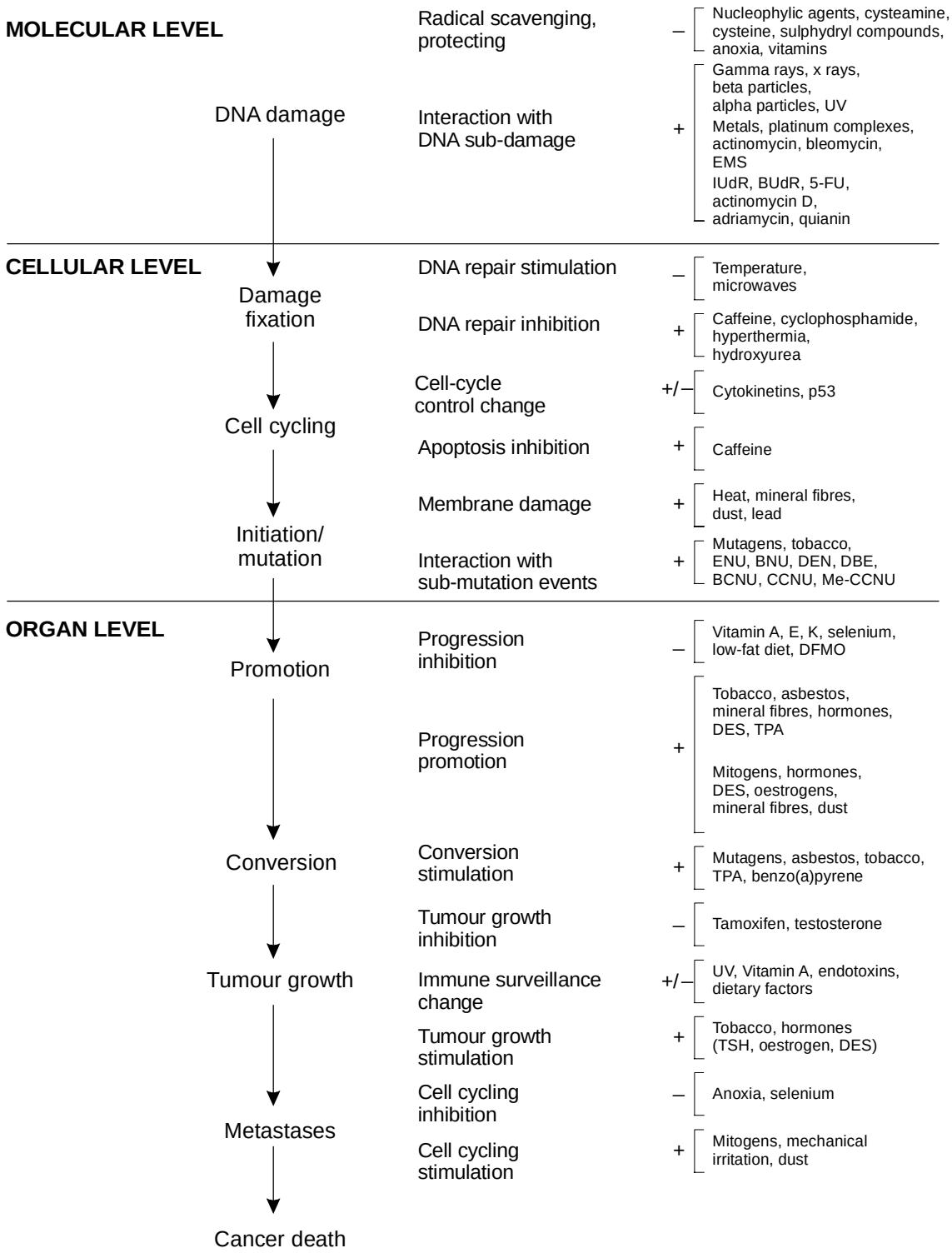

Figure V. Schematic representation of radiation-induced carcinogenesis and possible interaction mechanisms with examples of agents having shown a potential for more (+) or less (–) than additive effects for at least one tumour site.

III. SPECIFIC COMBINED EXPOSURES

109. Recent data on combined exposures to radiation and specific physical, chemical, or biological agents are reviewed in this Chapter. For each type of interaction, data from epidemiological studies of the adverse health effects in humans are generally reviewed first. Studies involving experimental animal models are considered next. Lastly, various effects observed in *in vitro* systems are reviewed. Carcinogenesis is the principal endpoint of interest; non-neoplastic endpoints are viewed mainly in relation to mechanistic considerations.

110. Only a minor fraction of the interacting agents described below are found in the human environment at potentially critical levels. An overview of agents known or suspected to affect human health on their own is given in Table 4. Details of the experimental and epidemiological conditions and results of studies on specified combined exposures may be found in the Appendix and are summarized in Table A.1.

Table 4
Exposure conditions and characteristics of prominent environmental and occupational agents and substances that may produce combined effects with them
[B6]

Agent	*Typical environmental exposure (E)*	*Occupational limit for chronic exposure (O)*	*Major health endpoint*	*Estimated contribution to total incidence/ mortality of endpoint (%)*	*Estimated lifetime risk* [a] *(%)*	*Substance with known or suspected combined effect*
Ionizing radiation	1.5-4 mSv a^{-1}	20 mSv a^{-1}	Cancer	3-8 (E) ~10 (O)	0.4-1 (E) ~4 (O)	Smoking, asbestos, hormones, arsenic?
UV radiation [W31]	Noontime intensity: UV-A: 40 W m^{-2} UV-B: 3 W m^{-2}	350 nm: 150 kJ m^{-2} in 8 h 300 nm: 100 J m^{-2} in 8 h	Skin aging Skin cancer Melanoma	Important (E) >50 (E) ?	- >20 (E) ?	Phototoxicity, allergy with UV sensitizers
Asbestos [I12]		Crocidolite: 0.2 fibers cm^{-3} Other forms: 2 fibres cm^{-3}	Lung cancer Mesothelioma	Low >50%		Smoking
Benzene [M60, W34]	14 µg m^{-3} in indoor air	3.2 mg m^{-3}	Leukaemia	2.5 (E)	0.01 (E)	Substrates for activation/ detoxification systems
Carbon tetra-chloride [W34]	3 µg m^{-3} in indoor air	65 mg m^{-3}	Cancer	0.05 (E)	0.01 (E)	Chloroform
Chloroform [I2]	1.2 µg kg^{-1} d^{-1} from tap water and showering	50 mg m^{-3}	Cancer	0.15 (E)	0.03 (E)	Carbon tetra-chloride
Dioxins/furans [F8]	1.3 µg kg^{-1} d^{-1} dietary intake	50 pg m^{-3}	Cancer	0.1 (E)	0.02 (E)	
Ethylenebisdithio-carbamates (EBDCs) [L49]	n.a.	n.a.	Cancer, adverse reproductive outcomes	0.17 (E)	0.034 (E)	
Polychlorinated biphenyls (PCBs) [G8]	14 ng kg^{-1} d^{-1}	1 mg m^{-3} (42% CI)	Cancer	0.06 (E)	0.01 (E)	

a Assuming 80 and 40 years of exposure for environmental (E) and occupational (O) levels, respectively. Risks are generally upper bound estimates based on linear extrapolations from high exposures.

A. RADIATION AND PHYSICAL AGENTS

1. Combinations of ionizing radiation

111. Many experiments have been undertaken to investigate the cellular effects of combined exposures of two types of ionizing radiations. In view of their potential radiotherapeutic applications, a wealth of data on the combined action of neutrons, heavy ions, and gamma or x rays was accumulated in the 1970s and early 1980s. This information was reviewed in Annex L, "*Biological effects of radiation in combination with other physical, chemical and biological agents*", in the UNSCEAR 1982 Report [U6]. The results generally indicate additive effects of combined exposures characterized by the so-called isoaddition of these agents at the cellular level [L10, L47], as described in Chapter II. A few data [E5, L26] were reported on radiation-induced tumours; these, in general, include exposure to internal emitters. The results indicate additive to slightly supra-additive effects for combined exposures, mainly because of the lower dose rates that are involved in the internal exposure to alpha radiation in these experiments. For estimating the risk of carcinogenesis, the effects of combined exposures to more than one type of ionizing radiation are expected to be isoadditive, i.e. to add up in the same way as effects of increments of the same agents, when at least one of the radiations is delivered at a low dose rate (chronic irradiation), as is generally the case for occupational and environ-mental exposure levels.

2. Ultraviolet radiation

112. Ultraviolet (UV) radiation is recognized as the most important initiator and co-factor for human skin carcinogenesis [S29]. It is mainly the skin that is exposed to UV radiation. A study of combined exposures to gamma radiation and UV radiation was presented by Shore, who analysed 12 studies on the incidence of skin cancer in populations irradiated with known skin doses [S29]. In the absence of a proper control (skin exposed to ionizing radiation but not to UV), it was concluded that, at least for combined exposures, the data are compatible with a linear dose-response relationship for ionizing radiation [S29] but that the interaction is unclear. The question of whether relative risk or absolute risk models are more appropriate remains open. From the mechanistic point of view, interaction at the cellular level may be expected, which results in a more or less additive effect for low exposure rates [L52]. The considerable variations in skin cancer among different populations and subgroups seem to reflect the large differences in UV exposures due to latitude and lifestyle and the differences in genetic predisposition to skin cancer due to skin type. The overwhelming dependence of skin cancer on extended exposures to UV prevents conclusive epidemiological data on the interaction of UV with ionizing radiation. Another important factor to take into account in possible interactions is UV-induced suppression of the immune system [B76, L58, N26]

3. Electromagnetic radiation

113. Neither low- nor high-frequency electromagnetic radiation have enough single photon energy to directly damage DNA and therefore cannot be cancer initiators. However, strong electromagnetic fields may modify and stimulate growth [K16, S33], and this has led to the hypothesis that electromagnetic fields may influence cancer development. However, no straightforward inferences from experimental results to exposure situations in occupational or environmental settings have been found at this stage for the combination of electromagnetic and ionizing radiation [B77, B78, B79, U19]. Moreover, there is at present little indication from a mechanistic standpoint for potentially harmful interactions between electromagnetic fields and ionizing radiation at controlled exposure levels in the workplace or the clinic. The possible modulation of radiation effects by heating produced by strong electromagnetic fields is considered in the next Section.

4. Temperature

114. Heat can kill mammalian cells in a predictable and stochastic way [D3]. Elevated temperature is used as a modifier of radiation sensitivity in many therapies to control tumour growth. In combination with ionizing radiation, heat can act synergistically on cell survival, cell proliferation, and cytogenetic damage by, for example, interfering with DNA repair. However, extremely high temperatures, which are generally not found in the workplace or in environmental conditions, are needed in the cells at risk, so heat is not considered as potentially enhancing radiation risk.

5. Ultrasound

115. Ultrasound has achieved widespread use in medical diagnostic and therapeutic procedures. Studies have shown that at the intensities used for diagnostic purposes, ultrasound does not interact with ionizing radiation to cause cytogenetic damage in treated cells, although the yield of sister chromatid exchanges was observed to be slightly increased in one study [K20]. Because cavitation-induced mechanical damage by ultrasound shows high thresholds, this mechanism is of little concern for environmental exposures. Such damage has to be prevented in other situations already caused by single-agent effects.

6. Dust, asbestos, and other mineral fibres

116. Mineral dust and fibres such as asbestos generally act through non-genotoxic mechanisms such as mechanical irritation and cell killing [B13]. The combination of radiation exposure and exposure to dusts and fibres is quite common in industrial settings and in the environment, and these agents are reported in both animal studies and *in vitro* studies to act synergistically at high exposures [B38, H11]. Silicosis was shown to be a risk factor for human lung cancer in metal miners in the 1940s [H9] and is implicated as a modifier of lung cancer risk in radon-exposed underground workers [K49]. Combined exposure to phosphate ore dust, gamma

radiation, and radon daughter products also resulted in elevated lung cancer risks in earlier practices [B74]. Although exposures and thus risks are considerably lower today, mineral dust and fibres still deserve attention because they may interact with radiation, including densely ionizing radiation such as alpha radiation in mining environments, to enhance the risk of cancer.

7. Space flight

117. A special form of combined exposure is experienced in space flights, where a multitude of stressors act in combination on astronauts. This problem has been investigated using animals [A13, A14, V3]. The most important environmental parameter is microgravity. Space radiation effects were comprehensively reviewed by Kiefer et al. [K54], the interaction of microgravity and radiation at the cellular level [H52, K55]. No synergistic actions were found. A very important aspect is a possible reduction of the immune response [S86], which could have an influence on cancer development. The changes of many parameters that are normally stable in experimental work on earth make well designed studies in space potentially important in addressing combined effects of physical agents.

B. RADIATION AND CHEMICAL AGENTS

118. A multitude of natural and man-made chemicals with cancer initiating and promoting potential are present in the human environment and may interact with radiation. Classification based on their mode of action is often difficult, as many have more than one type of action, but at least a crude separation can be made into substances that mainly act by damaging DNA (genotoxic substances) and those that act in other ways (non-genotoxic substances) [C48]. The former group includes chemically active species, or substances that can be activated, bind to or modify DNA directly, or indirectly via radicals. The non-genotoxic substances range from nonspecific irritants and cytotoxins to natural hormones, growth factors, and their analogues. They interact with the regulatory systems of cells and organs and cannot always be considered toxic by themselves. Some are clearly protective, e.g. they scavenge reactive species before they interact with DNA.

1. Genotoxic chemicals

119. Numerous examples of combined exposures to radiation and chemical genotoxic agents can be found in the literature, including studies on the improvement of radiation therapy by simultaneous treatment with a chemical (see Chapter IV). In many cases, supra-additive effects are reported, caused by interaction in the cellular phase and by the high exposure levels involved. The agents include 1,2-dimethylhydrazine (DMH) [S27], N-methyl-N-nitrosourea (MNU), butyl-nitrosourea (BNU), N-ethyl-N-nitrosourea (ENU) [H6, K15, S13, S20, S21, S22], diethylnitrosamine (DEN) [M8, P26], N-2-fluorenylacetamide (FAA), 4-nitroquinoline-1-oxide (4NQO) [H39], bleomycin [D6], and 1,2-dibromoethane (DBE) [L13]. The effects are dependent on the species, exposure conditions, time of exposure, etc., and sometimes the same chemical is involved in a supra-additive and a sub-additive result. In general, for short exposures to high concentrations and for low chronic concentrations, deviations from additivity are small, if at all existent. In most epidemiological and experimental studies, effects exceeding a level predicted from isoaddition have not specifically been demonstrated.

2. Non-genotoxic chemicals

120. Many chemicals in the human environment or their metabolites do not specifically attack DNA but influence cell proliferation and cell differentiation on an epigenetic level. These include the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) [H3, L24], carbon tetrachloride, and α-difluoromethylornithine (DFMO) [O12]. These agents act in combination with radiation on the cellular and tissue level of cancer development and can significantly enhance the induction of tumours. Specific mitogens may interfere with regulatory mechanisms and cell-cell signalling, but many substances with a high chemical reactivity act as non-specific irritants or toxicants via membranes or proteins. For example, toxin-induced cell death will induce proliferation in neighbouring cells, which may enhance the progression of premalignant cells. Substances acting in a non-specific manner, for example lipophilic solvents, quite often show highly non-linear dose-response relationships with apparent thresholds. Other agents may interfere with critical cellular processes involved in repairing damage to cellular constituents such as DNA. The assessment of possible synergistic effects at the exposure levels relevant for risk estimation remains very difficult because of the high exposures used in experimental systems and the apparent threshold levels. One important group of chemicals, which includes cysteamine and mexamine, has radioprotective effects; these chemicals scavenge radicals formed by ionizing radiation [M4]. A considerable number of agents may have both genotoxic and epigenetic functionalities such as the base analogue 5-bromo-2'-deoxyuridine (BrUdR) [A15].

3. Tobacco

121. Given the large collective dose from radon and its decay products in non-occupational and occupational settings and the prevalence of active smoking, the combined effect of these two exposures on human health deserves special attention [B27]. A large body of epidemiological evidence from uranium miner studies allows, at least for higher radiation doses, calculating risks and interaction coefficients directly from human data [C46]. However, the fact that tobacco smoke is itself a complex mixture of genotoxic and non-genotoxic substances and even contains some natural radionuclides (the long-lived radon progeny ^{210}Po and ^{210}Pb) makes a mechanistic assessment difficult.

122. Because of the complex composition of tobacco smoke, the issues surrounding combined exposures to radiation and

tobacco smoke are even more difficult to elaborate than for binary combinations. Some 4,000 individual chemical components of cigarette smoke have been identified, and there are probably a number of additional important but unidentified components, for example, extremely reactive, short-lived compounds or those present in very low concentrations [G1]. The complexity of tobacco smoke means that the action in combined exposures with radiation can take place in both the cellular and organ phase of cancer development. Tobacco smoke contains only relatively small amounts of DNA-reactive carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons, and pyrolysis products such as carbolines. Hence enhancing and promoting factors, e.g. catechols, other phenols and terpenes, are important. Discontinuation of smoking progressively reduces the relative risk of cancer development as time since withdrawal increases, probably because of reduced pressure from the action of promoters [W1].

123. In the last few years, joint analyses of original data sets [C1, L18] and meta-analyses of published results [T14] have yielded a detailed assessment of risk patterns and have allowed investigators to test risk models. The most comprehensive and complete analysis of radon-induced health risks was published by Lubin et al. [L18]. The review contains a joint analysis of original data from 11 studies of male underground miners. Data on smoking were available for 6 of the 11 cohorts, but the assessments were limited by incomplete data on lifetime tobacco consumption patterns and the sometimes exotic forms of tobacco use, such as water pipes in the Chinese study [L18]. Single studies for which smoking data could be analysed were generally not informative enough to allow choosing between an additive or a multiplicative joint relationship between radon progeny and smoking. The Chinese cohort seemed to suggest an association more consistent with additivity, while the Colorado cohort suggested a relationship more consistent with a multiplicative interaction. For all studies taken together, the combined influence of smoking and radon progeny exposures on lung cancer was clearly more than purely additive but less than multiplicative and compatible with isoadditivity [B69]. The most recent analyses of the BEIR VI Committee [C46], which were based on an update of these data, suggested synergism between the two agents that is statistically most consistent with a slightly sub-multiplicative interaction. A best estimate from miner data indicates that the lung cancer risk for smokers expressed in absolute terms is higher by a factor of at least 3. To further characterize the association, more detailed data on tobacco use would be needed. Age of starting to smoke, amount and duration of smoking, and type of tobacco were recognized as important determinants of risk. A further handicap of present studies is that the sub-cohorts of lifetime non-smokers exposed only to radon are very small. The statistical power of the conclusions on the radon-tobacco smoke interaction is correspondingly low. Data are available from a study by Finch et al. [F28] of smoke exposure and alpha-particle lung irradiation over the lifespan of exposed rats. The pulmonary retention of inhaled ^{239}Pu was higher, increasing with the concentration of the ^{239}Pu, in smoke-exposed rats than in sham-smoke-exposed rats. This effect on retention resulted in increased alpha-radiation doses to the lung. Assuming an approximately linear dose-response relationship between radiation dose and lung neoplasm incidence, approximate increases of 20% and 80% in tumour incidence over controls would be expected in rats exposed to $^{239}PuO_2$ + low-level cigarette smoke and $^{239}PuO_2$ + high-level cigarette smoke, respectively.

124. Hypotheses on the mechanistic interaction between tobacco smoke and radon were tested by applying the two-mutation clonal expansion model of carcinogenesis of Moolgavkar to data from the Colorado plateau miners [M39]. No interaction between radon and tobacco smoke in any of the three steps (the two mutation steps and clonal expansion) is needed to fit the data, which are clearly supra-additive for radon and smoking combined. The model, however, shows a significant dependence on age at exposure. Quantitatively similar results were obtained by Leenhouts and Chadwick [L9, L57]. A highly significant decrease in excess relative risk with time since exposure is found in miner studies in contrast to findings on lung cancer in survivors of the atomic bombings. This may be explained by microdosimetric considerations. In the case of high-LET alpha radiation from radon progeny, the minimal local dose from one single alpha track averaged over a cell nucleus is already in the range of several hundred milligray, whereas one electron track yields a dose to the nucleus of only 1–3 mGy. This means that even at the lowest possible nuclear dose from alpha exposure, stem cells that are hit carry a multitude of DNA lesions, which may considerably impair long-term cell survival and maintenance of proliferative capacity [B25, B27].

125. Smoking is also of great importance for non-occupational radon exposures in the indoor environment. Until now, little quantitative evidence has come from indoor radon studies. Most of the case-control studies published are inconclusive [A28, K53, P11]. Only one larger study [P11] was indicative of an indoor radon risk and its modification by tobacco that is comparable to what is predicted from miner studies. It remains doubtful whether the results from the many case-control studies under way will in the near future allow narrowing the uncertainties that surround indoor radon risk and the possible interactions with smoking. Emerging study results from Europe based on much longer residence times may offer better statistical power. Several large indoor case-control studies under way will narrow uncertainties in the next few years. First results from the United Kingdom [D33] and Germany [K53, W35] are indicating a lung cancer risk in the range of ICRP projections. However, confidence intervals are relatively large and still include zero risk in most analyses. Because of the limitation of the indoor radon studies, risk estimates based on miner data remain the main basis for predicting lung cancer from indoor radon exposure. A best linear estimate of the risk coefficients found in the joint analysis of Lubin et al. [L18, L35] for the indoor environment indicates that in the United States, some 10%–12%, or 10,000 cases, of the lung cancer deaths among smokers and 28%–31%, or 5,000 cases, of the lung cancer deaths among never-

smokers are caused by radon progeny. About half of these 15,000 lung cancer deaths traceable to radon would then be the result of overadditivity, i.e. synergistic interactions between radon and tobacco. Based on the same risk model, Steindorf et al. [S47] estimated an attributable risk for indoor radon of 4%–7% for smokers and 14%–22% for non-smokers. Because of the many differences between exposed persons and exposure situations in mines and homes and the additional carcinogens such as arsenic, dust, and diesel exhaust in mine air, these figures should be interpreted with caution.

4. Metals

126. Toxic metals are important trace pollutants in the human environment (Table 5). They interact in many ways with cellular constituents and may produce oxidative DNA damage or influence enzyme activity at low concentrations, e.g. by competing with essential metal ions [H38]. Carcinogenic transition metals are capable of causing promutagenic damage, such as DNA base modifications, DNA-protein cross-links, and strand breaks [K7]. The underlying mechanism seems to involve active oxygen and other radicals arising from metal-catalysed redox reactions. Cadmium, nickel, cobalt, lead, and arsenic may also disturb DNA repair processes [H48]. Only a few data are available from combined exposures of radiation and metals in human populations; no firm evidence of interactions was observed. However, metals and ionizing radiation have been shown to produce combined effects in many other biological systems (see the Appendix). Especially in underground mining, possible effects from the epidemiologically proven lung carcinogens arsenic, cadmium, chromium, nickel, and antimony [M65] have to be assessed together with high-LET radiation from radon. Arsenic in particular is a major risk factor in combined exposures to mineral dust, radon, metals, and diesel fumes [K48, T5]. The risk-enhancing effects of iron dust seem to be limited to very high dust concentrations, leading to changes in lung function [B74]. The significance of these data for radiation risk estimation at low dose levels remains unclear.

Table 5
Metals in the environment and effects on humans
[M38, N14, S48]

Metal	*Release* [a] *(10^9 g a^{-1})*	*Main sources of intake and typical levels in the body*	*Characteristics affecting health*
Arsenic	31 (61)	*Source:* food (seafood up to 120 mg kg^{-1}) and drinking water *Concentration in body:* 0.3 mg kg^{-1}	Mutagenic, teratogenic, co-carcinogenic, As^{3+} causes skin cancer
Cadmium	8.9 (85)	*Source:* inhalation (2 µg $cigarette^{-1}$) and food (0.025 mg kg^{-1})	Mutagenic, teratogenic, co-carcinogenic, causes cancer at multiple sites
Mercury	6.1 (59)	*Source:* metal vapours, food (up to 1 mg kg^{-1} $MeHg^+$ naturally in fish), tooth fillings *Intake:* by inhalation and ingestion 0.2 and 25 µg d^{-1}, respectively	Mutagenic, teratogenic (brain damage), co-carcinogenic, causes sarcomas and renal tumours
Nickel	86 (65)	*Source:* food intake (0.2 mg d^{-1}) *Concentration in body:* 0.007 mg kg^{-1}	Essential element; allergenic, comutagenic, cocarcinogenic, causes nasal sinus cancer
Lead	12 (96)	*Source:* Food, dust, air (0.15 mg d^{-1}) *Amount in body:* steady increase to about 200 mg at age of 60 years	Substitutes for Ca^{2+}, neurobehavioural deficits (decrease in fertility, abortifacient) Low mutagenic and carcinogenic potential (may cause renal adenocarcinoma)
Antimony	5.9 (59)	*Source:* food and tobacco (0.005 mg d^{-1})	Mutagenic as Sb^{3+}, organic antimony compounds used as emetics
Vanadium	114 (75)	*Source:* food (0.01-0.05 mg d^{-1})	Essential element Inhibits Na^+/K^+ ATPase and drug detoxification enzymes at low concentration Mutagenic, teratogenic, carcinogenic
Zinc	177 (66)	*Source:* food intake (10-50 mg d^{-1})	Essential element with small window of tolerance Clastogenic (causes chromosome aberrations) Causes growth of some tumours at elevated concentrations

a Global values; percentage of anthropogenic contribution is given in parentheses.

5. Mitogens and cytotoxicants

127. Although many mitogenic and cytotoxic compounds could have been considered above with genotoxic or non-genotoxic agents, they should be mentioned separately because of their potential to interact with radiation,

principally by virtue of their ability to stimulate cell proliferation. From a mechanistic standpoint, they can be expected to interact in the organ phase of radiation carcinogenesis, but the resulting interaction (sub- or supra-additivity) is not always predictable. Examples of such agents include N-methylformamide (NMF) [L15], caffeine [M11], theobromine, theophylline [Z4], 2-aminopurine, and tributyl phosphate. Many studies assessing deviations from additivity in combined exposures of mitogens/cytotoxicants and ionizing radiation are found in the literature (see Appendix), but the high exposure levels applied and the biological endpoint studied generally do not allow directly transferring the results to carcinogenesis in humans. However, any endogenous or dietary levels of agents influencing stem-cell population size or kinetics will have the potential to modulate response to radiation.

6. Antioxidants, vitamins, and other dietary factors

128. Diet can modify the effectiveness of chemical carcinogens, sometimes by a large factor, and interactions with radiation are found as well [B24, C26, H11, W29]. All classes of substances described in the five preceding Sections III.B.1–5 are found in human food supplies. Actions ranging from subadditive to supra-additive may occur, depending on the specific agent. The radiation risk may be reduced when growth stimuli are reduced as a result of nutritional deficiency or when repair possibilities are optimized. Synergism can be expected where lower levels of radical scavengers or the coenzymes needed for repair increase the yield of effective damage from ionizing radiation or impair the speed and accuracy of cellular recovery from damage. Some of the underlying mechanisms of specific agents have been identified in animal experiments. Tumour-incidence-enhancing effects have been noticed with elevated consumption of, for example, riboflavin, ethanol, and marihuana. Tumour-incidence-reducing effects are found for low-caloric diets, vitamins A, C, K, and E, retinoic acid derivatives (but enhancing effects of artificial beta carotin in some smoker cohorts), selenium, and 3-aminobenzamide. Very important in view of population health are behavioural changes and a tendency to malnutrition in alcohol addicts, which may increase the susceptibility to toxicants in the environment or at the workplace [U18]. In general, the combined action is not specific for radiation but is also found for other carcinogens, and the interaction is dependent on the dosage.

129. In summary, dietary factors are proven modifiers of risk from diverse agents at levels found in human populations and probably also influence the production and repair of endogenously arising lesions. Absence or deficiency of important coenzymes and nutrients on the one side and high levels of directly or indirectly acting mitogens on the other interfere with molecular, cellular, and tissue responses to ionizing radiation. A modulation in the radiation risk may occur in situations where growth stimuli are reduced or increased, owing to nutritional deficiency or surplus or where the number of stem cells at risk is changed. Synergisms are also to be expected where reduced levels of radical scavengers or coenzymes needed for repair increase the yield of primary damage from ionizing radiation or impair the speed and accuracy of cellular responses to damage. In general, these mechanisms apply to most deleterious agents in the human environment.

C. RADIATION AND BIOLOGICAL AGENTS

130. Many hormones are potent growth stimulators, and there is considerable evidence that they may modify cancer risk. They include thyroid-stimulating hormone (TSH), oestradiol-17 beta (E_2), prolactin, diethylstilbestrol (DES), and androgens in general. Their effect is dependent on tissue, type of hormone, and dosage and is important enough to be kept in mind when analysing radiation risks. Tamoxifen, a synthetic anti-oestrogen, has both cancer risk-enhancing functions (endometrium) and protective properties (breast), depending on the organ [J9]. An important consequence of interaction with hormones is the sex difference in tumour sensitivity, mainly of organs of the reproductive system.

131. Viruses, bacteria and microbial genetic sequences have been shown to play an important role in the development of tumours. Cancer viruses may interact with radiation by mutation or translocation of dormant viral sequences. Experiments so far give no clear indication of any interaction with radiation that influences cancer development. Viruses may induce genotypic and functional changes, i.e. they may act as highly site-specific genotoxic agents in multi-step mechanisms. Highly synergistic effects due to increased sensitivity may arise for some endpoints. Little information is available at present on the mechanism of the induction of gastric cancer by bacteria (Helicobacter pylori).

132. The interaction of several miscellaneous factors with radiation exposure and its role in carcinogenesis has been investigated. Some of these factors are reviewed in the Appendix. The role of others, such as psychosocial factors, remains unclear and is outside the scope of this Annex.

D. SUMMARY

133. Combinations of different types of ionizing radiation show mainly isoadditive effects. For decreasing doses and chronic exposure, the quadratic terms of the dose-effect relationships tend to vanish and the linear terms to prevail, indicating additivity for low-level exposures. Also, for the combination of UV radiation and ionizing radiation, additive effects are expected for low exposure levels. Temperature and ultrasound are not considered to significantly modify radiation risk. The temperature range and the ultrasound intensities necessary for an interaction with radiation are too high to be of relevance for environmental or occupational settings. Mineral dust and fibres, including asbestos, tend to show supra-additive interaction with radiation at high exposure levels. These levels were reached in workplaces in the 1950s and earlier. Today the occupational exposures are lower, but these agents still deserve attention for their potential to enhance risks after combined exposure.

134. At high exposures, a wealth of supra-additive effects between genotoxic chemicals (e.g. alkylating agents) and radiation were recorded. For low-level exposures, there is no mechanistic evidence of combined effects at the cellular level greater than those predicted from isoadditivity. Nor are these agents expected to show a more-than-additive effect at the organ level. However, non-genotoxic agents with mitogenic, cytotoxic, or hormonal activity may interact with radiation in an additive to highly supra-additive manner. High exposures clearly have a considerable potential for enhancing radiation risk during the organ phase of radiation-induced cancer. Since most of these substances show highly non-linear dose-effect relationships with sometimes considerable thresholds, the combined effects with radiation at low concentrations could be expected not to deviate much from additivity, i.e. to be additive to slightly supra-additive. Special attention has to be given to the combined effects of radiation and tobacco smoke. Tobacco smoke itself is a complex mixture of different genotoxic and non-genotoxic chemicals. Combined exposures to radiation and tobacco smoke show clearly supra-additive effects. Heavy metals and arsenic may generate free radicals or disturb DNA repair mechanisms and therefore may also cause more-than-additive effects. Many human cancers show considerable dependence on lifestyle, nutrition, and other dietary factors. Tumour- incidence-enhancing effects have been reported for riboflavin, ethanol, and high fat diets and incidence-reducing effects for low fat diets and some vitamins. In general, these combination effects have been found not just for radiation but also for other carcinogens.

IV. COMBINED EXPOSURES IN CANCER THERAPY

135. Many modern cancer treatment regimens combine surgery, radiotherapy, chemotherapy, and/or immunotherapy. Generally, combining the different treatments does not mean that the different therapeutic agents interact in a mechanistic manner. Central to the discussion of cancer therapy is, therefore, the distinction between non-interactive and interactive combinations, with the latter being of interest in this Annex. From the rapidly emerging understanding of the action and interaction mechanisms of different agents in combined modality therapy, information relevant to possible interaction mechanisms in environmental and occupational exposure situations may be obtained.

136. Central to cancer therapy is the relationship between the desired and undesired effects of the therapies chosen. This relationship is defined as the therapeutic index ratio or gain [G21, H41]. The gap between the sigmoid curves of tumour cure (tumour control probability) and dose-limiting toxicity to normal tissue (normal tissue complication probability) is the therapeutic index (Figure VI). The goal of cancer therapy is to increase the therapeutic index by separating the two curves. The therapeutic index is increased when the tumour control probability curve is displaced to the left of the normal tissue complication probability curve. This can be achieved in radiotherapy by altering the exposure schedule. Important techniques are hyperfractionation, accelerated fractionation, split-course techniques, interstitial irradiation, manipulation of target volumes, shrinking field techniques and others. Another approach to increasing the therapeutic index is to combine radiotherapy with chemotherapy. Drug-ionizing radiation interaction in therapy is useful only when it leads to a further separation of the curves, not just to their displacement [K43].

137. It should, however, be clearly noted here that the final goal of tumour therapy is tumour control and therefore cell death (apoptosis, necrosis) or blockage of cellular growth (loss of proliferative capacity, differentiation, senescence). These effects are mostly deterministic and often mechanistically different from the stochastic radiation effects that are of concern in radiation protection. Emphasis is therefore placed on the mechanisms of interaction between the drugs and radiation that reveal possible mechanisms of interaction between chemical agents and radiation under environmental and normal occupational settings. Clinical results will be mentioned only if mechanistic information with relevance for low dose effects can be provided.

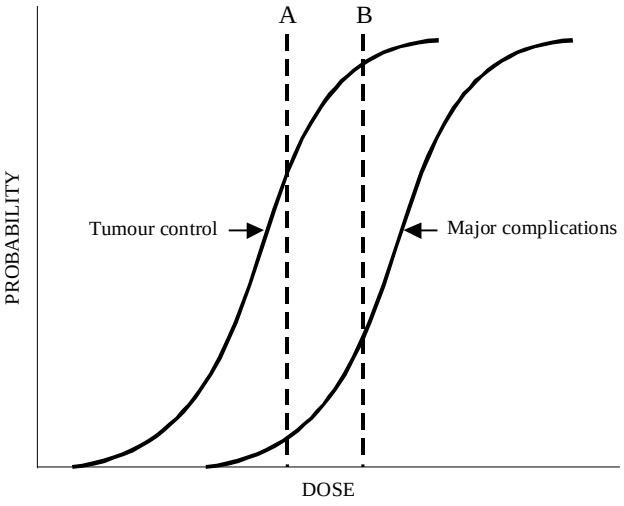

Figure VI. Sigmoid curves of tumour control and complications [H41].
A: Dose for tumour control with minimum complications.
B: Maximum tumour dose with significant complications.

A. MECHANISMS OF INTERACTIONS

138. Publications on the mechanisms of interaction between radiation and drugs are numerous but often lack precise and quantitative information. Factors on which the interaction of these two treatment modalities depends include the type of tumour and normal tissue involved, the endpoints studied, the drug and its dose level, the radiation dose, dose rate, and

fractionation, and the intervals between and sequencing of the combined treatments. Chemotherapy could slow the process of cell repopulation after radiotherapy or it could synchronize the cell cycle. Moreover, tumour reduction by chemotherapy could improve tumour oxygenation, thus increasing the effect of radiotherapy. At the cellular level, inhibition of repair of sublethal and potentially lethal radiation damage by anticancer drugs is probably the most important mechanism of radiosensitization. Exploitable mechanisms in combined chemotherapy and radiotherapy treatment can be described under four headings, as was originally done by Steel and Peckham [S1, S23, S46]: spatial cooperation, independent cytotoxicity, protection of normal tissues, and enhancement of tumour response.

139. Spatial cooperation describes a non-interactive combination of radiotherapy, chemotherapy, surgery, and other therapeutic strategies that act at different anatomical sites. The commonest situation is where surgery and/or radiation is used to treat the primary tumour and chemotherapy is added as an adjuvant to attack remaining local tumour cells and distant metastases. There is an analogous situation in the treatment of leukaemia, where chemotherapy is the mainline treatment and radiotherapy is added to deal with the disease in anatomical sites, e.g. the brain, protected from chemotherapeutic attack by vascular constraints or by blood barriers. Spatial cooperation still appears to be one of the main clinical benefits of combination modality treatment. This mechanism does not require interactive processes between drugs and radiation.

140. Independent cytotoxicity describes another form of non-interactive combination of therapeutic modalities. If two modalities can both be given at full dose, then even in the absence of interactive processes the tumour response should be greater than that achieved with either modality alone. The cost of this improvement on tumour response is that the patient has to tolerate a wider range of toxic reactions in normal tissues (within and outside of the radiation field). As with spatial cooperation, the mechanism of independent cytotoxicity does not require interactive processes between drugs and radiation. Independent cytotoxicity can even tolerate a subadditive interaction of the modalities and still produce an increase in therapeutic gain. The relative extent of reduction in toxicity to normal tissue within the radiation field is the critical parameter of this mechanism.

141. The protection of normal tissues requires an antagonistic interaction of the combined modalities. Since two toxic agents usually tend to produce more damage than either agent alone, it would seem rather unlikely that chemotherapy in conjunction with radiation could reduce the damage to dose-limiting normal tissue. However, there are well-documented situations in which certain cytotoxic drugs increase the resistance of normal tissue to radiation or to a second cytotoxic treatment.

142. Studies of this seemingly contradictory mechanism have concentrated on the bone marrow and the intestinal epithelium. It has been shown by Millar et al. [M51, M52] that in the bone marrow, the most effective cytotoxic agent, cytarabine, does not modify stem-cell radiosensitivity; instead, it stimulates enhanced repopulation by the surviving stem cells. This phenomenon is highly dependent on the timing of the two modalities. Maximal radioprotection is achieved when the drug is given two days before radiation. In the small intestine, microcolony survival was increased when cytosine arabinoside was given 12 hours before irradiation [P24]. Other cytotoxic drugs with radioprotective action are cyclophosphamide, chlorambucil, and methotrexate [M51]. Recently, a topoisomerase II inhibitor (etoposide) was shown to increase the radioresistance of the bone marrow when given one day before whole-body irradiation [Y1].

143. Normal tissue can be protected from radiation effects by radioprotective agents. Increasing the differential between tumour and normal tissue radiosensitivities would give a therapeutic advantage. Radioprotectors can thus be used as selective protectors against radiation damage to normal tissue, allowing higher curative doses of radiation to be delivered to tumours.

144. Chemical radioprotectors target the detoxifying mechanisms of the cell, in particular the antioxidant enzymes that are available for removal or detoxification of the reactive oxygen species and their products formed by the action of ionizing radiation. By far the most widely studied class of radioprotective agents is the thiols, and the most important non-protein thiol present in cells is glutathione. Other classes of agents conferring radioresistance to normal tissue are the eicosanoids, which are biologically active compounds derived from arachidonic acid, the lipoic acids, and calcium antagonists (reviewed in [M51, M56]). The effects of biological response modifiers such as the cytokines IL-1 and TNF_{α} as radioprotectors in normal tissue have been discussed in recent reviews [M53, M54, N12, N13, Z11, Z12].

145. Relative enhancement of tumour response is commonly perceived to be the principal aim of adding chemotherapy to radiotherapy. A wide variety of biological mechanisms have been proposed to explain interactions between radiation and therapeutic agents. In the context of this Annex, this kind of interaction is the most important mechanism with respect to environmental and normal occupational settings.

146. DNA adduct repair regularly involves strand scissions by repair enzymes. Conversion of repairable into lethal DNA damage may occur if a DNA-repair-associated single-strand break combines with a radiation-induced single-strand break to produce new DNA double-strand breaks. This mechanism has been suggested for the interaction of cisplatin and radiation. A similar mechanism, the production of double-strand breaks by combining single-strand breaks, may occur when topoisomerase I or II inhibitors and radiation are combined.

147. Many drugs inhibit the repair of radiation damage. Antitumour antibiotics (e.g. dactinomycin and doxorubicin), antimetabolites (e.g. hydroxyurea, cytarabine, and arabinofuranosyl-adenine), and alkylating agents and platinum analogues (e.g. cisplatin) have been shown to inhibit radiation-

induced DNA damage repair. Repair inhibition has been detected in a number of ways, including removal of the shoulder on the cell survival curve, inhibition of split-dose recovery, and inhibition of delayed plating recovery.

148. Cell-cycle synchronization exploits the fact that many cytotoxic drugs and radiation show some degree of selectivity in cell killing at certain phases of the cell cycle. Antimetabolites show a maximum effect on cells undergoing the S phase. Radiation sensitivity is highest in the G_2/M phase. There is, therefore, an attractive possibility of complementary action between drugs and radiation. The most attractive possibility seem to be the interaction between microtubule and topoisomerase poisons and primary DNA-damaging agents such as radiation.

149. Activation of apoptosis by differential pathways increases cell killing during tumour therapy and is therefore another possibility for combined action of radiation and chemotherapeutic drugs. Ionizing radiation may activate the apoptotic process by a DNA damage-p53 dependent pathway, whereas taxoids like paclitaxel may activate a pathway downstream of p53 by phosphorylation of Bcl-2. There is, therefore, a possibility that radiation-induced cell killing can increase, even in p53-deficient tumours. The involvement of apoptosis in radiation-induced cell killing has recently been studied extensively [B75, D34, H44, H49, M69, O19].

150. Reduction of the hypoxic fraction by bioreductive drugs targeted at hypoxic tumour cells increases tumour radiosensitivity. Most promising here is the development of dual-function drugs specific to hypoxic cells and with intrinsic cytotoxic activity (e.g. alkylating activity).

B. SECONDARY CANCERS FOLLOWING COMBINED MODALITY TREATMENT

151. The successful treatment of cancers involves radiation therapy and/or multi-agent chemotherapy, each of which is used either as primary therapy or as an adjunct to therapy of the primary tumour, and it often includes surgery. With further improvements in modern cancer therapy, the duration of survival and the curability of many patients has increased up to 45%. However, along with this progress has come a recognition of the long-term complications of therapy, such as secondary cancers (reviewed in [T30]). Although other clinical consequences in non-target tissues are known, the main focus in this Section is on secondary cancers after combined modality treatments. Secondary cancers resulting from the combined effect of radiotherapy and tobacco smoke are discussed in the Appendix, Section B.3.

152. No one specific type of secondary cancer is seen after therapeutic irradiation. Secondary cancers can occur after any initial cancer, when survival surpasses the latent period. Radiation-induced leukaemias begin to appear after 3–5 years. Solid cancers typically emerge more than 10 years after treatment but may occur earlier in particularly susceptible individuals [F9, G32, T31, V10]. When the risk of secondary solid cancer is elevated, it rises with increasing radiation dose to the site and with increasing time since treatment and persists as long as 20 years.

153. The predominant secondary cancer associated with chemotherapy is acute non-lymphocytic leukaemia (ANL). Most ANLs have occurred after treatment with alkylating agents or nitrosoureas. The findings are similar for Hodgkin's disease, paediatric cancers, ovarian cancer, multiple myeloma, polycythemia vera, gastrointestinal cancers, small-cell lung cancer, and breast cancer [B22, B36, B63, B65, C14, F9, G32, G33, R22, T31, T32, V10]. The risk for leukaemia rises with increasing cumulative dose of the alkylating agent or nitrosourea. A few ANL cases were reported following combination chemotherapy, including teniposide or etoposide. The leukaemias differ from those that follow alkylating agents in that they occur sooner and that specific chromosomal abnormalities are induced [P9, P29, P30, W32]. Few solid tumours have been linked to chemotherapy. Bladder cancer has been associated with cyclophosphamide treatment, and risk is dependent on the cumulative dose of cyclophosphamide [P4, T33, T36, W33]. Excess bladder cancer risk following treatment with both radiotherapy and cyclophosphamide was as expected from a summation of the individual risks. Bone sarcomas have also followed treatment with alkylating agents [T34]. In general, the risk for solid tumours after chemotherapy alone has been difficult to evaluate because too few patients survived long enough after treatment by chemotherapy alone. At present, several cohort studies are under way to assess this risk.

154. Earlier reports indicated a distinctive pattern of secondary cancers after treatment of childhood malignancies [M1]. The most common secondary cancer was bone sarcoma, followed by soft tissue sarcomas, leukaemias, and cancer of the brain, thyroid, and breast. The cancers showing the highest increases compared with the usual distribution of childhood cancers were retinoblastoma, followed by Hodgkin's disease, soft tissue sarcomas, Wilms' tumour, and brain cancer. This difference may reflect both the genetic predisposition to develop multiple tumours in the case of heritable retinoblastoma, soft tissue sarcoma of Li-Fraumeni syndrome, and possibly the immune dysfunction associated with Hodgkin's disease. To summarize the findings from recent study results [B68, O17, S6, S7], there is little indication that heritable sensitivity to treatment is a significant component of secondary cancer, but intensive multiple agent therapy used in childhood cancer treatment acts as an independent aetiological factor for a second tumour. The risk for a second malignant neoplasm after cancer in childhood is considerable. Absolute risks up to 7% over 15 years following diagnosis of the primary cancer were found for Hodgkin's disease [B68]. This amounts to an excess relative risk (ERR) of about 17, with breast cancer contributing most. A follow-up study in the Nordic countries showed a significant increase in the ERR from a low of 2.6 in patients first diagnosed in the 1940s and 1950s to 5.9 for cohort members included in the late 1970s and 1980s, indicating that newer treatments are not only more successful but also carry a higher long-term risk [O17].

155. In patients with bone sarcomas as the secondary tumour following childhood cancer therapy, the effects of radiation therapy, chemotherapy, and combined modality treatment have been analysed [H43, T34]. The risk for bone sarcoma rose dramatically with increasing doses of radiation with a linear trend. Patients with heritable retinoblastoma had a much higher risk for secondary bone sarcoma, but their response to radiation was similar to that of patients with other childhood cancers. In addition to the radiation dose, the exposure to chemotherapy was evaluated. There was an independent effect of exposure to alkylating agents in the risk for bone cancer, i.e. radiation and alkylating agents acted additively. The risk rose with increasing cumulative dose of the alkylating agents. The effect of alkylating agents was much smaller than that of radiation, and in the presence of radiation at the site of the bone sarcoma, the alkylating agents added little to the risk.

156. Thyroid cancer risk after treatment of childhood cancer is increased 53-fold compared with general population rates [T35]. The risk for thyroid cancer rose with increasing radiation dose. There was no increased risk of thyroid cancer associated with alkylating-agent chemotherapy.

157. There was a sevenfold increased risk of secondary cancers after treatment of acute lymphoblastic leukaemia (ALL) [N22]. Most of this risk was due to a 22-fold increase in brain cancers. The brain cancers occurred in patients diagnosed with ALL before the age of five years and who received cranial or whole-body irradiation.

158. Among 29,552 patients with Hodgkin's lymphoma, 163 cases of secondary leukaemia after treatment of the primary disease indicated a considerable risk [K44]. There was no difference in the relative risk of secondary leukaemias from chemotherapy alone (MOPP regimen) and chemotherapy plus radiotherapy. A relatively small risk for leukaemia was seen after radiation alone, and this risk increased with radiation dose. The risk did not vary significantly or consistently across radiation doses for any given number of chemotherapy cycles but increased consistently with more cycles of chemotherapy in each radiation dose range.

159. Significantly elevated risks for secondary solid tumours (lung, non-Hodgkin's lymphoma, stomach, melanoma, bone, and connective tissue) were reported in patients treated for Hodgkin's disease [T31]. The pattern of secondary tumours was distinctive and was similar to the distribution of cancers seen in immunosuppressed populations, such as renal transplantation patients or patients with non-Hodgkin's lymphoma. All cancers of the stomach, bone, and connective tissue occurred within areas previously treated with radiation therapy. All those who developed lung cancer had received radiation therapy and smoked. For breast cancer, a fourfold elevated risk was reported in Hodgkin's disease patients after 15 years of follow-up. The highest risk was in women irradiated before the age of 30 [H19]. Comparable results were reported from a Dutch study [V11]. These authors reported an overall relative risk of 3.5 for secondary cancers after Hodgkin's disease. Significant increases in relative risk of 34.7, 20.6, 8.8, 4.9, 3.7, 2.4, and 2.0 were reported for leukaemia, non-Hodgkin's lymphoma, soft tissue sarcoma, melanoma, lung cancer, urogenital cancers, and gastrointestinal cancers, respectively. Risk factors for leukaemia were chemotherapy and host factors; for non-Hodgkin's lymphoma they were combined modality treatment rather than single modality treatment or host factors. For lung cancer the risk factors were strongly related to radiation therapy, while an additional role for chemotherapy could not be demonstrated.

160. Significant excesses of ANL followed therapy for non-Hodgkin's disease with either prednimustine, a derivative of nitrogen mustard, or with regimens containing mechlorethamine and procarbazine, for example MOPP therapy, (nitrogen mustard, vincristine, and procarbazine prednisone) [T6]. Chlorambucil and cyclophosphamide were associated with smaller increased risk of ANL. In this study, radiotherapy did not add to the leukaemogenicity of alkylating agents. This finding should be interpreted cautiously, however, because of the small number of patients and the large number of parameters evaluated.

161. Few studies have evaluated the late effects of adjuvant chemotherapy and radiotherapy in breast cancer. The interaction of alkylating agents with radiation in producing leukaemia in women treated for breast cancer was investigated in a cohort of 82,700 patients in the United States [C29]. Based on 74 cases, the risk of ANL was significantly increased after radiotherapy alone (relative risk = 2.4, 7.5 Gy mean dose to the active marrow) and alkylating agents (melphalan and cyclophosphamide) alone (relative risk = 10). Combined therapy resulted in a more-than-additive relative risk of 17.4. The most common solid cancer that occurs after breast cancer is contralateral breast cancer, but fewer than 3% of these tumours could be attributed to radiation [B2]. The risk was highest in women treated at young age (under 45 years). The usefulness of such studies is still hampered by the fact that an important proportion of patients developing primary tumours might already belong to a genetically more sensitive subpopulation [E11]. In addition, combined treatments might be more often used in more advanced stages of tumours needing higher total doses or more cycles for cure.

C. SUMMARY

162. A large number of chemotherapeutic drugs are used in clinical cancer therapy in combination with radiation. The main ones in use or proposed for use are described in the Appendix, with emphasis on the mechanisms of interaction between the drugs and radiation that may have relevance for combined effects between chemical agents and radiation even at the low exposure levels found in controlled environmental and occupational settings. The main findings on modes of action and combined effects are summarized in Table 6.

163. The predominant secondary cancer associated with chemotherapy is ANL and, to a lesser degree, bladder cancer. No one specific type of secondary cancer follows

Table 6
Combined modality treatments in tumour therapy

Class of agent	*Agent(s)*	*Mode of action*	*Critical target(s)*	*Main effects*	*Combined effects with radiation*
Adduct forming agents [a]					
Alkylating agents	Nitrogen mustards (mechlorethamine, melphalan, chlorambucil, cyclophosphamide)	Addition of alkyl group to nucleophilic sites in biomolecules	DNA, proteins	DNA adducts, cross-links	Mainly additive (isoadditive) to borderline supra-additive
Nitrosoureas (Chloroethylnitrosoureas)	BCNU, CCNU, MeCCNU	Bifunctional with two reactive intermediates: isocyanate reacts with amine groups (carbamoylation reaction) and chloroethyl carbonium ion with nucleophilic sites (alkylation)	DNA	DNA adducts, cross-links, glutathione depletion	Additive to borderline Supra-additive for alkylation Supra-additive for glutathione effects
Platinum coordination complexes	Cisplatin [cisdiamino-dichloroplatinum (II)], carboplatin [diaminecyclobutane-dicarboxylatoplatinum (II)]	Bifunctional cross-linker	DNA, RNA, proteins	Cross-links, DNA adducts	Supra-additive, double-strand breaks result from the combination of strand breaks associated with DNA-platinum repair and radiation-induced strand breaks
Antimetabolites [b]					
Antifolates	Methotrexate	Depletion of intracellular nucleotide pools	DNA	Impaired DNA repair and synthesis	Supra-additive
Pyrimidine analogs and precursors	BUdR, IUdR (thymidine analogs)	Replacement of thymidine in DNA	DNA	Impaired DNA repair, synthesis and transcription	Supra-additive
	5-FU, precursor for FUdR	Depletion of nucleotide pools by thymidylate synthase inhibition	DNA, RNA	Depletion of dTTP pool	Supra-additive
	FUdR (uridine analog)	Replacement of uridine in RNA and thymidine in DNA	DNA, RNA	Impaired DNA repair and synthesis, impaired transcription	Supra-additive
Hydroxyurea		Depletion of nucleotide pools by ribo-nucleoside diphosphate reductase inhibition	DNA	Inhibition of DNA synthesis and repair	Supra-additive
Natural products					
Antitumour antibiotics	Anthracyclines (doxorubicin, daunomycin, epirubicin, idarubicin)	Free-radical formation and/or topoisomerase II inhibition Increased tumour oxygenation by inhibition of respiration (doxorubicin)	DNA, mitochondria	Protein-associated DNA breaks	Additive for free radical formation; supra-additive for topoisomerase II inhibition; supra-additive for respiratory chain inhibition

Table 6 (continued)

Class of agent	*Agent(s)*	*Mode of action*	*Critical target(s)*	*Main effects*	*Combined effects with radiation*
Antitumour antibiotics (continued)	Bleomycin	Produces active oxygen species after intercalating with DNA	DNA	Radiomimetic drug	Isoaddition
	Mitomycin C	Bifunctional alkylating activity after reduction of quinone entity; highest activity in reducing environment	DNA	DNA adducts, cross-links	Supra-additive when given before radiation; pronounced cytotoxic effect of the drug against radioresistant hypoxic cells
	Actinomycin D	Intercalates into DNA	DNA	Inhibition of transcription	Additive to supra-additive
Microtubule poisons	Vinca alkaloids (vincristine, vinblastine, desacetyl-vinblastine)	Inhibition of tubulin polymerization, induction of microtubule disassembly	Spindle apparatus	Cells blocked in mitotic phase	Additive to supra-additive when given before radiation
Topoisomerase poisons	Epipodophyllotoxins (etoposide, teniposide)	Inhibition of topoisomeras-II	DNA	Protein-associated DNA breaks	Supra-additive when applied together, repairable radiation-induced DNA damage is transformed into lethal damage
	Camptothecin, topotecan	Inhibition of topoisomeras-I	DNA	Blocking of replication forks (S-phase specific), single-strand breaks	Supra-additive
Oxygen	Hyperbaric oxygen	Increased oxygenation of tumour			Additive to supra-additive
Bioreductive drugs	Quinone alkylating agents (EO9, porfiromycin)	Metabolic reduction in anoxic tumour cells to alkylating agents, mechanism similar to mitomycin C	DNA	DNA adducts, cross-links	Supra-additive when given before radiation, pronounced cytotoxic effect of the drug against radio-resistant hypoxic cells
	Nitroimidazoles (metronidazole, misonidazole, etanidazole, RSU 1096)	Metabolic reduction in anoxic tumour cells to cytotoxic agents; additional alkylating function in RSU 1069	DNA	Oxidative stress under aerobic conditions, DNA-covalent reaction products under hypoxic conditions	Supra-additive
	Benzotrizine di-N-oxides (tirapazamine)	Metabolic reduction in anoxic tumour cells to cytotoxic agents with production of free radicals	DNA	DNA damage by free radicals	Supra-additive

a Effects cell-cycle independent.
b Effects mainly cell-cycle dependent.

radiotherapy. In general, there are independent effects of exposure to alkylating agents and radiotherapy. For secondary solid tumours, radiation is the main risk factor, while a role for chemotherapy has been demonstrated in some cases. For lung cancer, an additional role of smoking was reported. Host factors, for example, age of diagnosis and treatment for breast cancer, are additional risk factors. For secondary leukaemias the main risk factors are chemotherapy and host factors. There is only a small increase in this risk due to radiation. The effect on secondary cancers of increasingly used adjuvant treatments with topoisomerase I or II inhibitors, microtubule poisons (discussed in the Appendix), and hormone treatment is as yet unknown. In summary, secondary, treatment-related cancers are observed increasingly because of the long-term success of the initial treatments. At present, no important synergistic effects between ionizing radiation and other agents are known. Further investigations are needed to assess and to develop strategies to reduce this potential complication.

V. EFFECTS OTHER THAN CANCER

164. Given that there is an overlap in the development of radiation-induced cancer and other biological effects, such as cellular effects, deterministic effects, and teratogenic effects (see Chapter II), results of combined exposures to radiation and other agents for these other effects might give some information on the mechanistic aspects of possible interactions for radiation carcinogenesis. In this Chapter effects other than cancer following combined exposures are reviewed with the aim of concluding whether the agents and low-level radiation interact. It must be kept in mind that most deterministic effects and many aspects of teratogenesis are a result of cytotoxicity and cytolethality having apparent threshold levels in tissue. Qualitatively, the results reported in this Chapter can be considered as interactions occurring at the cellular and tissue/organ level of radiation-induced cancer. In view of the many data available and the aim of this Annex, only effects in humans and mammalian organisms are reviewed.

165. Especially in earlier occupational situations, concomitant exposures to other agents may have caused pathological changes in organs such as the lung, with considerable implications for exposure-dose conversion coefficients and possibly also for target sensitivity towards stochastic effects from ionizing radiation. For example, in the studies of miners, reduced pulmonary function and early onset of silicosis from exposure to dust is also correlated with end points of interest in the context of this Annex [K21, K49, N25]. Although these combined exposures have little relevance at present, they contribute to the uncertainties involved in drawing inferences from historic occupational risks and applying them to modern-day working environments and non-occupational settings.

A. PRE- AND POST-NATAL EFFECTS

166. The effects of x-irradiation and hyperthermia at 43°C both individually and in combination on mouse embryos were investigated by Nakashima et al. [N1]. Cultured eight-day $B6C3F_1$ embryos were exposed to 0.3–2 Gy from x rays, 5–20 minutes of heating, or 5 minutes of heating and irradiation at 0.3, 0.6, and 0.9 Gy. Irradiation alone at 0.3 Gy showed no apparent effect on embryonic development, but irradiation at 0.6–2 Gy caused a dose-dependent increase in malformed embryos. Heating alone for 5 minutes produced no malformed embryos, while heating for 10–20 minutes caused malformations as a function of heating time. Combined treatments produced higher frequencies (22%–100%) of malformations than would have been expected from considering the sum of the separate treatments (0%–42%). The malformations observed were primarily microphthalmia, microcephaly, and open neural tubes. The results indicate that in cultured mouse embryos irradiation combined with a non-teratogenic dose of hyperthermia increases the formation of malformed embryos. The interaction is most probably in the cellular phase of effects development (see Section II.B).

167. The interaction of exposures to heavy metals with radiation was studied during the pre-implantation stage in mice by Müller and Streffer [M3]. At this stage, placental protection against chemical attack is lacking, and low cell numbers limit replacement of damaged cells. Of the metals arsenic, cadmium, lead, and mercury tested in micromolar concentrations, arsenic showed no interaction with radiation [M59] and cadmium and lead showed supra-additivity only for single endpoints: morphological development for cadmium and micronuclei formation for lead. Mercury, however, showed considerable interaction for morphological development and cell proliferation. A classical construction of the envelope of additivity in the range 0–3 Gy from x rays and 0–8 μM of mercury chloride showed synergism, i.e. an interaction effect exceeding isoadditivity. However, there was no effect on micronuclei formation by mercury. Time factors were shown to play an important role in these experiments [M57, M58]. For an enhancement of radiation risk, exposure to mercury (3 μM) had to start immediately after irradiation and to last for an extended time period afterwards (112 hours). The interaction is probably in the cellular phase, but the fact that a 24-hour exposure has little effect speaks against inhibition of repair of radiation-induced DNA damage as the only mechanism of mercury toxicity in this system.

168. The interaction of ionizing radiation with cadmium, which at higher concentrations is teratogenic by itself, was studied by Michel and Balla [M37]. Metal exposure (2 mg kg^{-1}) on day 8 of gestation significantly increased exencephaly and eye anomalies. They found considerable antagonistic effects on survival, growth retardation, and developmental malformations for combined exposures to $CdCl_2$ and x rays (0.5 and 1 Gy) in NMRI mouse embryos. Since the metal exposure had to precede radiation for an

antagonistic effect, induction of maternal metallothionein was proposed as a protective mechanism. However, application of metallothionein shortly before $CdCl_2$ exposure exerted no protective effect. $HgCl_2$ alone induced a low rate of exencephaly, and combined treatment with x rays resulted in additivity of single-exposure effects in the range tested (0.5 and 1 Gy, 2 mg kg^{-1}). No conclusion on possible implications for chronic, low-level exposures and radiation carcinogenesis is possible.

B. GENETIC AND MULTI-GENERATION EFFECTS

169. An understanding of mutations in germ cells and of carcinogenic and teratogenic effects from germ-cell exposure is of great importance for assessing health risks in future generations [N19, N24]. The same holds true for potential combined effects in these exposure situations. The hereditary effects of radiation have been considered in most previous reports of the Committee. Experimental studies in animals and emerging human evidence from epidemiology considered here deal mainly with combined modalities in tumour therapy. The combined effects of cytotoxic substances used in tumour therapy on mouse stem cells and gamma-ray doses of 5 and 9 Gy were studied using the spermatocyte test [D7]. Most of the chemicals tested showed additive effects when combined with doses in the ascending part of the dose-response curve and potentiating effects when combined with doses in the curve's descending part. This has generally been considered additional confirmation that any kind of spermatogonia depletion is sufficient to modify the genetic response of stem cells. The chemicals mitomycin C and N,N',N''-triethylenethiophosphoramide (thiotepa) induced very low yields of translocations after single treatments. In combined treatments with a dose of 5 Gy, mitomycin C was found to have a subadditive effect and thiotepa, an additive effect. Combined with a dose of 9 Gy, the compounds potentiated the effect of radiation.

170. Based on the generally accepted hypothesis that most cancers are multifactorial in origin, perinatal and multi-generation carcinogenesis should be considered in depth. Nevertheless, the consequences of prenatal exposures and of prenatal events are often ignored [T15], partially because it is not possible at present to quantify the role of prenatal exposures to carcinogens/mutagens in determining or modulating the risk of cancer in humans. Tomatis [T15] listed prenatal events important to the occurrence of cancer as the consequence of one of the following:

(a) the direct exposure of embryonal or fetal cells to a carcinogenic agent;
(b) a prezygotic exposure of the germ cells of one or both parents to a carcinogen/mutagen before mating; or
(c) a genetic instability and/or a genetic rearrangement resulting from selective breeding, which may favour a deregulation of cellular growth and differentiation.

Because they involve both germ and somatic cells, studies of prenatal carcinogenesis are sometimes difficult to interpret but are essential for a more accurate estimation of the risks attributable to environmental agents. At the same time they may contribute to an understanding of some of the mechanisms underlying individual variability in the genetic predisposition to cancer. With regard to combined effects, no new or additional mechanisms are apparent for genetic and multi-generation effects.

C. DETERMINISTIC EFFECTS

171. Deterministic effects of ionizing radiation are the result of exposures that cause sufficient cell damage or loss of proliferative capacity in stem cells to impair function in the irradiated tissue or organ. For a given deterministic effect, a large proportion of cells must generally be affected, so that in most cases there are considerable thresholds in the range from tenths of a sievert to several sievert. Deterministic effects were reviewed by the Committee in Annex I, "Late deterministic effects in children", of the UNSCEAR 1993 Report [U3]. Although deterministic effects are practically excluded in controlled settings, side effects in tissue adjacent to treated tumours and localized effects in skin, eyes, and lungs must still be considered when assessing human health risks. Since loss of the ability to divide is also a result of DNA damage, many of the molecular mechanisms that modulate combined effects in carcinogenesis also modulate deterministic effects. In this context, the scavenging of radiation-induced radicals by scavengers such as cysteamine will also exert antagonistic effects for deterministic endpoints. Because this field is of limited relevance for this Annex, only a few examples from this poorly explored field are given below.

172. There are suggestions from clinical findings that pre-existing diabetes exacerbates radiation injury to the retinal vasculature. Gardiner et al. [G2] studied this phenomenon in streptozotocin-induced diabetic rats. In both diabetic and control rats, the right eye was irradiated with 90 kVp x rays to 10 Gy and the prevalence of acellular capillaries in trypsin digests of the retinal vasculature was quantified 6.5 months after irradiation. Diabetes as well as irradiation led to a statistically significant higher prevalence of acellular capillaries. The net increase in acellular capillaries following irradiation was much greater in rats with an eight-month term of pre-existing diabetes (180%) than in those that had been diabetic for only three months (36%). These results suggest a synergistic relationship between pre-existing diabetes and ionizing radiation in the development of retinal vasculopathy that seems to depend on the duration of diabetes before radiation exposure.

173. Ivanitskaia [I4] studied the reduction of spermatogenesis and of activities of key enzymes as a result of single or combined action of ionizing radiation and mercury in rats. The combined biological effects seemed to be close to the sum of the effects caused by the single agents.

174. Higher acute radiation doses are known to impair immune functions at least temporarily. Generally, immune

deficiencies are the condition being considered. However, overstimulation of the immune functions or the emergence of new antigenic sites as a secondary effect of radiation damage have to be considered as well. A stimulation effect at high doses was described by Lehnert et al. [L14] in inbred C57BL mice. The mice were irradiated with 10 Gy delivered to the thorax 24 hours prior to the induction of graft-versus-host disease by the injection of allogeneic lymphoid cells ($2\ 10^7$ cells). In mice only irradiated or only injected, survival was 100% at 250 days. In contrast, a combination of the two treatments, graft-versus-host disease and partial-body irradiation, resulted in a mortality of 83% and a mean survival time of only 29 days, indicating strong synergy between graft-versus-host disease and partial-body irradiation. From histological studies of the lung, it appeared that about 40% of the deaths occurring after combined graft-versus-host disease and partial-body irradiation (PBI) treatment might be attributable to pneumonia. The cause of death in the remaining mice that received combined treatment is unknown. Mice receiving combined PBI/lymphoid cell treatment also develop a characteristic skin lesion that is not seen in non-irradiated mice and that is confined to the irradiated area. A first indication of the mechanism involved is the fact that the amplifying effect of pre-induction partial-body irradiation on the timing and severity of graft-versus-host disease is similar to the effect that would be produced by an increase in the number of effector cells. Such a proliferative response should display a highly non-linear dose-response relationship with an apparent threshold similar to immune deficiencies based on widespread stem-cell killing. Therefore, no direct relevance of these findings for much lower occupational or environmental exposures is apparent.

175. Guadagny et al. [G18] described an increase in immunogenicity of murine lymphoma cells following exposure to gamma rays *in vivo*. On the basis that mutagenic compounds such as 5'(3,3'-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) cause a marked increase in immunogenicity in murine lymphoma cells *in vivo* or *in vitro*, they then conducted further experiments to test whether ionizing radiation would be able to affect the immunogenic properties of cancer cells in a mouse leukaemia model. Male $CD2F_1$ mice were inoculated with histocompatible L1210 Ha leukaemia cells and treated with 4 Gy of whole-body irradiation. A number of transplant generations were carried out with leukaemic cells collected from irradiated donors, generating a radiation-treated line. The immunogenicity of radiation-treated cell lines increased significantly compared with that of the L1210 Ha line as early as after three passages *in vivo*. However, no strong transplantation antigens comparable to those elicited by treatment with DTIC were found in radiation-treated cell lines, even after a number of transplant generations. The combination of bis-chloroethyl-nitrosourea and the weakly antigenic radiation-treated cell line elicited a strongly synergistic immune response of the host. Moreover, lymphoma induced with radiation-treated cell lines acquired strong immunogenic properties after a single cycle of DTIC treatment *in vivo*. Again, these results may well provide an experimental model for the exploitation of a radiation-induced increase of tumour cell immunogenicity for combined radioimmunochemotherapy in cancer treatment, but no direct relevance for the risk of radiation carcinogenesis is evident. As with other combined modalities in tumour therapy that also enhance and modulate deterministic effects (described in depth in the Appendix), the above examples do not indicate mechanisms leading to marked supra-additivity for effects from low-level exposures to multiple agents.

EXTENDED SUMMARY

176. In this Annex, the effects of combined exposures to radiation and other agents are considered particularly with respect to the induction of stochastic effects at low doses. A large amount of information on the combined exposures of radiation and other physical, chemical, and biological agents is reviewed. In many situations agents can interact with radiation and may significantly modify the biological processes and outcomes. The implications for radiation risk assessment and limitation of individual and collective health risks are considered.

177. For ionizing radiation, the main potential risk to humans from exposures at low doses, i.e. at the level of background radiation or a few times that level, is the enhanced incidence of stochastic effects, i.e. carcinogenesis and heritable genetic effects. In this Annex the effects of combined exposures to radiation and other agents are considered, particularly with respect to the possibility of enhanced radiation carcinogenesis caused by chronic low doses. Many radiobiological experiments, however, used acute, high radiation doses and high exposures to the other agent. It is usually not clear how these results might be extrapolated to low and chronic irradiation conditions and to humans. Such data may, however, be informative on the possible mechanisms of the interactions between various agents and radiation, and in that sense they may be of relevance for combined, low-dose exposures leading to carcinogenesis.

178. For assessing the effects of chemical agents, the situation is somewhat more complex than for ionizing radiation. For genotoxic chemicals, the main biological effects from low and chronic exposures are comparable to effects from low levels of radiation, i.e. stochastic in nature. However, as with radiation, most experimental data are from high, acute exposures. The wealth of epidemiological data and risk estimates based on such information, is much greater for radiation protection than for toxicology. With only a few exceptions (e.g. asbestos, smoking, and arsenic) for which human data are available, chemical

carcinogenesis data are based solely on biochemical, cell biological, and/or animal data. The general assumption about the dose-effect relationships for radiation and genotoxic chemicals in the low-dose region for chronic exposures and for stochastic endpoints is, however, the same. In general, it is assumed that these relationships are linear from high-dose ranges with observed effects down to zero dose. For non-genotoxic chemicals, non-linear dose-effect relationships are the norm, since higher order enzyme reactions are involved in most cases (uptake of agents, incorporation, metabolization, cell physiological reactions, etc.). These reactions are dominated by sigmoidal dose-effect relationships with apparent thresholds, related to the biochemical Michaelis-Menten kinetics.

179. The starting point for an analysis of combined exposures is to specify the doses of the agents at the site of interaction. For ionizing radiation, absorbed dose is the quantity most generally applied to characterize the exposure, and methods have been developed to calculate the dose in target cells from the irradiation conditions. For other agents, different methods are used, depending on the characteristics of the agent involved. Unfortunately, the exposure of the cells at risk for carcinogenesis is not always clear. No unifying concept of dose exists. In this Annex, methods of biochemical and biological monitoring and dosimetric evaluation are reviewed. The conclusion is that physiologically based parameters, such as concentration of toxic agents in blood or urine, are often not specific and sensitive enough to be generally applicable for the analysis of the biological action of a chemical agent. Therefore, biological endpoints at the cellular level, which are more directly related to stochastic health effects, have been developed and used as measures for genetic changes in somatic as well as germ cells. To these endpoints belong biochemical parameters such as DNA adducts, and gene mutation parameters such as mutation frequency and spectrum, and stable chromosomal alterations. A generally applicable method for analysing combined effects is still lacking, but taking these endpoints as a measure of the genotoxic burden of radiation or of chemical agents, a unifying risk concept based on genetic burden can be envisaged.

180. Carcinogenesis is, in general, a slowly developing process extending over years and even decades. From a mechanistic standpoint, different phases in the development of the effects of an agent can be considered. These may be characterized broadly as changes on the molecular, cellular, and tissue/organ levels. Agents can interact with radiation in each phase to produce an effect. Radiobiological research has turned up numerous agents potentially capable of influencing the progression of early radiation effects towards adverse health effects. General conclusions are hindered by the multitude and complexity of the possible interactions and the dependence of the combined effect on the sequence of the exposures. More explicitly, because of the long time period between the initial radiation event and the final effect, a combined exposure to radiation and another agent may occur after simultaneous exposure but also from exposures hours or even years apart.

181. In the early, molecular phase of the development of the radiation effect, interactions of chemicals with the primary radiation process can occur that are important for the fixation of the primary molecular radiation damage. For an interaction to occur, the active agents must be in close proximity to the DNA, which is the most important molecule for radiation carcinogenesis, at the time of irradiation or during repair and in a sufficiently high concentration. Molecular interactions are studied particularly to investigate the early radiation mechanism, and changes in the radiation effect from interactions have been seen. However, because of the high concentrations needed to observe a significant effect, the results from these investigations are not of direct relevance for the low levels of exposure found in occupational or environmental settings.

182. An impressive amount of information concerning interactions in the cellular phase can be found in the literature. For acute exposures, many agents can interact with radiation in this phase, including physical (e.g. UV) and chemical (e.g. alkylating and other genotoxic) agents. Toxic chemicals have been evaluated, using the isobolic method of analysis. Different interaction mechanisms are involved, ranging from an accumulation of DNA (sub)lesions, sometimes enhanced by repair inhibitors, to modulation of cell-cycle kinetics. The results of the interactions range from subadditive to supra-additive; however, for interaction to occur, the agents must generally be present during or shortly after irradiation, and the interaction effects decrease at low doses and dose rates. This mode of interaction may have implications for radiation carcinogenesis, but at low doses and for chronic irradiation, deviations from additivity are expected to be small.

183. In the organ phase of cancer development, interactions in combined exposures can be significant. The long duration of this phase creates many opportunities for interaction with other agents. As is also concluded for chemical carcinogenesis, these interactions are potentially important, but only a few data from human epidemiological studies are suitable for quantitative analysis. Radiation has been found to interact with physical agents such as UV radiation and mineral fibres; with chemical agents such as alkylating chemicals, tumour promoters, dietary factors, arsenic, and heavy metals; and with biological agents such as hormones and viruses. Well-defined effects are summarized in Table 7. Observation and identification of combination effects in this phase is difficult, because the duration of the interaction with the radiation damage may be long. In view of the many possibilities, it may well be that different interactions in the organ phase are largely responsible for the variations in background cancer incidence between populations.

184. A very important combined effect is the interaction of smoking and exposure to radon, although even in this case there is still no unambiguous conclusion on the interaction mechanism. Epidemiological data clearly indicate that combined exposure to radon and cigarette smoke leads to more-than-additive effects on lung cancer. These results warrant special consideration in estimating the radiation risks because a large proportion of the world's population

is exposed concomitantly to considerable levels of indoor radon and smoking. The combined analysis of 11 miner studies [L18] indicates that the effect of radon may be enhanced by a factor of about 3 by being combined with smoking.

185. Since the Committee's previous review of this subject [U6], there have been advances in modelling the multi-stage processes involved in carcinogenesis. The development and application of mechanistically based, multi-stage carcinogenesis models promise to give new insights into the interaction processes, especially because with these models it is possible to analyse interactions at the tissue/organ level of carcinogenesis. The results indicate, for example, that the effect of radiation is dependent on the background tumour incidence; they also show how the interaction of radiation with other agents might influence carcinogenesis.

186. Information is scarce on combined exposures of radiation and specific agents that might alter the radiation health risks caused by ambient exposures in the human environment. The possible relevance of the interaction of other agents with the radiation effect is obscured by the many sometimes poorly known or unknown sources of uncertainty surrounding radiation-induced carcinogenesis, such as variations in background cancer incidences, population characteristics and genetics, diet, and individual susceptibility.

Table 7
Agents that interact with ionizing radiation of importance in radiation carcinogenesis

Interacting agent	*Interaction*	*Endpoint*
Physical agents		
External ionizing radiation with internal emitters	Supra-additive	Bone cancer
Ultraviolet radiation (UV)	Possibly supra-additive	Skin cancer
Alpha emitters with mineral fibres, including asbestos	Supra-additive	Lung cancer
Chemical agents		
Nitroso compounds, such as MNU, DEN, 4NQO	Supra-additive	
Tumour promoters, such as TPA	Supra-additive	Effects shown only in animal experiments
Smoking	Supra-additive	Lung cancer
Vitamins	Subadditive	
Diet/fat	Sub- to supra-additive	Interaction dependent on comparing level
Arsenic	Supra-additive	Extrapolated from chemical carcinogenesis
Biological agents		
DES	Supra-additive	Breast cancer
Testosterone	Supra-additive	Prostate cancer

CONCLUSIONS

187. Combined exposures are a characteristic of life. The environment in which organisms reside and the organisms themselves are complex systems in which a multitude of interactions between physical, chemical, and biological factors occur. The specific agents involved in exposures in the environment and in occupational settings vary widely, but almost all physical and chemical agents, both natural and man-made, are capable of producing adverse effects under some exposure conditions, although individual agents differ considerably in their capacity to do so. In general, for many agents essential for life, there is a spectrum of effects associated with exposure, ranging from deficiency through sufficiency to adverse effects with increasing levels of exposure.

188. Although both synergistic and antagonistic combined effects are common at high exposures, there is no firm evidence for large deviations from additivity at controlled occupational or environmental exposures. This holds for mechanistic considerations, animal studies, and epidemiology-based assessments. Therefore, in spite of the potential importance of combined effects, results from assessments of the effects of single agents on human health are generally deemed applicable to exposure situations involving multiple agents.

189. With the exception of radiation and smoking, there is little indication from epidemiological data for a need to adjust for strong antagonistic or synergistic combined effects. The lack of pertinent data on combined effects does not imply per se that interactions between radiation and other agents do not occur. Indeed, substances with tumour promoter and/or inhibitor activities are found in the daily diet, and cancer risk therefore depends on lifestyle, particularly eating habits. Not only can these agents modify the natural or spontaneous cancer incidence, but they may also modify the carcinogenic potential of radiation. Such modifications would influence the outcome particularly when radiation

risks are projected relative to the spontaneous cancer incidence.

190. The analysis of small effects of combined exposures of other agents with radiation is also inhibited by the lack of well defined and pertinent harmonized measures of the exposures to radiation and the other agent. A generally applicable method for use in the analysis of combined effects is still lacking, but taking end points such as measures of the genotoxic effects of radiation or chemical agents, a unifying risk concept based on genetic burden could be envisaged for combinations of genotoxicants. At this stage, the uncertainties in the data permit the possible interactions from combined exposures of radiation with other agents to be only qualitatively recognized. A quantitative assessment of the radiation risks at low doses is not yet possible. In other words, even possible deviations from additivity at lower exposures are generally too low to show up in experimental studies or population cohorts.

191. The extent to which the effects of combined exposures can be elucidated is highly dependent on clarification of the carcinogenesis process itself and its dependence on environmental and lifestyle factors. Interactions of agents with radiation can be broadly grouped into three different levels (molecular, cellular, and tissue/organ levels) of the radiation effect. The molecular phase lasts only a fraction of a second until the primary radiation damage to DNA has occurred. For interactions during this phase, the other agent has to be present concomitantly and in a high enough concentration.

192. The cellular phase of the radiation effect lasts for one or a few cell cycles until the primary radiation damage in DNA has been repaired or the remaining damage has been fixed into heritable genetic damage (mutation in somatic and germ cells). For low doses and dose rates of radiation and low doses and chronic exposures to genotoxic chemical agents, the supralinear or quadratic terms of dose-effect relationships tend to vanish, and the linear terms dominate for single-agent effects. In the absence of target specificity, this implies that interaction at the cellular level during long-term low-level exposures to radiation and chemicals is of limited importance.

193. During the tissue/organ phase of radiation-induced carcinogenesis, which lasts from the first fixed genetic alteration to the clinically manifested tumour and which may include several genetic and epigenetic changes, combined effects can occur from exposures of two and more agents spread over days or decades, giving a large potential for combined effects. Besides genotoxic chemicals, many non-genotoxic agents may interact during the organ phase. Tumour promotion, mitogenic stimulation, and hormonal activation are a few of the important examples of processes with the potential for more-than-additive effects. Also, radiation- or chemical-induced genetic instability, which leads to new genetic damage after many cell generations, may be prone to more-than-additive effects. An overview of possible interaction processes and groups of agents involved in these processes is given in Figure V.

194. Within the framework of the multi-stage mechanism of carcinogenesis, the following general conclusions can be drawn for the combined action of different carcinogenic and co-carcinogenic agents:

(a) genotoxic agents with similar biological and mechanistic behaviour and acting at the same time will interact in an isoadditive or concentration-additive manner. This means that concurrent exposures to ionizing radiation and other DNA-damaging agents with no specific affinity to those DNA sequences that are critically involved in carcinogenesis will generally result in effects not far from isoadditive. Isoadditivity at this point includes “apparent synergisms” or “autosynergisms” resulting from non-linear dose-effect relationships of the single-agent effects. Supra-additivity of this quality generally does not exceed the expectation value derived from high-exposure, single-agent effects combined with linear dose-effect models;

(b) for genotoxic agents acting on different rate-limiting steps of multi-stage stochastic diseases like cancer, strong deviations from additivity might result. Deviation from additivity can depend on the specificity of the agents for the different steps, sequence specificity, and the sequence of exposures. Highly synergistic effects are, however, only to be expected in cases where both agents are responsible for a large fraction of the total transitions through the respective rate-limiting steps;

(c) in combinations of radiation and non-genotoxic agents in which the second agent causes promotion, i.e. the multiplication of premalignant cells, highly synergistic effects may arise. This combined effect is dependent on the exposure schedule. Thresholds for such combined effects are generally implicated from the highly non-linear dose-effect relationship for the non-genotoxic agent acting alone;

(d) for agents acting independently and through different mechanisms and pathways, heteroadditivity or effect additivity is predicted. Apparent thresholds will not interfere with each other, and possible conservatisms in linear dose-effect extrapolations from high exposures will not be affected;

(e) in combinations of agents, in which one agent induces adaptive mechanisms, e.g. increased DNA repair capacity or increased radical scavenger function, and the other agent induces DNA damage, antagonistic effects may arise. Owing to the generally short half-times of adaptive mechanisms, the exposures have to occur concurrently or nearly concurrently.

195. In summary, the following parameters need to be considered to address and assess potential combined effects: the mode of action of the agent (genotoxic or non-genotoxic); the shape of the dose-effect relationship for single-agent effects; the dose or concentration involved (low or high); the type of exposure (chronic or acute); and the sequence and time interval between exposures (simultaneous or before or after radiation exposure).

196. There has been little systematic research on possible interactions of radiation with other agents. An exception is

the use of combined modalities in tumour therapy, but the high doses and deterministic effects involved cannot be easily related to stochastic, low-level combined effects. Considerable progress in the biological sciences and the many radiological and toxicological disciplines involved will be needed to allow predicting the potential presence of combined effects at low exposure levels and negative health outcomes. It can be stated, however, that the conclusion of the Committee's previous review on combined effects [U6] still holds: except for radiation and smoking, there is no evidence that low-level exposures to multiple agents yield combined effects far from additivity, or above the estimates resulting from linear extrapolation of single agent effects to lower doses.

FURTHER RESEARCH NEEDS

197. The lack of systematic mechanistic understanding and quantitative assessments of combined exposures and the resulting possible interactions urgently needs to be resolved. This elucidation of interactions of agents in combined effects critically depends on both a qualitative (mechanistic) and a quantitative knowledge of the action of any single exogenous or endogenous agent involved. The basic tenets of experimental toxicology and radiobiology will have to be applied in studies of the adverse health effects of combinations of exposures. In view of convincing evidence that the critical stochastic endpoint, cancer, is multifactorial, the many studies concentrating on single carcinogenic agents and attempting to quantify cancer risks as if they were due to single factors have to be supplemented and extended to address potential modifications from joint effects.

198. Present knowledge of the many qualitatively different interactions already found in biological systems speaks against the emergence of simple unifying concepts to predict modifications of risk from combined exposures. However, mechanistically based classifications of interactions may be helpful in predicting effects. At present, relevant knowledge is being gained on interaction mechanisms in different parts of the long process of radiation carcinogenesis. A better understanding of how important these separate physical, chemical, and biological interaction mechanisms are for the ultimate endpoint will help to create a basis for risk assessment, and a better understanding of the carcinogenesis process itself and the rate-limiting steps involved may contribute to an understanding of the interactions as well. The development of mechanistically based cancer models could greatly improve the estimation of quantitative risks.

199. Individual genetic susceptibility is already a concern for the assessment of radiation risks. In addition to those parts of the genome susceptible to radiation-induced effects, e.g. repair and proofreading genes, heterozygosity for oncogenes and tumour suppressor genes, many additional gene products determining the biokinetics and biotransformation of chemical agents will have to be considered in the individual response to combined exposures involving chemicals.

200. Progress in the analysis of interactions between ionizing radiation and toxicants is often hampered by a lack of scientific data that quantitatively relates chemical exposure to health risk or experimental endpoints. The implementation of standard protocols and dosimetry to harmonize reported research is urgently needed to allow comparison of data from studies on different agents. Data will also have to be extended over a sufficiently large exposure range to allow extrapolating to the doses relevant in environmental health.

201. Epidemiological studies have already revealed important combined effects for carcinogenesis, particularly for the joint effect of cigarette smoke with either radiation or asbestos. New tools in molecular biology point the way to the field of molecular epidemiology and will provide investigators with markers of exposure and damage that are much more sensitive than the cruder incidence measures of clinical diseases. Such approaches, if successful, can be expected to yield significant new information on interactions between agents at the cellular and molecular levels. Markers of this kind can also be used in more classical human epidemiological studies of cancer, some of which may help in probing potential interactions between agents that may have induced the disease.

202. A mechanistic assessment of combined effects is dependent on progress in the scientific understanding of other generic issues, such as extrapolation from high to low levels, transfer of data from laboratory animals to humans, and age dependence of the radiation risk, that are central to the general risk assessment process. Current approaches to the risk assessment of complex exposures rely heavily on linear dose-effect relationships and additivity models. However, in the dose and concentration range of interest for human exposures, dose-effect relationships other than linear (sigmoidal and even U-shaped curves in the case of partially stimulatory or essential agents) are reported as well. This issue must be fully addressed in assessing the risks of combined effects.

203. Finally, a comprehensive approach for the study and quantitative assessment of combined effects must be developed. The gap between different conceptual approaches in the assessment of risk in chemical toxicology and radiological protection has to be bridged urgently. Multidisciplinary approaches to research (radiobiology, toxicology, cell and molecular biology, biostatistics, epidemiology) have to be forged. In some instances, recasting and combining results from recent studies and on-going work into more refined models that take into account additional mechanisms of responses and that take advantage of the multidisciplinary approach may improve the understanding and quantification of specific interactions. This, together with the application of refined multi-stage models, will help to reduce uncertainties at the low exposure levels found in the human environment.

APPENDIX

Combined effects of specific physical, chemical, and biological agents with ionizing radiation

1. Studies of the combined effects of specific physical, chemical, and biological agents in association with radiation exposure are reviewed in detail in this Appendix. The intention is to provide an overview of the available literature in support of the more general findings, summaries, and conclusions of this Annex. First, data from epidemiological studies of the adverse health effects in humans of each group of agents are reviewed. Studies involving experimental animal models are then considered. Finally, various effects observed using *in vitro* systems are reviewed. Carcinogenesis is the principal endpoint of interest, but non-neoplastic endpoints are also discussed. Only a minor fraction of the interacting agents described below are found in the human environment at potentially critical levels. A few such critical agents already known or suspected to affect human health on their own are listed in Table 4. The findings of specific combined exposures and effects described in depth in this Appendix are summarized in Table A.1.

A. RADIATION AND PHYSICAL AGENTS

1. Combinations of different types of ionizing radiation

2. Understanding how cellular damage produced by high linear-energy-transfer (LET) radiation interacts with that produced by low-LET radiation is important both in radiation therapy and in evaluating risk. In view of the possible radiotherapeutic applications, a wealth of data on the combined action of neutrons, heavy ions, and gamma or x rays was accumulated in the 1970s and early 1980s. This information was reviewed in Annex L, "Biological effects of radiation in combination with other physical, chemical and biological agents", in the UNSCEAR 1982 Report [U6]. Because the underlying damage mechanism is similar, the interaction between different types of ionizing radiation is, in general, of the isoadditive type in the case of cell killing. Deviations from additivity are generally not very large and can be explained in most cases by concomitant changes in exposure rates and in exposures of critical target structures. For example, survival of Chinese hamster V79 cells *in vitro* after irradiation first with neon ions (LET = 180 keV μm^{-1}) and then with x rays (225 kVp) was additive, as predicted from independent action. The system also showed no dependence on the order of application [N5].

3. In extreme emergency situations, localized exposures to the skin or other organs in the presence of elevated external radiation fields is of considerable concern. Randall and Coggle [R3] studied the deterministic effects of concomitant whole-body irradiation and localized radiation trauma from beta activity on the skin. They modelled the immunosuppressive effects of whole-body gamma radiation in the sublethal to lethal range (1–11 Gy) on skin reactions produced by 50 Gy from superficial beta radiation from ^{171}Tm in male mice. For gamma doses below 4 Gy, no interaction effects were detectable. For gamma doses in the range 4–8 Gy, the skin reaction developed more slowly, but it was not much more severe. The overall time for the resolution of the skin reaction, about 45 days, was also unaffected by high-dose whole-body irradiation. The authors ascribed the absence of any considerable deviation from additivity in this system to the mismatch in time between maximal immunosuppression and localized severe beta burns ranging from 2 to 10 days and 10 to 25 days, respectively. Although such beneficial mismatches in time are species-specific, these mechanisms may also be important in humans. Deterministic combined effects in Chernobyl power plant staff and emergency workers are discussed in Annex J, "*Exposures and effects of the Chernobyl accident*".

4. A strong antagonistic combined effect was found in an experimental study of deterministic effects in rats. When the animals received high external gamma (about 6 Gy) or beta (about 24 Gy surface dose) radiation, lethality was lower by a factor of 5 when the animals received a concomitant exposure to the thyroid gland of 0.3 kBq g^{-1} from ^{131}I given orally [M13]. The protective influence of the combined treatment was attributed to ^{131}I-induced changes in the hormonal state in the course of acute radiation sickness. In another study by the same author with lower sublethal external doses of up to 3 Gy and additional orally administered ^{131}I, there was an increased yield of mammary tumours in the combined treatment group receiving low exposures from iodine (0.04–0.8 kBq g^{-1}). For higher iodine exposures, however, the reverse was true [M30]. The combined effects observed seem to be deterministic and can be attributed mainly to different organ doses rather than different radiation qualities.

5. Changes in the haematopoietic bone marrow, i.e. in the number of colony-forming units (CFU), of rats were observed by Brezani et al. [B1] after a single whole-body neutron dose of 2 Gy and combined single neutron (2 Gy) and continuous gamma irradiation (6 Gy, daily dose rate of 0.57 Gy). Neutron irradiation alone significantly reduced the number of karyocytes, including CFU-S in the bone marrow and induced extensive cytogenetic damage. When followed by continuous gamma irradiation, the primary damage from neutrons was not enhanced, however CFU-S remained at a decreased level for the whole time of irradiation. Recovery from damage began only after termination of the continuous irradiation; its course was similar to that after single neutron irradiation. A long-lasting supra-additive influence of the combined

exposure to neutrons and gamma rays is nevertheless manifested in later periods after irradiation by a reduction in the total CFU-S number in the bone marrow.

6. Most studies of stochastic effects from combined irradiations are undertaken in connection with cancer. For bone-seeking radionuclides, a synergistic effect was found in mice for osteosarcomas after combined exposure to short-lived ^{227}Th (190 Bq g^{-1}, corresponding to about 10 Gy mean skeletal alpha dose) and to longer-lived ^{227}Ac (1.9 Bq g^{-1}) bone-seeking radionuclides. The beta emitter ^{227}Ac produces protracted internal alpha exposures through ingrowth of the decay product ^{227}Th. At 700 days after intraperitoneal (ip) injection of pure ^{227}Th or ^{227}Th contaminated with 1% ^{227}Ac (combined exposure) in the form of citrate, an osteosarcoma incidence higher than additive was found for the combined exposure. With incidences of less than 1% for controls, 8% for ^{227}Ac alone, and 36% for ^{227}Th alone, the combined effect of 62% amounted to an interaction factor of 1.7 [L26]. In terms of the time for 50% tumour appearance, the interaction factor was reduced to a barely significant 1.3. The authors speculated that the increased oncogenetic effectiveness of ^{227}Th contaminated with 1% ^{227}Ac may be caused by the continuous stimulation of cell proliferation or by the activation of retroviruses by protracted low-level alpha irradiation from ^{227}Ac/^{227}Th.

7. Bukhtoiarova and Spirina [B33] studied the combined effect of external gamma irradiation and ^{239}Pu on the incidence of osteosarcomas in inbred male rats. Osteosarcomas occurred more frequently and at earlier times and displayed a more pronounced multicentric pattern of growth and metastatic spreading than the malignancies induced by exposure to only one of the two agents. The differences resulted from increased development of tumours and decreased osteogenesis. A quantitative evaluation of the combined effect of the same radiation mix on biochemical parameters of the rat immune system was undertaken by Elkina and Lumpov [E5]. The combined effect of external gamma radiation (^{137}Cs, 1–4 Gy) and incorporated alpha radiation (^{239}Pu nitrate, 9.3–93 kBq kg^{-1} body mass) was estimated by determining changes in nucleic acid metabolism and the number of cells in rat thymus, spleen, and bone marrow. The data obtained for the lower end of exposures were consistent with an additive model. The same researchers also studied aminotransferase and lactate dehydrogenase activity in the blood of dogs exposed to the joint action of external gamma and internal alpha radiation [E6]. After the effect of external gamma radiation (0.25–2 Gy) and inhaled ^{239}Pu submicron oxide containing 25% ^{241}Am (approximately 7–10 kBq kg^{-1}) delivered separately and in combination, activities of alanine-aspartate aminotransferase and lactate dehydrogenase changed in an undulatory manner, tending to increase at later times. The change was a function of type and level of radiation as well as time elapsed from the onset of exposure. Even at the relatively high exposures used in these experiments, the combined effect of gamma and alpha radiation did not exceed the additive effect of the two factors delivered separately. In view of the deterministic nature of the endpoints studied, no inferences for controlled exposures are apparent.

8. Several authors have shown that large radiation doses influence biokinetics and hence exposure from incorporated radionuclides. The influence of external gamma radiation on ^{239}Pu redistribution in pregnant and lactating rats was described by Ovcharenko and Fomina [O1]. A quite high dose range of acute external gamma radiation, from 0.5 to 4 Gy, was investigated. Transplacental transfer of ^{239}Pu to the embryo increased with dose to a maximum at 1 Gy and then declined. However, transfer of ^{239}Pu via milk to newborn rats was decreased by external gamma irradiation of lactating rats with the dose of 0.5 Gy. The nature of the biological mechanisms responsible for the changes in biokinetics remains elusive. There are no suggestions by the authors that these radiation effects on metabolism are stochastic in nature and would extend to low doses and dose rates.

9. Lundgren et al. [L19, L20] examined the carcinogenicity of a single, acute pernasal inhalation exposure of 3,201 male and female F344 rats to $^{239}PuO_2$, followed one and two months later by whole-body x-irradiation. Plutonium lung burdens were 56 or 170 Bq, and the x-ray exposure was fractionated into two exposures totalling either 3.8 or 11.5 Gy. Other groups of rats received control (sham) exposures. Minor x-ray-dependent differences in ^{239}Pu lung retention were observed; however, exposure to x rays significantly reduced the median survival times in rats of both sexes [L19]. For a given level of x-ray exposure (0, 3.8, or 11.5 Gy), the level of ^{239}Pu exposure (0, 56, or 170 Bq) had no effect on median survival time. A preliminary histological evaluation of primary lung tumours produced has been reported for approximately two thirds of the rats in this study. The authors noted an apparently antagonistic interaction between the two agents in producing lung tumours; for example, crude tumour incidences were 10.8% in rats receiving 11.5 Gy x-irradiation alone, 9.2% in rats receiving a 170 Bq lung burden of $^{239}PuO_2$ alone, but only 11.7% in rats receiving a combined exposure at these levels [L20]. The authors cautioned, however, that a simple evaluation of the crude tumour incidence is insufficient because of the effect of exposure on lifespan. They further state that analysis of this study is not yet complete.

10. An apparent synergism was described in an *in vitro* study of the combined effect of alpha particles and x rays on cell killing and micronucleus induction in rat lung epithelial cells (LEC) [B39]. The cells were grown on Mylar films and exposed to both x rays and alpha particles, separately or simultaneously. X rays and alpha particles given separately caused dose-related increases in cell cycle time, with alpha particles producing greater mitotic delay than x rays. Damage from alpha particles and x rays given simultaneously did not interact to further alter the cell cycle. Cell survival data following exposure to x rays and alpha particles, combined or individually, were fitted by linear-quadratic models. Survival curves following exposure to alpha particles only, or to 1 Gy from alpha particles plus graded x-ray doses, were adequately described using only the linear (alpha) terms with values of the coefficients of 0.9±0.04 and 1.03±0.18 Gy^{-1}, respectively. Survival following exposure to x rays only or to 0.06 Gy from alpha particles combined with x rays was

best fitted using both alpha and beta terms $(0.12\pm0.03)D + (0.007\pm0.002)D^{2,}$ and $(0.57\pm0.08)D + (0.3\pm0.02)D^2$, respectively. The numbers of micronuclei in binucleated cells produced by exposure to alpha particles or x rays alone increased linearly with dose, with slopes of 0.48± 0.07 and 0.19±0.05 micronuclei per binucleated cell per Gy for alpha particles and x rays, respectively. Simultaneous exposure to graded levels of x rays and a constant alpha dose of either 1.0 or 0.06 Gy increased micronuclei frequency, with a slope of 0.74±0.05 or 0.58±0.04 micronuclei per binucleated cell and Gy, respectively. These slopes are similar to that produced by alpha particles alone. These studies demonstrated that both cell killing and the induction of micronuclei were greater with combined exposures than with separate exposures.

11. A refined model for the combined effects of mixtures of ionizing radiations was recently published by Lam [L4]. Assuming that ionizing radiation is a special group of toxic agents whose general interaction can be calculated, the model postulates the existence of a common intermediate lesion and the relative action of lesions before, at, and after this common stage. General quantitative dose-effect relationships of mixed radiations can be derived from the dose-effect relationships of the components in the mixture. Again, only small deviations from isoadditivity are predicted by this damage function, which allows treating mixed irradiation as two different increments of dose from the same radiation source.

12. A unifying concept to predict the expected combined stochastic radiobiological effects of different ionizing radiations was presented by Scott [S15]. Additive-damage dose-effect models were developed for predicting the radiobiological effects of sequential and simultaneous exposures. These additive-damage dose-effect models assume that

(a) each type of radiation in the combined exposure produces initial damage, called critical damage, that could lead to the radiobiological effect of interest; and
(b) doses of different radiations that lead to the same level of radiobiological effect (or risk) can be viewed as producing the same amount of critical damage, which is indistinguishable as far as the effects of subsequently administered radiation are concerned.

The methodologies allow the use of known radiation-specific risk functions to derive risk functions for the combined effects of different radiations, called global risk functions. For sequential exposures to different ionizing radiations, the global risk functions derived depend on how individual radiation doses are ordered. Global risk functions can also differ for sequential and simultaneous exposures. The methodologies are used to account for some previously unexplained radiobiological effects of combined exposures to high- and low-LET radiations. Since all radiation effects are traced to a common initial damage mainly occurring in DNA, the model is basically additive.

13. At doses lower than those that induce deterministic effects, no large deviations from additivity are found in the interaction of different radiation qualities (see also Table A.1). Although the mathematical modelling of mixed radiation showing non-linear dose-response relationships with a single radiation quality yields apparent synergistic interactions when the analysis of endpoints like survival is based on some current definitions [Z2], these definitions are clearly inappropriate for the approach used in this Annex. This point is also made by Suzuki, who stressed the need for definitions based on biological mechanisms [S3].

14. In summary, it can be stated that when dose rates and other possible confounders are taken into account, practically all the results from mixed radiation yielding more than the sum of the single agents can be explained by isoaddition, so that general quantitative dose-response relationships for mixed radiations can be derived from the dose-response relationships of the components in the mixture [L4, L47]. There is no indication that the influence of external radiation on the biokinetics of radionuclides found at high doses is relevant at occupational or environmental exposure levels.

2. Ultraviolet radiation

15. Ultraviolet (UV) radiation is recognized as an important initiator and co-factor for human skin carcinogenesis. Genetic predisposition, i.e. skin type, age at exposure, and duration of exposures are important determinants of risk for UV radiation-induced skin cancer. Shore analysed 12 studies on the incidence of skin cancer in irradiated populations with known skin doses [S29]. In the absence of a proper control (skin exposed to ionizing radiation but not to UV), it was concluded that at least for combined exposures, there was no evidence of a dose threshold for radiation-induced skin cancer. The data are compatible with a linear dose-response relationship [S29]. The question whether relative risk or absolute risk models are more appropriate remains open. Considerable variations in sensitivity to skin cancer induction among demographic and genetic subgroups may be mainly a reflection of the large differences in UV exposures because of lifestyle, skin type, and tanning.

16. Combined exposure to UV and x rays leads to synergistic interaction in killing mammalian cells [H53], confirming previous studies in yeast [S87]. Only a small interaction was found for mutations at the *hprt* locus in Chinese hamster cells [K56]. A recent study by Spitkovsky et al. [S82] in human peripheral lymphocytes on the interaction between x-ray doses of 5–250 mGy and 20 J m^{-2} of 254 nm UV light in DNA repair, measured by unscheduled DNA synthesis (UDS), indicated that the repair of UV-induced damage was modulated by previous x-ray exposures. For radiation alone, UDS was highest for 20–30 mGy and 150–200 mGy and lowest at 100 mGy. For combined exposures, i.e. ionizing radiation followed by UV, UDS was highest in cells previously exposed to 100 mGy and lower than in UV-only controls for cells previously exposed to 20–30 or 150–200 mGy. The mechanism of this proposed adaptive response remains to be elucidated.

Table A.1
Combined effects of ionizing radiation and other agents

Radiation	*Combining agent*	*Study system*	*Endpoint studied*	*Nature of effect*	*Comments*	*Ref.*
Physical agents						
Beta to skin	Gamma, whole body	Male mice	Deterministic effect	Additive		[R3]
Beta, ^{131}I to thyroid	Gamma, whole body	Rats	Deterministic effect; change in hormone status	Subadditive		[M13]
Alpha	Gamma	Rat lung epithelial cells *in vitro*	Cell killing and micronuclei formation	Supra-additive		[B39]
Alpha, ^{227}Th	Beta, ^{227}Ac	Mice	Osteosarcoma	Supra-additive		[L26]
Alpha, ^{239}Pu	Gamma	Male rats	Osteosarcoma	Supra-additive	Additive at low exposure	[E5]
Low LET	UV radiation	Humans	Skin cancer	?	Meta-analysis of 12 epidemiological studies; controls with ionizing radiation only were not available	[S29]
Alpha	Laser (633 nm)	Bacteria (*E. Coli*)	Survival	Subadditive	Mechanism only found in bacteria	[V6]
X rays (5.5 Gy)	Microwave field (200 µW cm^{-2})	Rats	Survival	Subadditive		[G13, G16]
X rays	Static field (58 mT)	Cell culture	Growth and survival rate	Supra-additive		[K16]
Gamma (3-9 Gy)	Static field (10-400 mT)	Mice	Survival	Subadditive	Field has to precede irradiation by 7-30 days for full protection	[S33]
X rays	Temperature	Human, mammals	Cell killing, suppression of proliferation	Supra-additive	Complex adaptive processes, important for tumour control	[G6, M15, Z3]
X rays	Ultrasound	C3HT½ cells	Transformation	None		[H10]
Alpha (radon, 6 000 WLM)	Mineral fibres (intrapleurally)	Rats	Lung carcinomas and mesotheliomas	Supra-additive		[B38]
Alpha (radon)	Mineral fibres	Rats	Thoracic tumours	Supra-additive		[B38]
Alpha (radon)	Mineral fibres	C3HT½ cells	Transformation	Supra-additive		[H11]
Chemical toxicants (genotoxic chemicals)						
X rays (7.5 Gy)	Alkylating agents	Human	Secondary non-lymphocytic leukaemia after breast cancer therapy	Supra-additive		[C29]

Table A.1 (continued)

Radiation	*Combining agent*	*Study system*	*Endpoint studied*	*Nature of effect*	*Comments*	*Ref.*
X rays	Cyclophosphamide	Mice	Mutations in germ cells	Supra-additive	Interference of cyclophosphamide with DNA repair	[E7]
X rays (9 Gy)	DMH, 1,2-dimethylhydrazine (0.15 g kg^{-1})	Rats	Colon carcinogenesis	Supra-additive		[S27]
X rays (0.25-3 Gy)	Methyl, ethyl, butylnitrosourea (10 mg kg^{-1})	Rats Mice Rats Rats C57Bl/6N mice BDF_1 mice	Gastrointestinal tumours T-cell lymphomas Neural tumours Brain tumours Lymphomas Thymic lymphomas	Supra-additive Supra-additive Subadditive Subadditive Supra-additive Subadditive to supra-additive	Deterministic effect suggested by enhanced risk in generating lympho-haemopoiesis Killing of stem cells? (Radiation suppresses schwannoma induction by ENU and also spontaneous squamous-cell carcinomas) Probably due to radiation-induced alkyl-transferase Effect traced to cellular kinetics and clonal expansion Lower radiation doses enhanced, higher doses delayed leukaemogenesis	[M32] [S22] [H6, K15] [S13] [S21] [S20]
Gamma (0.75-3 Gy)	Diethylnitrosamine (DEN) (100 mg kg^{-1})	Rats	Liver foci	Supra-additive	Large sex differences, different histo-chemically characterized foci show divergent response	[P26]
Neutrons (0.125-0.5 Gy)	Diethylnitrosamine (DEN) (100 mg kg^{-1})	Mice	Liver carcinomas and foci	Supra-additive	Effect mainly of increased foci appearance	[M8]
$^{90}Sr/^{90}Y$ (27 Gy, skin surface)	4-Nitroquinoline 1-oxide	ICR female mice	Skin tumour	Supra-additive	No tumours from single-agent exposure despite acute effects	[H39]
Gamma (1, 9 Gy)	Bleomycin (60 mg kg^{-1})	Mouse germ cells	Reciprocal translocations	Additive to supra-additive		[D6]
X rays	1,2-Diethylnitrosamine (DEN)	Tradescantia	Stamen hair cell mutations	Supra-additive	Interaction of single-strand lesions, covered by isoaddition	[L13]
X rays (3 Gy)	Paraquat (superoxide generating agent)	C3HT½ cells	Survival, transformation	Supra-additive	Additive for sister chromatid exchange	[G5]
Chemical toxicants (non-genotoxic chemicals)						
X rays	TPA	see Table A.3		Synergism	TPA not present in natural environment	
X rays	Sulfhydryl-carrying radioprotectors	Male BALB/c and C57Bl mice	Survival, tumour induction	Antagonism	Many systems and endpoints studied with similar results	[M6, M7]
$^{90}Sr/^{90}Y$ (3 × 3 Gy per week)	alpha-Difloormethylornithine (DFMO) antipromotor (1% in drinking water)	ICR mice	Skin and bone tumours	Antagonism	Tumour emergence delayed	[O12]

Table A.1 (continued)

Radiation	*Combining agent*	*Study system*	*Endpoint studied*	*Nature of effect*	*Comments*	*Ref.*
Neutrons (1.7-3.3 Gy)	CCl_4	C57Bl6 mice	Liver carcinomas	Supra-additive	No effect with chloroform	[B16]
Alpha (PuO_2)	CCl_4	Rats and hamsters	Modification of biokinetics		Results not yet known	[B16]
X rays (1.5 Gy)	Bromo-2'-deoxyuridine (BrdUrd) (3.2 mg per rat)	Rats	Total tumour yield, latency	Supra-additive	Potential clinical problem	[A15]
X rays (4-10 Gy)	Nicorandil, inhibitor of free radical production	Chinese hamster V79 cells	Survival	Subadditive		[N20]
X rays (2-8 Gy)	Calyculin A, protein phosphatase inhibitor (2.5-10 mM)	BHK21 cells	Cell killing	Supra-additive	Disruption of protein kinase - mediated signal transduction?	[N21]
Tobacco						
Alpha (^{222}Rn)	Active smoking [a]	Humans (miners)	Lung cancer	Supra-additive	Confounders: other air pollutants; transfer to modern workplaces and indoor exposure	[C1, L18]
Alpha (^{222}Rn)	Active smoking [b]	Humans (miners)	Lung cancer	Additive to supra-additive	More-than-multiplicative only for smoking preceding radon	[T18]
Alpha (^{222}Rn)	Active smoking [a]	Humans (home)	Lung cancer	Supra-additive	Little statistical power	[P11]
Alpha (^{222}Rn)	Active smoking [a]	Humans (thorotrast patients)	Lug cancer	None	Proper lung dosimetry lacking, therefore little statistical power	[I5]
Alpha (^{222}Rn)	Mainstream smoke [b]	Rats	Lung cancer	Supra-additive	Interaction only for radon	[C9]
Alpha (^{222}Rn)	Mainstream smoke [b]	Beagle dogs	Lung cancer	Subadditive	Reduced dose to critical cells by thickening of bronchial mucus?	[C21]
Alpha (^{222}Rn)	Diesel fumes [b]	Sprague-Dawley rats	Lung cancer	None	Diesel fumes alone had no effect	[M61]
Alpha (^{239}Pu)	Mainstream smoke [a]	Mice	Lung dose, histopathology	Supra-additive	Lung clearance reduced	[T2]
Alpha (^{239}Pu)	Mainstream smoke [a]	F344 rats	Lung lesions, lung cancer	Supra-additive	Lung clearance reduced	[F17]
X rays (>9 Gy to target)	Active smoking [b]	Humans (Hodgkin's disease patients)	Lung cancer	Supra-additive	Clinical problem	[V7]
X rays	Active smoking [b]	Humans (breast cancer patients)	Lung cancer	Subadditive to supra-additive	Possible bias in positive study	[I9, I10, N4]
Metals						
Alpha (^{222}Rn)	Arsenic	Humans (miners)	Lung cancer	None	Adjustment for arsenic exposure reduced the radon risk estimate (ERR WLM^{-1})	[L18, T5]

Table A.1 (continued)

Radiation	*Combining agent*	*Study system*	*Endpoint studied*	*Nature of effect*	*Comments*	*Ref.*
Alpha (150-6 700 Bq ^{239}Pu)	Beryllium (1-91 µg lung burden)	Rats	Lung dose Lung tumours	Supra-additive None	Lung clearance reduced	[S42]
Alpha (60-170 Bq ^{239}Pu)	Beryllium (50-450 µg lung burden)	F344 rats	Lung dose Lung tumours	Supra-additive Supra-additive	Lung clearance reduced	[F12]
X rays (1, 2 Gy)	Beryllium (0.2, 1 mM)	CHO cells	Cell-cycle delay Chromosome aberrations	Supra-additive Supra-additive	Multiplicative interaction probably limited to S and G_2 cells	[B37]
Gamma	Cadmium (1 mg kg^{-1})	Mice	DNA damage in peripheral lymphocytes	Subadditive	Cadmium exposure has to precede radiation by several hours	[P27]
$^{134, 137}$Cs (~2 500 Bq kg^{-})	Lead (16-320 mg kg^{-1})	Arabidopsis thaliana	Mutations	Subadditive to supra-additive		[K42]
^{137}Cs (-0.012 Gy)	Zinc (10-100 mg kg $^{-1}$) cadmium (0.5-16 mg kg^{-1})	Soil microbes	Nitrogen fixation, dentrification, CO_2 flux	Supra-additive	Catalase activity reduced	[E19, E20]
Mitogens and cytotoxicants						
X rays (0.24, 0.94 Gy)	Caffeine (1-7 mM)	Pre-implantation mouse embryos	Micronuclei induction Embryonal development	Supra-additive Supra-additive	Apparent threshold below 1 mM	[M11]
X rays	Caffeine	*In vitro* chemistry	Oxygen radical concentrations	Subadditive		[K29]
X rays	Caffeine, theobromine, theophylline	T lymphoma cells TKG cells	Apoptosis Apoptosis	Supra-additive Subadditive		[Z4]
X rays	N-methylformamide (0-170 mM)	Human colon tumour line	Survival	Supra-additive	Effect on alpha term, i.e. stronger at lower exposures	[L15]
Antioxidants, vitamins and other dietary factors						
X rays	Restricted food intake [a]	Animals	Tumour incidence	Subadditive	Probably via reduced growth stimuli	[C26]
X rays (10 Gy)	NaCl (1% in diet), ethanol (10%) [b]	CD(SD):Crj rats	Intestinal metaplasia	Supra-additive	No effect on incidence of gastric tumours	[W29]
Gamma rays (1.5-4 Gy)	Tetrahydrocannabinol [b]	Rats	Tumour incidence	Supra-additive	Breast adenocarcinoma increased by a factor 5	[M24]
Gamma rays (4 Gy)	Vitamin A, E, selenium, 3-aminobenzamide [a]	C3H10T½ cells	Transformation	Subadditive	Epidemiological studies indicate importance of dietary form	[B24, H11]
Biological agents (hormones, viruses, other)						
Gamma, neutrons	Oestradiol-17 beta [b]	Rats	Mammary tumours	Questionable	High hormone levels applied	[B32]

Table A.1 (continued)

Radiation	*Combining agent*	*Study system*	*Endpoint studied*	*Nature of effect*	*Comments*	*Ref.*
Neutrons	Prolactin [a]	Rats	Mammary tumours	Supra-additive		[Y6]
Neutrons (0.064 Gy)	Diethylstilbestrol (DES) (12.5 mg kg^{-1}) [a]	Rats	Mammary tumours	Supra-additive	Increased time span between radiation and hormone did not reduce interaction	[S24]
Gamma rays (2.6 Gy)	DES pellet (release 1 µg d^{-1}) [a]	Rats	Mammary tumours	Supra-additive	Irradiation in late pregnancy or during late lactation clearly more effective	[I11, S40]
Beta rays (1.5 MBq ^{131}I)	Castration ± testosterone [b]	Male rates	Thyroid neoplasms	Supra-additive		[H12]
X rays (2 × 10 Gy)	Testosterone and DES dimethyl-estradiol (0.2-2.5 mg implant) [b]	Normal and gonadecto-mized CD(SD);Cjr rats	Intestinal metaplasia	Subadditive Supra-additive	DES in females Testosterone in females; DES in males	[W28]
X rays	Retrovirus T1223/B [a]	C57Bl mice	Leukaemogenesis	Supra-additive	Recombinant viral DNA found in the genome of every tumour	[A25]
X rays	Microbial substances [a]	Mammals	Survival	Subadditive	Stimulation of immune system?	[A12]
Gamma rays (1-4 Gy)	Urethane [a]	Athymic nude and euthymic mice	Radiation enhancement of urethane-induced lung tumours	Additive	Immunosurveillance by T cells not important in this system	[K41]
X rays	Dimethylhydrazine-induced inflammation of bowel [a]	BALB/c mice	Colon cancer	Additive		[W2]

a Relative for low doses.
b Uncertain whether relative for low doses.

17. In the early study of molecular genetics, a wealth of data were accumulated on interactions between UV and ionizing radiation in bacterial systems. For a review, see Annex L, "Biological effects of radiation in combination with other physical, chemical, and biological agents", in the UNSCEAR 1982 Report [U6]. Recently, laser applications have become important in industrial settings. A sparing effect from visible light on irradiated bacteria was reported by Voskanian et al. [V6]. The study measured the combined effect of laser (helium-neon laser, 633 nm) and alpha radiation on the survival of *Escherichia coli* K-12 cells of different genotypes. Pre- and post-irradiation exposures to laser radiation diminished the damaging effect of alpha particles. The increase in survival was more pronounced for post-irradiation exposure. There is a well-known molecular basis for enhanced DNA repair and hence for survival: photoreactivation with visible light after UV irradiation. The protective mechanism involved in the repair of damage from alpha irradiation, especially the one involved in pre-irradiation exposure to laser light, remains to be elucidated. However, at this stage there are no such mechanisms known in mammalian cells. Despite large human populations with considerable combined exposures to ionizing and UV radiation to parts of the skin, no indications of a critical interaction are apparent.

3. Low- and high-frequency electromagnetic radiation

18. The photon energies of all frequencies of electromagnetic radiation below infrared are clearly too low to produce direct chemical damage to DNA. However, there is a large body of published data suggesting the presence of effects at exposure levels below those from critical thermal effects, i.e. local heating by several degrees Celsius. (Heat stress is discussed in Section A.4.) The epigenetic influences of heat stress could only act on later stages of cancer development. Whether so-called athermic levels of high-frequency non-ionizing radiation may interfere with cell signalling, levels of cellular calcium, or systemic melatonin remains disputed on the level of the single agent.

19. Tyndall undertook an investigation [T3] to ascertain the combined effects of magnetic resonance imaging fields and x-irradiation on the developing eye in mice from the strain C57Bl/6J. Dams in groups were subjected to absorbed doses of 50, 150, and 300 mGy. Other dams were exposed to T2 spin-echo magnetic resonance imaging fields under clinically realistic conditions following exposure to 300 mGy from x-irradiation. It was found that the 300 mGy dose had significant teratogenic effects on the eye of C57BL/6J mice. Groups exposed to both types of radiation fields demonstrated malformation levels similar to those in animals irradiated only with 300 mGy from ionizing radiation. The results confirmed the teratogenic effects of low-level x rays but gave no evidence for an enhancement of the teratogenicity of x-irradiation on eye malformations in the mouse system tested.

20. Somewhat unexpected results of combined effect of microwave exposures of non-thermal intensity and ionizing radiation were reported in rats and chicken embryos by Grigor'ev et al. [G13, G16]. Rats were pre-exposed to electromagnetic radiation of power flux density (PFD) 200 $\mu W\ cm^{-2}$ 30 minutes daily for 8 days, followed the next day by single whole-body gamma irradiation at 5.5 Gy. Pre-exposure to microwave radiation reduced the mortality rate of the test animals by 33% compared with the controls. Immuno-biological examinations revealed a significant increase in the stimulation index in mitogen (phytohemagglutinin, PHA) induced lymphocytes. The imprinting of chicks was disrupted when they were irradiated in early embryogenesis for 5 minutes with microwaves (PFD = 40 $\mu W\ cm^{-2}$) and then with gamma rays at a dose of 0.36 Gy.

21. The same group also described changes in humoral immunity and in autoimmune processes under the combined action of microwave, infrasonic, and gamma irradiation [G13, G16]. The exposure regimens for rats and rabbits were 9.3 GHz and 0.1 GHz (200 and 1,530 $\mu W\ cm^{-2}$, respectively), infrasound (8 Hz, 115 db), and gamma radiation (cumulative dose of 5.5 Gy). It was shown that pre-irradiation with microwaves increased the resistance of the animal to gamma radiation, but microwaves combined with infrasound enhanced the biological effect of gamma radiation. Since no hypotheses on possible mechanisms are suggested, no inferences applicable to controlled human environments can be drawn at this stage from the extremely high exposure levels in this study.

22. A very strong radioprotective effect of static magnetic fields of 10, 120, and 350 mT on the survival of mice (CBA × C57Bl/6) after acute ^{60}Co irradiation with a dose of 9 Gy was described by Schein [S33]. The adaptive effect of an exposure of 6 hours in a static field increased with time and was strongest in animals irradiated 30 days later. The weakest field, 10 mT, led to a survival of up to 60% of the animals, whereas controls had survival rates of only 0%–4%. The mechanisms behind this antagonism are speculated to be unspecific stress-induced stimulation of endocrine systems by magnetic fields, an increase in surviving stem cells after ionizing radiation, or a faster proliferation and differentiation of bone marrow stem cells in adapted animals.

23. Growth and survival rates of cultured cells (FM3A) were investigated in a static gradient magnetic field with a strength of 58 mT at the center and a mean gradient of 0.6 $T\ m^{-1}$ [K16]. The magnetic field alone reduced the growth rate by 5% and survival by 20%. The combined effect of ^{60}Co irradiation followed by exposure to the magnetic field showed synergism.

24. Magnetic fields have been shown under certain reaction conditions to perturb the rates at which radical pairs recombine. An example is catalase-catalysed decomposition of H_2O_2, which is increased by 20% in an extremely high magnetic field of 0.8 T [M35]. In theory, this could lead to changes in the kinetics of free-radical production and recombination [S60]. To measure the interaction potential of this indirect genotoxic effect of magnetic fields with ionizing radiation, the exposures

would have to be simultaneous and not sequential, as described in the preceding paragraphs.

25. In assessing the association between exposure to electromagnetic fields and cancer, Koifman [K17] defined the elements necessary for quantitative analysis. Obtaining more accurate measurements of exposure to electromagnetic fields is a key to understanding any possible association. In certain circumstances, strong electromagnetic fields may stimulate growth and hence fulfill the characteristics of a cancer-promoter in biomechanistic models of carcinogenesis. This leads to the hypothesis that electromagnetic fields do not act alone to affect health, as is assumed in many epidemiological studies, but only where their action is combined with that of other initiator agents.

26. In summary, no straightforward inferences from experimental results to exposures in occupational settings are possible at this stage for the combination of electromagnetic and ionizing radiation. From the standpoint of mechanistic considerations, there is little evidence for potentially harmful interactions between the two radiation modes for controlled exposure levels in the workplace or in the clinic.

4. Temperature

27. Heat kills mammalian cells in a predictable and stochastic way [D3]. Heat stress at the cell and tissue level may disrupt energy metabolism (local depletion of oxygen and ATP) as a result of the enhanced reactivity of most enzymes, the production of heat shock proteins, and finally denaturation and cell death. Critical changes leading to a loss of proliferative capacity involve cell membrane blebbing, probably owing to detachment of the cytoskeleton from the plasma membrane [R23]. A slow mode of cell killing by hyperthermia in CHO cells involves the formation of multi-nucleated cells from damage to centrioles [D3]. Above 42.5°C, cell-survival curves for Chinese hamster ovary cells in culture where the abscissa is the duration of heat treatment are similar to the curves for x rays. At 42°C and below, the survival curves tend to flatten out with time as tolerance to the elevated temperature develops. The cell-cycle dependence of sensitivity to heat contrasts with that of x rays, with late S-phase cells being the most sensitive to hyperthermia treatment. Cells at low pH or deficient in nutrients also show elevated heat sensitivity. Temperature is therefore an important modifier of radiation sensitivity in many therapies to control tumour growth. In general, hyperthermia increases the relative susceptibility of tumour cells to radiation compared with healthy tissue. Very hot or very cold ambient temperatures are rarely encountered in the modern workplace and the temperatures that do prevail generally do not change the body core temperature. No correlation with elevated radiation exposure is apparent in such workplace settings. The same is true for recreational settings and even for hot spas with elevated radon levels. Therefore the combined action of high and low temperatures remains in the realm of clinical research, and the following paragraphs give only some cursory remarks on recent *in vitro* work.

28. At the mechanistic level, it is important to note that the large effects found in hyperthermia treatments cannot be attributed solely to changes in blood flow and concomitant changes in local oxygen pressure alone. The disruption of energy metabolism due to considerably accelerated biochemical reactions and a decrease in molecular stability are important far below the threshold of protein denaturation. Dauncey and Buttle [D2] found a tendency towards elevated plasma concentrations of growth hormone and prolactin in 14-week-old pigs acclimated to 35° or 10°C, respectively. In mammalian cell culture (L5178Y), protease inhibitors such as phenylmethylsulfonyl fluoride were shown to potentiate hyperthermic cell killing [Z13]. It is suggested that protease inhibitors sensitize by inhibiting the proteases that are needed to degrade denatured proteins induced by heat. In response to heat, cells and tissue produce proteins of mainly 70 and 90 kilodaltons. These proteins are called heat-shock proteins, although many other agents such as arsenite and ethanol also induce them. Their appearance coincides with the development of thermotolerance, an important effect that can influence the slope of the survival curve by a factor of up to 10. The development of thermotolerance and the production of heat-shock proteins occur during heating at temperatures up to 42°C (CHO cells) but are delayed by several hours for heat treatment with higher temperatures [H36].

29. Skin is the only tissue whose temperature might differ considerably from the core temperature. Therefore, Zölzer et al. [Z3] studied the influence of radiation and/or hyperthermia on the proliferation of human melanoma cells *in vitro*. DNA synthesis and content were both determined with two-parameter flow cytometry. In controls, most of the S-phase cells showed incorporation of BrUdR. The fraction of quiescent S-phase cells increased after irradiation (up to 8 Gy from x rays) and/or hyperthermia (up to 6 hours at 42°C or up to 2 hours at 43°C). There was a clear dose dependence for radiation and hyperthermia alone or in combination. In general, the combined effect seemed to be additive.

30. Combination effects of radiation and hyperthermia were found, however, in several other *in vitro* cell systems. Matsumoto et al. [M15] treated cultured human retinal pigment epithelial cells by radiation, hyperthermia, or a combination of the two. The effect on cell proliferation was evaluated by counting the cell number and measuring the uptake of bromodeoxyuridine. x-irradiation with a dose of 1 Gy or 3 Gy was not effective in suppressing proliferation of the retinal pigment epithelial cells. Similarly, heat treatment at 42°C for 30 minutes did not suppress proliferation. However, combining hyperthermia at 42°C for 30 minutes with 3 Gy irradiation suppressed cellular growth of the retinal pigment epithelial cells to 36% of the control, as estimated by cell counting, and to 48% by the bromodeoxyuridine uptake assay. The effect of radiation combined with heat on three human prostatic carcinoma cell lines was investigated by Kaver et al. [K6]. Cells were exposed to different radiation

doses followed by heat treatment at 43°C for 1 hour. Heat treatment given 10 minutes after radiation significantly reduced the survival rate of all the cell lines studied. The combined effect of radiation and heat produced greater cytotoxicity than predicted from the additive effects of the two individual treatment modalities alone. Impairment of DNA repair with elevated temperature is considered an important mechanism [W36].

31. Growth, cell proliferation, and morphological alterations *in vivo* in mammary carcinomas of C57 mice exposed to x rays and hyperthermia were followed by George et al. [G6]. Radiation doses of 10, 20, or 30 Gy from x rays or heating to 43°C for 30 minutes preceded or not by exposure to 10 Gy were studied. Tumour growth, cell proliferation kinetics, induction of micronuclei, and morphological changes in necrosis and vascular density were simultaneously determined. These showed very complex adaptive responses. Treatment with radiation and/or hyperthermia produced only a delay in tumour growth of between 1 and 3.8 days. However, the effects of the treatments became more apparent when the amounts of muscle and necrosis were deducted from the originally measured tumour volume. Radiation-induced G_2 block of the cells was observed 12 hours after radiation alone. After combined treatment, however, the G_2 block was delayed beyond 12 hours. Whereas the amount of necrosis was markedly enhanced five days after treatment with 10 Gy plus heat, as well as after 30 Gy, no changes in the density of small blood vessels could be observed during this period. These results clearly demonstrate that the apparent changes in tumour volume after x rays and hyperthermia do not truly reflect the response of the constituent cells and that there are many other factors, for instance cell proliferation and morphological alterations, that influence the combined effects of radiation and hyperthermia.

32. Heat shock before, during, or immediately after exposure to ionizing radiation can increase cell killing in a supra-additive manner [B70]. The heat-shock treatment was shown to inactivate the Ku auto-antigen binding to DNA, and this binding capacity of Ku was directly related to the hyperthermic radiosensitizing effect. The Ku auto-antigen is the regulatory subunit of the DNA-dependent protein kinase and is directly involved in DNA double-strand break repair and V(D)J recombination.

33. In general, it can be said that because of the high temperatures and exposures needed to produce enhanced cell killing in poorly oxygenated tissue, combined effects from hyperthermia and ionizing radiation are not relevant outside the realm of tumour therapy. Temperature in combination with ionizing radiation can act synergistically on cell survival, cell proliferation, and cytogenetic damage. However, temperatures higher than those found in the human body are needed to cause these effects.

5. Ultrasound

34. Possible effects from ultrasound exposures alone or in combination with ionizing radiation are of some concern because ultrasound is so widely used in diagnostic procedures. Above a threshold level, ultrasound by itself may induce cavitation, leading to mechanical damage to cellular structures and to microlesions. Kuwabara et al. [K20] studied the effects of ionizing radiation and ultrasound at exposure levels typical for diagnostic purposes on the induction of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes. No statistically significant increases in the frequencies of dicentric and ring chromosomes or sister chromatid exchanges were discovered after ultrasound exposure alone at the diagnostic level (Table A.2). Nor could elevated frequencies of these phenomena be found following exposure to ultrasound before or after ionizing radiation, compared with the frequencies found after the same dose of ionizing radiation alone. However, simultaneous exposure to ultrasound and ionizing radiation seemed to induce a slight enhancement of sister chromatid exchanges, although no significant changes were noted in the yields of dicentric and ring chromosomes.

Table A.2
Effects of combined exposures to ionizing radiation and ultrasound in peripheral human lymphocytes [K20]

Exposure		*Dicentrics and rings*	*Sister chromatid exchanges*
Radiation	*Ultrasound*		
None (control)	None (control)		6.64±0.40
3 Gy	None	0.61±0.08	7.92±0.54
	40 min (immediately following)		6.31±0.53
	80 min (immediately following)		7.00±0.47
	30 min (simultaneous)	0.52±0.07	9.80±0.91
4 Gy	None	1.12±0.11	
	30 min (simultaneous)	1.10±0.11	9.96±0.50

35. Continuous-wave ultrasound and neoplastic transformation was assayed *in vitro* by Harrison and Balcer-Kubiczek [H10] in C3H10T½ cells in suspension. An initiation-promotion protocol for neoplastic transformation induced by continuous-wave ultrasound was used. Cells were insonated at 1.8 MHz for 40 minutes. Two ultrasonic

intensities were used: 1.3 and 2.6 W cm^{-2} spatial average. The first intensity was found to be non-cytotoxic; the second was above the threshold level for cavitation and resulted in immediate lysis of 20% of the cells (cavitation-induced cell killing), followed by the clonogenic survival of 64% of the remaining cells. Ultrasound was delivered alone or in combination with x rays (2 Gy, 240 kVp given before ultrasound) and/or TPA (0.1 µg ml^{-1} after irradiation). Under all treatment conditions, ultrasound had no effect on transformation at the 95% confidence level. The effects of high-energy shock waves, i.e. therapeutic levels of ultrasound generated by a lithotripter in combination with ^{137}Cs gamma rays were shown to act additively or slightly supra-additively in colony-forming assays and cell-cycle analysis [F29]. Both pellets of single cells and multicellular spheroids of the bladder cancer cell line RT4 gave similar results.

36. In conclusion, it can be said that the ultrasound intensities used for diagnostic purposes and ionizing radiation did not interact to cause cytogenetic damage in treated cells. However, sister chromatid exchanges were slightly increased in one study. *In vitro* transformation rates caused by ionizing radiation were not changed by ultrasound.

6. Dust, asbestos, and other mineral fibres

37. The combination of radiation exposure and exposure to dusts and fibres is quite common in important industrial environments such as mining, metallurgical industries, and power plants. Some dusts and fibres are pathogenic or carcinogenic by themselves. Both experimental results from mammals and epidemiological evidence are available [B9, B13, C22, K13, P1, P5]. In cases where the main biological effect results from soluble toxicants that dissolve from the surface of dust particles to interact with biological structures, the interaction is basically between radiation and a chemical, which is dealt with in Section B of this Appendix.

38. Silica is often considered to be a co-carcinogen through the route of silicosis. Harlan and Costello [H9] studied 9,912 metal miners (369 silicotics and 9,543 non-silicotics) to investigate the association between silicosis and lung cancer mortality. When lung cancer mortality in silicotics and non-silicotics was compared, the age-adjusted rate ratio was 1.56 (95% CI: 0.91–2.68). Further adjustment for smoking yielded a rate ratio of 1.96 (95% CI: 0.98–3.67), and the value for employment in mines with low levels of radon was 2.59 (95% CI: 1.44–4.68). The statistical power of the study was too weak to quantify single contributions and interactions between metal, radon, silica, and smoking. For high dust loads and concomitant exposures to gamma radiation and radon in earlier times, there is indication for an increased lung cancer risk (standardized mortality ratio = 2.5 with 20 years of employment and hired before 1960) in the phosphate industry [B74].

39. The molecular mode of action of mineral fibres is quite distinct from radiation and genotoxic chemicals interacting directly with nuclear DNA. They are relatively ineffective as mutagens but quite powerful inducers of human mesotheliomas and bronchial cancers. Fibre dimensions, fibre durability, and surface characteristics are important properties affecting their carcinogenicity. In the case of asbestos, there is clear evidence for the induction of chromosomal aberrations and aneuploidy [B13]. A possible mechanism of asbestos cell toxicity is phagocytosis and accumulation of the fibres in the perinuclear region of cells. During mitosis, the fibres would then interfere with chromosome segregation, and chromosomal abnormalities would result. In addition, mechanical irritation and cell killing may lead to growth stimulation and transcellular epigenetic promotion. The production of active oxygen species on fibre surfaces was proposed as a directly acting genotoxic mechanism; however, the relatively long diffusion length from the site of radical production outside the nucleus to the target structures argues against the importance of this pathway.

40. Recent reviews of mortality and cancer morbidity in asbestos worker cohorts with large cumulative exposures showed an ERR for pleural mesothelioma of about 1 for each fibre-year ml^{-1} of air [A3]. For lung cancer, an ERR from 0.0009 to 0.08 per fibre-year ml^{-1} has been found [N9], which, in absolute terms, is considerably higher than the mesothelioma risk. The ratio of the number of mesotheliomas to the excess number of cases of lung cancer ranges from 0.06 to 0.78.

41. Few epidemiological data exist describing potential interactions between mineral fibres and radiation. In a case-control analysis of deaths from lung cancer among persons employed at the Portsmouth Naval Shipyard at Kittery, Maine, in the United States, elevated odds ratios for exposures to ionizing radiation, asbestos, and welding by-products were found in a first crude assessment. Further analysis of data on radiation exposure, controlling for exposures to asbestos and welding, found no evidence for a risk related to radiation exposure. The low cumulative radiation doses and the absence of data on cigarette smoking and socioeconomic status precluded an assessment of possible interactions among the three toxic agents [R1].

42. The synergistic effects of the combined exposures to asbestos and smoking in the causation of human lung cancer was one of the first examples of a supra-additive interaction of importance for protection in the workplace [S19]. In most studies, very high risk ratios were observed in asbestos-exposed subjects who were heavy smokers. The interaction observed in most cases conforms more closely to a multiplicative model than an additive one. Brown et al. [B7] were able to show in organ cultures derived from Fischer F344 rats that the ability to metabolize benzo(a)pyrene was significantly reduced after *in vivo* exposure to crocidolite, thus suggesting possible mechanisms leading to a departure from linearity. Work by Fasske [F2] showed that after the combined instillation of 1 mg chrysotile and 0.5 mg benzo(a)pyrene, lung tumours arose much earlier than after the instillation of only one of the carcinogens.

43. Regarding animal experimentation, Bignon et al. [B38] inoculated radon-exposed Sprague-Dawley rats intrapleurally with asbestos fibres, glass fibres, or quartz. In rats given mineral materials, bronchopulmonary carcinomas and mixed carcinomas were observed, as well as typical mesotheliomas and combined pulmonary pleural tumours, whereas in rats inhaling radon alone, only bronchopulmonary carcinomas occurred. A clear co-carcinogenic effect of the insult from the minerals was established for malignant thoracic tumours. Significant differences in survival time were found for exposures to different types of dust, depending on the additional tumour types induced. The same group also studied whether similar co-carcinogenic effects would take place over longer distances, i.e. from subcutaneous injection of chrysotile fibres. Neither mesotheliomas nor evidence of co-carcinogenic effects were found in the animals treated with both radon and asbestos fibres [M19]. Three groups of animals were used: 109 rats that inhaled radon only (dose = 1,600 working-level months [WLM]); 109 rats given a subcutaneous injection in the sacrococcygeal region of 20 mg of chrysotile fibres after inhalation of radon resulting in the same dose; and 105 rats injected with fibres only. As already stated, no mesotheliomas occurred in any of the three groups. The incidence of lung cancer was 55% in the second group, 49% in the first, and 1% in the third group. Statistical analysis using the Pike model showed that the carcinogenic insult was slightly higher in the second group than in the first group. Electron microscopy analysis of fibre translocation from the injection site showed that less than 1% of injected fibres migrated to the regional lymph nodes and only about 0.01% to the lungs. After injection, the mean length of the fibres recovered in lung parenchyma increased with time, suggesting that short fibres are cleared by pulmonary macrophages, whereas long fibres remain trapped in the alveolar walls. Kushneva [K49] studied pathological processes in the lungs of white rats exposed intratrachealy to 50 mg of finely dispersed quartz dust and to 3 hours of $3\ 10^8$ Bq m^{-3} radon. Supra-additivity is clearly implied but only described qualitatively.

44. To assess the possible co-carcinogenic effects of mineral dust in radon-prone mines, five groups of 30 Sprague-Dawley rats received minerals typically found in metal mines (nemalite; biotite, present in many granites; iron pyrite; chlorite) by intratracheal instillations one month after the end of a 1,000 WLM radon exposure. No or only slight co-carcinogenic effects were found [M62]. In earlier work with the same experimental system to investigate the effect of intrapleural injection of asbestos fibres (chrysotile), glass fibres, and quartz on the yield of radon-induced thoracic tumours, a clear promoting effect was noted [B38].

45. Densely ionizing alpha particles, similar to those emitted by radon progeny, are highly effective in inducing transformations in cell cultures such as CH310T½ cells. The yield of foci from combined alpha/asbestos exposure is clearly greater than would be predicted from the sum of the effects found with single-agent exposures. Figure A.I shows a clearly supra-additive interaction with asbestos fibres [H11].

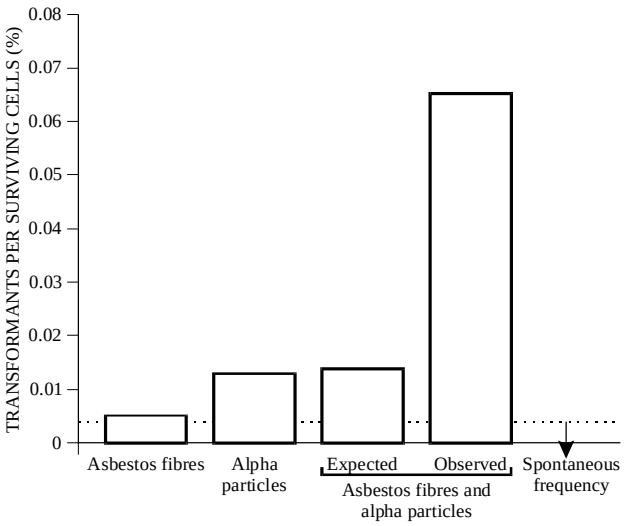

Figure A.I. *In vitro* transformation of C3H10T½ cells exposed to asbestos fibres and alpha particles alone and in combination [H11].

46. In an experimental study, Donham et al. [D10] studied possible combined effects of asbestos ingestion and localized x-irradiation of the colon in rats based on the hypothesis that the mucous produced by goblet cells that normally coats the normal bowel surface protects against tissue penetration by ingested asbestos. X-ray treatment results in localized damage to the colonic mucosa and theoretically disrupts the normal mucous coating, allowing increased tissue penetration by the fibres. To study this, segments of the colons of laboratory rats were exposed to x-irradiation. The animals were then divided into three groups, which were fed a diet containing 10% chrysotile asbestos, a diet containing 10% non-nutritive cellulose fibre, or a standard laboratory diet. Autopsies and histopathology were performed on all animals that died spontaneously and those that were killed at 350 days. Various types of inflammatory and degenerative lesions were commonly seen, but there was little difference in frequency between the diet groups. Five adenocarcinomas and two sarcomas were seen in the fibre groups (three tumours in the asbestos group and four tumours in the cellulose group), but no tumours were seen in animals on the standard diet. There was no significant difference in tumour rates between the asbestos and cellulose groups, nor was there a significant difference between the combined fibre groups and the standard diet group. Ingested asbestos did not increase the risk of tumour development and does not, therefore, seem to be co-carcinogenic or to promote tumours by disrupting the mucous coating.

47. In summary, it can be stated that mineral dust and fibres such as asbestos generally act through non-genotoxic mechanisms. These include mechanical irritation and cell killing. However, chromosomal aberrations, especially aneuploidy, can be induced by interfering with the spindle apparatus of mitotic cells. At exposure levels found in

workplaces until the early 1940s, there was a clearly supra-additive interaction between asbestos and tobacco smoke exposure in the causation of lung cancers, with a concomitant shift in the cancer spectrum from mesotheliomas to bronchopulmonary carcinomas. A similar supra-additive interaction and shift in the cancer spectrum was observed in animals exposed to both asbestos and radon. The much lower occupational exposures experienced today considerably decrease the risk for potential detrimental interactions between dust/fibres and radiation. However, in view of the proven interaction effects in humans, any stochastic and/or genotoxic effects of these agents merit further consideration.

7. Space flight

48. In space flight, which involves an extreme situation of controlled exposures, a multitude of stressors act in combination on astronauts, the most important being microgravity. Its biological and medical role has been extensively reviewed [M71]. Microgravity effects may occur at all levels of biological organization, and in principle can also lead to modifications of radiation action. From an experimental point of view there are no clear-cut results at the organ and tissue level. With simple organisms, a synergistic action of microgravity and radiation has been reported for teratogenic effects [B80]. Antipov et al. [A14] analysed structural and functional changes in the central nervous system of experimental animals exposed to the isolated and combined effects of space flights. They evaluated the significance of ionizing and non-ionizing radiation, hyperoxia, hypoxia, acceleration, vibration, and combined effects of some of these factors for anatomic and physiological changes in the rat brain. Neuronal functions were found to be sensitive to ionizing radiation and hypoxia, but these synapses were shown to be highly resistant to short-term hyperoxia and electromagnetic radiation [A13]. Along with radiation, the investigated stressors had additive, synergistic, and antagonistic effects on the central nervous system. However, as significant effects and deviations from the sum of effects from exposure to isolated stressors were always linked to high exposures and exposure rates, they have little relevance for exposure situations on the ground.

49. In radiobiological experiments in space, a more-than-additive interaction between microgravity and radiation was reported in several cases (reviewed in [H47]). Insect embryos in particular appear to be susceptible. Conflicting results were reported for cellular systems. In human lymphocytes that were exposed to ^{32}P-irradiation in space, chromosomal aberrations were significantly increased compared with ground controls [B18]. However, the follow-up experiment by the same authors did not show this interaction [B19]. More recently, experiments on the interaction of space microgravity and DNA repair were performed by Hornek et al. [H14]. Microgravity had no measurable effect on strand rejoining of x-ray-induced DNA strand breaks in *Escherichia coli* (120 Gy) and in human fibroblasts (5 and 10 Gy) or on the induction of SOS reponse in *E. coli* (300 Gy). In yeast no microgravity related effects on the repair of DNA double-strand breaks were found both for cells irradiated previously on ground [P31] or during flight using a ^{63}Ni beta source [P32]. Therefore, repair of radiation-induced DNA damage seems not to be disturbed by microgravity, and other mechanisms must be involved in the reported interaction between radiation and space gravity.

50. At similarly high exposures, Vasin and Semenova [V3] showed synergistic effects for combined stress from radiation and vibration or normobaric hyperoxia. A study was made of the combined effect of normobaric hyperoxia and vibration on the sensitivity of hybrid mice (CBA × C57Bl)F_1 and F_2(CBWA) to gamma radiation. Both single and protracted (for five days, daily) vibration before irradiation aggravated acute radiation sickness. Hyperoxia also enhanced the development of the intestinal form of radiation sickness. The combined effect of the two additional factors aggravated the intestinal syndrome of acute radiation sickness. These deterministic effects have no direct implication for present-day controlled exposure situations. Nevertheless, the changes of many parameters that are normally stable in experimental work on earth make well-designed studies in space potentially important in addressing the combined effects of physical agents.

B. RADIATION AND CHEMICAL TOXICANTS

51. A multitude of natural and man-made chemicals with cancer-initiating and -promoting potential are present in the human environment and may interact with radiation. Classification based on their mode of action is often difficult, but at least a crude separation can be made into substances that mainly act by damaging DNA directly (genotoxic substances) and non-genotoxic substances [C50]. The former group includes chemically active species (activation-independent chemicals) or species dependent on biotransformation and their active metabolites (activation-dependent chemicals). The mode of action is either direct, by forming covalent links with DNA, or indirect, via radical attack of DNA. The latter group comprises chemicals ranging from nonspecific irritants and cytotoxins to natural hormones and growth factors and their analogues that interact with the regulatory systems of cells and organs. At this point, chemicals that protect against ionizing radiation should also be mentioned. Many endogenous and exogenous sulfhydryl-carrying molecules as well as other radical-scavenging agents considerably reduce the primary damage and hence the clinical effects caused by radiation [M6, M7]. A wealth of experimental data is available to describe the action of single chemical agents, but the literature on interactions between these substances and other agents is far more sketchy. It is important to note that recent efforts to quantify tissue doses of chemical toxicants and their metabolites showed the decisive importance of interactions in activation and deactivation/excretion processes. For example, an assessment of the toxicity of benzene and its metabolites was

shown to depend crucially on the presence of other toxicants such as toluene, and this effect extended to concentrations found in human exposures [M60].

1. Genotoxic chemicals

52. The large group of genotoxic chemicals may be further subdivided on the basis of their need to be activated by metabolism. Most chemicals require metabolic activation through the generation of highly reactive electrophiles, which form DNA adducts by binding covalently to nucleic acids. The metabolism of any individual chemical can be very complex, because the chemical can be the substrate of several metabolizing enzymes. Genotoxic chemicals can also be subdivided based on whether the reactive compound acts directly by covalent binding to DNA or indirectly by the generation of free radicals. In the latter case, effects similar to those of radiation can be envisaged.

(a) Activation-independent alkylating agents

53. Modern cancer therapy involves many combined treatments using radiation and genotoxic drugs. Although exposures are well known and strong interactions exist, this human experience is of limited direct importance for risk assessment at low doses, because with therapy, cell killing is the main endpoint envisaged. Therefore this subject is considered separately in Section D of this Appendix. The occurrence of second primary tumours in healthy tissue adjacent to treated tumours is of great direct relevance.

54. Morishita et al. [M32] examined the effects of x rays on N-methyl-N-nitrosourea (MNU)-induced multi-organ carcinogenesis in both sexes of ACI rats. Rats were treated with MNU (25 or 50 mg kg^{-1}) at 6 weeks of age and/or with x rays (3 Gy) at 10 weeks of age. The incidence of adenocarcinomas in the small and large intestines of male rats treated with 50 mg kg^{-1} MNU and x-irradiation (small intestine, 48%; large intestine, 32%) was significantly higher than the sum of the incidences resulting from 50 mg kg^{-1} MNU alone (small intestine, 17%; large intestine, 8%) and with radiation only (small intestine, 0%; large intestine, 0%) and also higher than the frequency of adenocarcinomas in the large intestine of males treated with 25 mg kg^{-1} MNU alone (0%). Strongly synergistic effects in these high-exposure studies were restricted to the gastrointestinal system. When MNU or 1,2-dimethylhydrazine (DMH) treatment was started two months after x-irradiation, no induction of gastric tumours was observed with MNU [W3], and only a low incidence was observed with DMH [A7]. Surprisingly, an inverse relationship between incidences of gastric tumours and intestinal metaplasias was apparent. These findings again indicate the importance of the order and timing of the exposures in the induction of combined effects. It comes as a further surprise that the presence of intestinal metaplasia, long considered a basis for further malignant growth, does not exert a positive influence on the induction of gastric neoplasia by MNU in the rat.

55. Seidel [S22] studied the effects of radiation on chemically induced T-cell lymphomas (thymomas) in BDF_1 mice. N-methyl-N-nitrosourea or butylnitrosourea (BNU) were the main inducers, and x rays in various dose schedules were applied. The radiation was seen to shorten the latency period between induction and lymphoma emergence in protocols of 12 exposures of 0.25 Gy. This effect was most pronounced compared with chemically induced non-irradiated controls with a prolonged median induction time as a result of a dose reduction of the chemical (median induction time 27–36 weeks instead of 16–18 weeks under optimal conditions using 50 mg kg^{-1} of MNU). Irradiation 2–5 weeks before administrating 40 mg kg^{-1} of MNU also enhanced leukaemogenesis. Again, mice with regenerating lymphohaemopoiesis after lethal irradiation and bone marrow transplantation were more sensitive to both chemicals than were the controls. Combined effects from radiation and N-ethyl-N-nitrosourea (ENU) on neural tumours in Wistar rats were reported by Hasgekar et al. [H6]. The animals received 2 Gy whole-body irradiation, followed immediately by 10 mg kg^{-1} of ENU on the day of birth. Of 33 rats given ENU alone, 14 developed 22 tumours of the nervous system, of which 15 (68%) were gliomas and 7 (32%) were schwannomas. Of 34 rats given both irradiation and ENU, 12 were found to harbour 15 neural tumours, of which 14 (93%) were gliomas and 1 (7.1%) was a schwannoma. The pretreatment with irradiation seems to have resulted in selective suppression of schwannoma induction. Whether this antagonistic relationship is a result of overkill or whether it may be relevant for lower radiation doses remains to be elucidated.

56. The combined effects of radiation and BNU on murine T-cell leukaemogenesis was studied by Seidel and Bischof [S20] in BDF_1 mice. The animals were exposed to BNU (0.02% in drinking water) for 12 weeks, and they died of thymic lymphomas with median latency periods of 12–20 weeks. Groups of mice received weekly radiation doses of 0.06–1.0 Gy in addition to BNU. Lower doses (12 × 0.25 Gy) enhanced leukaemogenesis, high doses (12 × 0.75 Gy) delayed it, and intermediate doses (12 × 0.50 Gy) had no effect. Doses lower than 12 × 0.25 Gy had marginal enhancing effects. After a dose of 12 × 1.0 Gy, the mice died earlier than after treatment with BNU alone, and as with the dose of 12 × 0.75 Gy, some extrathymic lymphomas were observed. The numbers of CFU-S in the femur and the spleen showed a dose-dependent depression, in addition to the decrease from BNU alone. In lymphocyte stimulation assays with Con A and LPS and also in the mixed lymphocyte reaction, a reduced proliferation was found, again dependent on the radiation dose. Thus, there was an inverse correlation between leukaemogenesis and the degree of stem-cell reduction or depression of these immune parameters.

57. Stammberger et al. [S13] analysed the activity of O^6-alkylguanine-DNA alkyltransferase (AT) in the fetal brain and liver and made long-term observations of Wistar rats that were treated *in utero* either with x-irradiation

(1 or 2 Gy), with ENU (50 mg kg^{-1}), or with both in combination. They hoped to reveal any relationship between the O^6-alkylguanine repair capability and tumour incidence in the organs of the offspring. The AT activity in the brain was affected to the same extent in the fetuses as in the dams. There was a 61% decrease in AT activity in fetuses 24 hours after ENU treatment. This correlated with a significant increase in the incidence of brain tumours in the treated offspring (44%) compared with control animals. The inductive effects of x-irradiation on AT activity (131% for 1 Gy and 202% for 2 Gy) corresponded with a reduction in the incidence of tumours after the combined treatment (27% and 8.3% tumour incidence, 103% and 158% AT activity). Comparing biochemical and morphological results suggests that this antagonistic effect may be the result of the AT induction by x rays.

58. Yokoro et al. [Y6] found that whole-body irradiation facilitates chemically initiated T-cell lymphomagenesis in mice. This was attributed to the amplification of the cell population susceptible to a chemical carcinogen in the target tissues, bone marrow, and thymus during the recovery phase after irradiation. Split administration of ENU showed different effects in the different phases of carcinogenesis leading to T-cell lymphomas. Once more the authors emphasized that after a cell has been initiated by a genotoxic agent, its fate is determined by the presence of promoters and inhibitors and that modifiers of target cells play a crucial role in the induction yield of tumours. The possibility of synergistic effects in carcinogenesis due to changes in cellular kinetics brought about by combined treatment with radiation and ENU was studied by Seyama et al. [S21]. Lymphomas in female C57Bl/6N mice were used as a model system. A single intragastric administration of 5 mg (about 200 mg kg^{-1} body weight) of ENU was only slightly lymphomagenic, inducing thymic lymphomas in 20% of mice; the incidence was elevated to 92% if the ENU treatment was preceded (five days earlier) by 4 Gy from whole-body x-irradiation, which alone is seldom lymphomagenic. A high yield of lymphoma (84%–93%) was also obtained when 5 mg (about 200 mg kg^{-1}) of ENU was delivered in two split doses four days apart of 4 mg and 1 mg (160 and 40 mg kg^{-1}), indicating that cellular kinetics or clonal expansion, but not two agent-specific different initiation events in the combined treatment, is at the root of this apparent synergism. Drastic injury to both the thymus and bone marrow caused by either 4 Gy whole-body x-irradiation or the first dose of ENU (4 mg, or about 160 mg kg^{-1}) was followed by a vigorous regeneration within a few days. The maximum induction rate of lymphoma was obtained when the subsequent dose of ENU (1 mg, or 40 mg kg^{-1}) was given at the peak of DNA synthesis in the bone marrow and thymus following the first treatment. The data indicate that the principal effect of irradiation or the first dose of ENU was to provide a susceptible cell population, and that a high yield of lymphomas was brought about by the action of the subsequent dose of ENU on a larger number of potentially radiation-modified target cells engaged in heightened DNA synthesis.

59. A clear antagonistic effect of ENU and x-irradiation was observed by Knowles [K14, K15] for neurogenic tumours in neonatal rats. After neonatal injection of rats with 10 mg kg^{-1} of ENU, whole-body x-irradiation with 1.25 Gy caused a reduction in induced neurogenic tumours, which was greatest when radiation was given 1 day after ENU and progressively decreased with irradiation at 5 and 30 days. Although x-irradiation did not affect the range of histological appearances in the tumours, malignant schwannomas, particularly those of the trigeminal nerve, were significantly reduced by 1.25 Gy given after ENU (10 mg kg^{-1}). The mean latency for clinical signs of tumour appearance was not affected by radiation. Another important finding in this study also points to the importance of the size of stem cell pools in interactions: a significant reduction in the high spontaneous incidence of squamous-cell carcinomas of the mouth in the inbred strain used after 1.25 Gy from x-irradiation. The reduction was greater after irradiation at 5 days of age than at 30 days. A large study on the incidence rates of neural, pituitary, and mammary tumours in Sprague-Dawley rats treated with x-irradiation and ENU during the early post-natal period was undertaken by Mandybur et al. [M2]. These late effects of early post-natal treatment with ENU, preceded by x-irradiation to the head, were studied in 226 neonatal CD rats. The animals were divided into six groups, each receiving one of the following treatments: x-irradiation with 5 Gy to the head on the third post-natal day; ip injection with 30 mg kg^{-1} ENU on the fourth post-natal day; ip injection with 30 mg kg^{-1} ENU on the seventh post-natal day; a combination of x-irradiation with 5 Gy to the head on the third post-natal day, followed by ip 30 mg kg^{-1} ENU on the fourth post-natal day; a combination of x-irradiation of 5 Gy to the head on the third post-natal day, followed by ip 30 mg kg^{-1} ENU on the seventh post-natal day; and untreated controls. The results indicated that (a) x-irradiation to the head alone significantly extended the lifespan of females compared with that of control females and did not affect the survival of males; (b) x-irradiation did not influence the latency period or mortality from neurogenic tumours when ENU was given 1 or 3 days afterwards; (c) ENU itself was a factor in shortening latency periods for mammary tumours; (d) x-irradiation alone did not increase the incidence of mammary tumours and revealed no protective effect on the ENU-induced mammary carcinogenesis; (e) x-irradiation increased the prevalence of pituitary tumours in the females; (f) no enhancement of pituitary tumours by ENU was observed; and (g) there was a statistically significant association of pituitary and mammary tumours in females. Again, these widely divergent findings speak against the possibility of simple concepts for the interaction of different genotoxic agents.

60. Post-natal development and cancer patterns in NMRI mice after combined treatment with ENU and x-irradiation on different days of the fetal period were studied by Wiggenhauser and Schmahl [W10]. When mice were irradiated to 1 Gy on day 14, 15, or 16 of gestation, this did not result in an increased tumour frequency in the offspring until 12 months. Mice treated with ENU (45 mg kg^{-1}) on day 15 of gestation developed a significantly increased tumour frequency in the lungs and liver and in the ovaries. After

combined treatment in the sequence x rays plus ENU with an interval of 4 hours, a significantly increased incidence of animals with tumours was observed in the offspring treated on gestation day 14 or 16. Moreover, the treatment on day 16 exhibited the highest tumour frequency per examined animal (5.7) of all treatment groups. Although the result was due to a relatively uniform increase of all tumour types, the frequency of liver tumours was most marked. In the reverse sequence (ENU plus x rays), the total tumour outcome was not significantly altered compared with the effects of ENU alone. However, detailed analysis also showed a significant augmentation of the liver tumour frequency with treatment on day 15.

(b) Metabolism-dependent alkylating agents

61. Maisin et al. [M8] studied the effects of x rays alone or combined with the initiator diethylnitrosamine (DEN) on liver cancer induction in infant C57Bl/Cnb mice. The number of induced liver foci and carcinomas was found to depend essentially on the dose of DEN. X rays did not produce any combined effect on the induction of foci or carcinomas when given seven days before or after administration of DEN [M34]. Using the same system for exposures to DEN and neutrons (average energy = 3.1 MeV), it was shown that even high-LET irradiation (0.125–0.5 Gy) initiated only small numbers of nodular lesions, whereas DEN alone increased liver nodules significantly and proportional to dose (0.3–2.5 µg). A supra-additive interaction between the two initiating agents was found mainly in the increased rate of foci appearance after 1.25 µg of DEN and 0.125 Gy of neutrons, both given seven days before or after DEN exposure [M33]. Peraino et al. [P26] studied three altered hepatocyte foci (elevated gamma-glutamyl transpeptidase [GG+] and/or iron-exclusion [Fe–]) in Sprague-Dawley rats exposed to DEN (0.15 µmol g^{-1}) and/or gamma rays (0.75, 1.5, and 3 Gy) shortly after birth. The exposure was followed by a phenobarbital (0.05%) diet to promote focus expression. Radiation alone was a weak hepatocarcinogen. A strong synergism was seen at the lower radiation doses for the induction of [GG+] foci but not for other focus phenotypes. A qualitatively different type of genetic damage for DEN (point mutations) and for radiation (rearrangements) is postulated from the result. Large sex differences in the yield of DEN-induced [GG+/Fe–] foci by a factor of up to 10 are additional indicators of the complexity of this system.

62. The potential for pulmonary carcinogenic interactions between $^{239}PuO_2$ and the tobacco-specific nitrosamine 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a genotoxic lung carcinogen, was studied in 740 male rats [L17]. The animals received $^{239}PuO_2$ by inhalation to result in lung burdens of 0 or 470 Bq. The NNK was administered by multiple ip injection at doses of 0, 0.3, 1.0, or 50 mg kg^{-1}. The highest dose of NNK markedly reduced the median lifespan of the rats, whereas in the other treatment groups survival was minimally reduced in comparison with the controls. Results on carcinogenicity are not yet available from this study.

63. An apparent synergism between low-LET ionizing radiation and the carcinogen 1,2-dimethylhydrazine (DMH) in the induction of colonic tumours in rats has been described by Sharp and Crouse [S27]. They evaluated the interaction of radiation (9 Gy to the abdomen only) and DMH (150 mg kg^{-1}) with respect to colon carcinogenesis in male Fischer 344 rats. Radiation was administered 3.5 days before the DMH. At eight months post-treatment, the incidence of DMH-induced colon tumours was doubled by prior radiation exposure. When the protocol of radiation plus DMH was repeated three times at monthly intervals, a 15-fold increase in tumour incidence (from 5% to 74%) was observed at six months post-treatment. This finding demonstrated an apparent synergy between radiation and the chemical carcinogen. Throughout the study, the appearance of carcinomas was associated with pre-existing colonic lymphoid nodules. The reproducibility of tumour induction as well as the range of tumour incidence generated by treatment variations in this system appeared to be sensitive enough to allow the examination of combined effects of much lower doses of radiation and/or chemical carcinogens. The model could be used to evaluate the relationship between existing lymphoid aggregates, which alter local epithelial cell kinetics and are associated with fenestrations in the basement membrane. The quantification of the development of colon cancer in congruent sites may assist in defining dose-response curves for combined agents and may also provide a system for evaluating the mechanisms underlying their interactions. When DMH treatment was started two months after x-irradiation, only a slight increase in gastric tumour incidence was recorded [A7]. These tumours occurred on top of a background of radiation-induced gastrointestinal metaplasia.

64. Ehling and Neuhäuser-Klaus [E7] studied the induction of specific-locus and dominant-lethal mutations by combined cyclophosphamide (see also Section D.1.a for uses in combined modalities in tumour therapy) and radiation treatment in male mice. Unlike radiation, this widely used antineoplastic agent, used alone, induced recessive mutations in spermatozoa and spermatids but not in spermatocytes and spermatogonia. Pretreatment (with 60 mg kg^{-1}) 24 hours before radiation, however, enhanced the frequency of specific-locus mutations in spermatogonia. The mutational spectrum among seven loci remained the same as in animals treated only with radiation. The synergistic interaction was mechanistically explained by the interference of cyclophosphamide, a strong inhibitor of DNA and RNA synthesis, with repair of radiation-induced damage.

65. The effect of radiation on chemical hepatocarcinogenesis has also been examined in male ACI/N rats [M26]. The number of neoplastic nodules or hepatocellular carcinomas in rats given N 2-fluorenylacetamide (FAA) (0.02% in diet for 16 weeks) followed by x-irradiation (3 Gy) was significantly greater than in rats given FAA alone ($p < 0.001$). In addition, the incidence of hepatocellular carcinomas in rats given the combined treatment was also higher than in rats given FAA alone ($p < 0.003$). No liver lesions

were found in animals receiving only an x-ray dose of 3 Gy. The authors suggested that these highly supra-additive results indicate that ionizing radiation acts as a promoter in this model.

66. An inhibition of urethane(ethyl carbamate)-induced pulmonary adenomas by inhaled ^{239}Pu in random-bred male A2G mice was reported as far back as 1973 by Brightwell and Heppleston [B66, B67]. This early study of combined exposure to alpha radiation and a genotoxic chemical comprised four groups, each of 32 animals, receiving plutonium inhalation followed by urethane (PU), plutonium followed by saline (PS), mock inhalation followed by urethane (MU), and mock inhalation followed by saline (MS). Exposures consisted of initial lung burdens of 925 Bq ^{239}Pu and ip urethane injections of 1 mg g^{-1} body weight two weeks later. Eight weeks after the injections, PS-treated animals showed no increase in pulmonary tumours over control animals (MS), whereas practically all animals in the PU and MU groups had multiple tumours. The number of tumours per animal 8, 16, and 24 weeks after urethane treatment was clearly lower in the PU group, which had 4.2, 11.4, and 13 as compared with 8, 24.4, and 38 in the MU group. An earlier hypothesis, that this finding is the result of alpha irradiation counteracting immuno-suppression by urethane, is rejected on the basis of ultrastructural evidence. Severe morphological changes in mouse type-II cells in the vicinity of alpha particles indicate that functional impairment of the initiated cells is the main cause of the effect. The authors said, however, that this apparent antagonism needs to be viewed with caution; it remains to be determined, they concluded, if much smaller local plutonium doses would augment urethane tumorigenesis.

67. The transgenerational combined effects of x rays (2.2 Gy) and urethane were studied by Nomura [N23, N24] in three different mice strains (ICR, LZ, and N5). Urethane treatment of F_1 offspring of either irradiated males or females yielded an 18% incidence of tumour nodule clusters in the lung compared with only 2.8% in offspring of non-irradiated controls. Tumour clusters were defined as having 12 or more nodules. The transgenerational effect of radiation alone resulted in lung tumours (at least one tumour nodule) in 7.5% of the animals, whereas the value in unexposed controls was 4.7%.

68. The interaction of gamma rays with urethane in lung tumorigenesis in mice in relation to the immune status has been studied by Kobayashi et al. [K41]. Male athymic nude mice (nu/nu) and their female heterozygous litter mates (nu/+) were treated with 1–4 Gy of ^{137}Cs gamma rays and 0.5 mg g^{-1} of urethane. Gamma-ray exposure alone caused relatively few lung tumours (in up to 10% of animals); urethane alone caused tumours in 70%–80%. The combined effect was supra-additive. There was a tendency towards higher yields in nu/+ mice, suggesting that impaired immunosurveillance from T-cell deficiency does not increase lung tumorigenesis in this system. Since relatively radiation-resistant macrophages and natural killer cells had higher activities in nu/nu mice, the authors concluded that the influence of immunological status on tumorigenesis remained unresolved.

69. A strong synergism was found by Hoshino and Tanooka [H39] for skin tumours in beta-irradiated ICR mice painted later with 4-nitroquinoline 1-oxide (4NQO); 27 Gy of ^{90}Sr/^{90}Y radiation or 20 applications of 5 mg ml^{-1} 4NQO in benzene to the skin alone did not produce any skin tumours in groups of 50 mice. Radiation followed by 4NQO painting with an interval of 11–408 days between the two treatments resulted in an incidence of malignant skin tumours (squamous-cell carcinomas and papillomas) of up to 17%. There was no significant decrease of the synergistic effect with increasing interval, the greatest effect being seen with an interval of 234 days.

70. A notable finding indicating the considerable uncertainties and misinterpreting the results of experimental animal studies was described by Little et al. [L55], who studied the potential synergistic interactions between ^{210}Po (185 Bq, resulting in a lifetime lung dose of about 3 Gy) and benzo[a]pyrene (0.3 mg) in the induction of lung cancer in Syrian golden hamsters. It was shown that simultaneous administration by intratracheal instillation led to additive effects. A significant apparent synergism was found when benzo(a)pyrene was given 4 months after the ^{210}Po. Most of this effect could be ascribed, however, to a potentiating effect of the seemingly innocuous 0.9% NaCl instillation solution alone.

71. The effects of repeated low exposures at high dose rates such as used in some diagnostic radiologic procedures at the time of the study were published by Lurie and Cutler in 1979 [L56]. The induction of lingual tumours by 7,12-dimethylbenz[a]anthracene (DMBA) and radiation to the head and neck was studied in Syrian golden hamsters. Treatment schedules were topical application of 0.5% DMBA in acetone on the lateral middle third of the tongue three times a week for 15 consecutive weeks, about 200 mGy radiation exposures (x rays with 100 kV peak) of the head and neck once a week for 15 consecutive weeks, or concurrent radiation and DMBA treatments for 15 consecutive weeks. Histopathology was performed 35 weeks after the start of the treatment. Animals receiving radiation alone had no detectable changes. The combined treatment led to an excess of lingual papillomas compared with animals receiving only DMBA (35% versus 15%). In addition, an excess of non-lingual oral tumours (lip, gingiva, and floor of the mouth) was found in the animals receiving the combined treatment compared with the DMBA-treated animals. Whether this radiation enhancement of DMBA-induced tumorigenesis has implications for the lower combined exposures found for cigarette-smoke-derived carcinogens in the bucal cavity of humans and dental x rays, remains to be elucidated.

72. Studies on chromosome aberrations from the combined effect of gamma rays and the mutagen thiotepa on unstimulated human leukocytes showed no significant

difference from the sum of their separately induced effects. The sequence of treatment and the interval between them (up to 4 hours) did not affect the frequency of chromosome aberrations [B21].

73. Leenhouts et al. [L13] investigated the combined effect of 1,2-dibromoethane (DBE) and x rays on the induction of somatic mutations in the stamen hair cells of tradescantia KU 9. At low radiation doses, a synergistic interaction was found between the two agents for both DBE exposure followed by acute x rays and chronic simultaneous exposures. The synergism was considered to result from an interaction of single-strand lesions in the DNA. It was concluded that this type of interaction would not be too important for radiological protection. However, it could be of significance in evaluating the effects of chemicals at low exposure rates.

(c) Free-radical-generating chemicals

74. Superoxide (O_2^-) generating agents such as the dipyridilium compound paraquat might also interact directly with the fixation or repair of radiation-induced damage. Geard et al. [G5] investigated the combined effects of paraquat and radiation on mouse C3H10T½ cells. Effects on oncogenic transformation, chromosome alteration, cytokinetics, or cellular survival were the endpoints measured. Paraquat alone is a cytotoxic agent and is also a weak radiosensitizer. Treatment with 0.1 mM for 24 hours results in about 30% cell survival and enhances the cell-killing effects of ^{137}Cs gamma rays by a factor of about 1.2. The drug appears to function lethally by initiating interphase cell death and also by slowing cell cycling. In combination with radiation (3 Gy), paraquat acted either additively (sister chromatid exchanges) or with a greater-than-additive effect (cell survival and oncogenic transformation).

75. De Luca et al. [D6] studied the induction of reciprocal translocations in mouse germ cells (BALB/c) by bleomycin alone or combined with radiation (see also Section D.3 for bleomycin used in combined modalities in tumour therapy). The dose-response relationships after treatments with doses of 20, 40, and 60 mg kg^{-1} of bleomycin as well as the combined effect of bleomycin and gamma rays were studied. A positive, significant correlation between the dose of bleomycin and the frequency of translocations was found. Both potentiation and additivity were found when the yields of translocations induced after combined treatments, separated by a lapse of 24 hours, were compared with the sum of translocation frequencies induced after the corresponding single treatments. Potentiation occurred in the treatments with 1 Gy plus 9 Gy and 60 mg kg^{-1} of bleomycin plus 9 Gy, while additivity occurred in the treatments with 60 mg kg^{-1} of bleomycin plus 1 Gy and 1 Gy plus 60 mg kg^{-1} of bleomycin. In mice irradiated with 1 Gy plus 9 Gy and mice treated with 60 mg kg^{-1} of bleomycin plus 9 Gy, similar translocation yields were found. The potentiating effect of bleomycin was found to be similar to that obtained with non-radiomimetic compounds such as triethylenemelamine, cyclophosphamide, and adriamycin. The high doses involved and the erratic changes from synergistic to additive relationships preclude extending inferences from these experiments beyond cancer therapy to occupational or non-occupational settings.

76. In summary, there are many examples of strong deviations from hetero- and isoadditivity in the interactions between genotoxic chemicals and ionizing radiation (Table A.1). Owing to generally high exposures to both agents under study, deterministic effects were shown or suspected to be the cause of strong deviations from additivity in many studies. Thus, the several cases of synergism found seem to be mostly the result of modifications of the biokinetics and the metabolism of the chemical rather than of agent-specific genotoxicity at different stages of the pathological processes. Similar considerations hold for antagonistic effects, where depletion of stem cells and inhibition of cellular growth may be a factor in the high dose range. Additional risks, beyond the level predicted from isoaddition, from the combined effects of ionizing radiation and genotoxic chemicals at low exposures levels are, accordingly, not specifically demonstrated by the many epidemiological and experimental studies reviewed in this Section.

2. Non-genotoxic chemicals

77. Many chemicals in the human environment or their metabolites do not specifically attack DNA but influence cell proliferation and cell differentiation on an epigenetic level. Specific mitogens may interfere with regulatory mechanisms and cell-cell signaling, but many substances with a high chemical reactivity act as unspecific irritants or toxicants via membranes or proteins. Toxin-induced cell death will induce proliferation in neighbouring cells, which may enhance the progression of premalignant cells. Substances acting in a non-specific manner, for example lipophilic solvents, quite often show highly non-linear dose-response relationships with apparent thresholds. Other agents may interfere with the critical cellular processes involved in repairing damage to cellular constituents such as DNA. The assessment of possible synergistic effects at the exposure levels relevant to this Annex is very difficult, because of the high exposures used in experimental systems and the apparent threshold levels.

78. The tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) has the potential to enhance the yield of radiation-induced tumours. This has been well documented *in vitro* and in animal systems. The combined effects of paternal x-irradiation and TPA on skin tumours in two generations of descendants of male mice was studied by Vorobtsova et al. [V5]. Progeny of outbred SHR male mice non-irradiated or exposed to a single dose of whole-body x-irradiation (4.2 Gy) were skin-painted twice a week for 24 consecutive weeks from the age of four months onwards with acetone or with TPA in acetone (6.15 µg ml^{-1}). The incidence and number of skin papillomas were monitored between week 2 and week 20 after the last application of the promoter (TPA). Exposure to acetone was never followed by skin tumour development in the progeny of either irradiated or non-irradiated males. Two weeks after

TPA treatment, the incidence of skin tumours in the progeny of non-irradiated mice was 21% in males and 37% in females, and 20 weeks later it was 12% in males and 15% in females. The skin tumour incidence in the progeny of the irradiated male mice 2 and 20 weeks after the last painting was clearly elevated: 75% and 68% in males and 50% and 43% in females, respectively. Some of the F_1 offspring of irradiated male mice were mated before the start of TPA treatment, and F_2 progeny were exposed to acetone or TPA as F_1. The incidence of skin papilloma 2 weeks after the last TPA painting was 58% in males and 40% in females, whereas at 20 weeks after the last exposure to the promoter it was 53% and 36%, respectively. In the progeny of irradiated male mice there were more animals with multiple (>4) skin papillomas than in the progeny of non-irradiated mice. The incidence of other than skin tumours in offspring was also clearly increased in TPA-treated progeny from irradiated male mice. The authors suggested that irradiation of males before mating increases the susceptibility of progeny in at least two generations to promoters of carcinogenesis as a result of persisting genomic instability. On the other hand, Brandner et al. [B30] found no influence of ip-administered TPA on the incidence of radiation lymphomas in C57Bl/6 mice. Female C57Bl/6 mice, given four x-irradiations each with 1.7 Gy, developed lethal lymphomas in more than 90% of animals 270 days after irradiation. Intraperitoneal application of TPA, 30 ng g^{-1} twice weekly for 240 days, had no influence on survival of the animals or on incidence of the malignant lymphomas. However, the incidence in radiation-only treated animals was already so high that this test was highly insensitive to the promoting effects of TPA.

79. Jaffe et al. [J2] studied the effect of proliferation and promotion time on radiation-initiated tumour incidence in Sencar mice. In this system, a single subcarcinogenic dose of ionizing radiation followed by 60 weeks of TPA treatment led to the formation of squamous-cell carcinomas. Even TPA pretreatment before irradiation seemed to result in an overall increase in total tumour incidence, including both epidermal and non-epidermal tumours [J1]. Based on these findings, the effect of the proliferative state of the skin before irradiation and the promotion duration after irradiation on tumour incidence was further investigated in CD-1 mice. To examine the influence of the proliferative state of the skin, a 17 nmol TPA solution was applied to one half of the mice 24 hours before irradiation. The skin was irradiated using 4 MeV x rays at a dose rate of 0.31 Gy min^{-1}. Animals received a single dose of x rays of 0.5 or 11.3 Gy, followed by twice weekly applications of TPA (8 nmol). The animals were then promoted for either 10 or 60 weeks. All animals promoted with TPA for the same duration had a similar incidence of papillomas regardless of radiation or TPA pretreatment. Increasing the promotion duration did not significantly alter the incidence of squamous-cell carcinomas at either initiation dose. At the lower initiation dose, only animals that were promoted for 60 weeks developed squamous-cell carcinomas. TPA pretreatment at the higher dose resulted in a slight decrease in tumour incidence; however, this was not statistically significant. The incidence of basal-cell carcinomas was radiation-dose-dependent and appeared to be independent of TPA promotion. Again, as in many other cases, no common pattern emerged for the different tumour types.

80. The interaction between ionizing radiation and TPA has been studied using a three stage model of initiation, promotion, and progression. Ionizing radiation is well established as an initiator, whereas its potential for promotion and progression is less well known. Therefore, Jaffe and Bowden [J1] performed a three-stage experiment using ionizing radiation in the third stage of mouse skin carcinogenesis. CD-1 mice were initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), followed by biweekly promotion with TPA. After 20 weeks of promotion, the animals were treated with either acetone, TPA (twice a week for two weeks), or eight fractions of 1 MeV electrons (1 Gy per fraction over a period of 10 days). The conversion of papillomas to squamous-cell carcinomas was 80% for animals treated with ionizing radiation compared with 25% for tumour-bearing animals treated with TPA. Ionizing radiation increased the number of cumulative carcinomas per group. The absence of an increase in the number of cumulative papillomas per group due to late exposure to ionizing radiation suggests that the dose and fractionation protocol used in this study enhanced the progression of pre-existing papillomas.

81. The tumour-initiating and -promoting effects of ionizing radiation in mouse skin was also studied with TPA by Ootsuyama and Tanooka [O2]. Neither single 24 Gy $^{90}Sr/^{90}Y$ beta irradiation followed by repetitive treatment with TPA nor single pretreatment with 7,12-dimethylbenz-(alpha)-anthracene (DMBA), followed by repetitive 4.7 Gy beta irradiation, produced tumours above the level of significance within a period of 210 days, while a positive control, DMBA + TPA, yielded a high incidence of papilloma in a shorter period. In this system, DMBA seemed to exert an action antagonistic to beta particles in the induction of malignant tumours. It was concluded that the tumour-enhancing activity of repetitive radiation is qualitatively different from the promoting activity of TPA.

82. Nomura et al. [N6] were able to show that *in utero* irradiation at early stages of embryogenesis, which was not visibly carcinogenic by itself in a tester strain of mice (PT × HT F_1), followed by post-natal application of TPA, led to a high incidence of skin tumours. Radiation doses in this system were 0.3 and 1.0 Gy of 180 kVp x rays, respectively, at about 10.5 days after fertilization. Two dose rates, 0.54 and 0.0043 Gy min^{-1}, were used. The incidence of both embryonic mutations, determined as spots of different coat color, and tumours increased with *in utero* doses. Low-dose-rate irradiation led to a large (about 80%) reduction in tumour incidence.

83. TPA also causes enhanced transformation of irradiated mouse 10T½ cells (Figure A.II). For the loss-of-contact inhibition, two genetic steps and modulation by epigenetically acting substances were proposed by Little

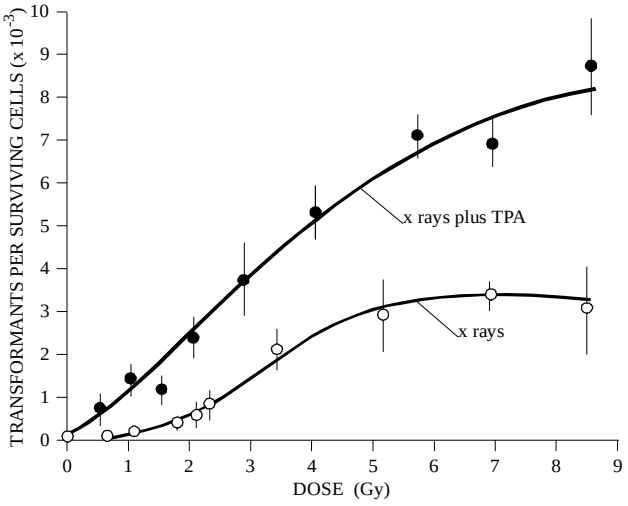

Figure A.II. *In vitro* transformation of C3H10T½ cells exposed to x rays (50 kV) with and without post-irradiation incubation in TPA (0.1 µg ml^{-1}) [H3].

[L24]. TPA promotes following exposure to x rays or to fission-spectrum neutrons without any effect on cell survival [H3]. However, treatment of unirradiated cells with 0.1 µg ml^{-1} of TPA resulted in a small increase in transformation frequency above background (i.e. from 1.1 10^{-5} to 1.0 10^{-4}). Thus, besides being a promoter, TPA seems to be also a weak initiator. The enhancement factor of TPA for radiation-induced transformation was greater after low doses than high doses of either radiation. In addition, TPA caused the RBE of neutrons as compared to x rays to increase with increasing dose. For x-ray doses from zero to approximately 1.2 Gy, TPA raised transformations to frequencies approximately equal to those due to neutrons alone. Analysis of TPA enhancement in the context of the combined effect of two inducing agents, TPA plus radiation, indicates that with either x rays or neutrons, TPA acts synergistically. The main mechanism of action of TPA is suggested by the finding that the dependence of transformation frequency on the density of viable cells is also altered by the tumour promoter. In contrast to the constant frequency of transformants per surviving (or viable) cell, which was observed after a fixed dose of x rays or neutrons for a range of cell inocula, the increase in the frequency of transformation caused by TPA and radiation was dependent on cell inocula. The frequency of transformation from combined treatment decreased with increasing size of the inoculum, from approximately 20 to 6,000 viable cells per 90-mm Petri dish, a result that the authors interpreted as an interference with cell-to-cell communication by TPA plus the fading of initiation events caused by radiation.

84. DNA base analogues are another group of substances with the potential to modify the effects of radiation and other genotoxic agents (see also Section D.2). 5-Bromo-2'-deoxyuridine (BrUdR) is an analogue for thymidine and widely used in tumour diagnosis, cytogenetics, and flow cytometry. Important examples of epigenetic and (indirect) genetic effects are the inhibition of differentiation in cultured myoblasts and photosensitivity of patients, respectively. Anisimov and Osipova [A15] investigated carcinogenesis induced by combined neonatal exposure to BrUdR and subsequent whole-body x-irradiation of rats. Outbred LIO rats at 1, 3, 7, and 21 days of post-natal life were exposed to subcutaneous injections of 3.2 mg of BrUdR per animal and/or at the age of 3 months to single whole-body x-irradiation at a dose of 1.5 Gy. In males, treatment with BrUdR alone decreased the latency of all tumours and increased the incidence of malignant tumours and the number of tumours per rat compared with controls. Combined exposure to BrUdR and x-irradiation increased total and malignant tumour yield and multiplicity over that in all other groups. More testicular Leydigomas, tumours of prostata, kidney, and adrenal cortex, and leukaemia were seen in male rats exposed to BrUdR plus x rays, compared with male rats treated with BrUdR or x-irradiation alone. In female rats, treatment with BrUdR alone decreased the latency for the total number of tumours and increased their incidence and number per rat, in comparison with controls. Combined exposure of females to BrUdR and x rays did not increase total tumour incidence in comparison with females that had only been irradiated; however, it shortened tumour latency. The incidence and multiplicity of malignant tumours and incidences of pituitary adenomas, mammary adenocarcinomas, and uterine polyps were significantly increased, whereas the latency of kidney tumours was decreased in females exposed to BrUdR plus x rays, compared with all other groups. The data from this experimental model provide, together with other studies, evidence that perturbation of DNA induced by the nucleoside analogue BrUdR contributes substantially to the spontaneous development of tumours and enhances the sensitivity of target cells to carcinogenesis induced by x-irradiation as well as by chemicals or hormones.

85. Information on the effects of the interaction of thorium and phenobarbital, an anticonvulsive drug inducing liver detoxification functions and showing promoting activity, may be available from earlier epileptic patients. Thorium exposure (thorotrast) resulting from angiographic procedures correlated with the use of anticonvulsive drugs. Olsen et al. [O15, O16] found considerably increased risks for liver cancer, but since thorotrast exposure was considered a confounder in both studies, no definitive quantitative information on combined effects from thorium and phenobarbital was given.

86. The potentially important interaction of phenobarbital, a widely used anticonvulsant and sedative, with x-irradiation was studied by Kitagawa et al. [K18]. Male newborn Wistar-Ms rats received whole-body x-irradiation of 0.5, 1, and 4 Gy at 8 or 22 days. After weaning they were fed either a basal diet or a diet containing 0.05% phenobarbital. The x rays induced numerous adenosine-triphosphatase-deficient islands appearing in the liver by week 22 of age. However, no hepatic tumours were observed by 22 months after radiation, even in phenobarbital-treated animals.

87. Supra-additivity was also found for a combination of fast-neutron irradiation and subcutaneously applied carbon

tetrachloride in male and female C57Bl6 mice. The animals received a single whole-body dose of 1.7 or 3.3 Gy from fast neutrons, followed nine weeks later by a single subcutaneous injection of carbon tetrachloride. Carbon tetrachloride markedly increased the incidence of radiation-induced liver carcinomas, whereas chloroform, which was also tested in this system, did not influence the incidence of radiation-induced tumours [B16].

88. The potential for carbon tetrachloride to modify the biokinetics of an inhaled, soluble form of plutonium is also being examined in both F344 rats and Syrian hamsters [B16]. Groups of animals were exposed to carbon tetrachloride in whole-body chambers at concentrations of 0, 5, 20, or 100 ppm for 6 hours per day, 5 days per week, for a total of 16 weeks. After 4 weeks of exposure, approximately one half of the animals were exposed by a single pernasal inhalation exposure to ^{239}Pu nitrate. Serial sacrifices of groups of animals were conducted at 4 hours and 2, 4, 6, or 13 weeks after plutonium exposure for the quantification of ^{239}Pu in lung, liver, kidney, and bone (femur) and for the evaluation of histologic changes in various tissues. Results describing possible carbon tetrachloride effects on plutonium disposition are not yet available from this study. Another subgroup of rats and hamsters was exposed to a radioactively labelled insoluble tracer particle. Tracer particle clearance was analysed for 13 weeks following exposure, and no significant clearance differences were observed between carbon-tetrachloride-treated and control groups.

89. Since ionizing radiation and tumour-promoting agents increase the level of ornithine decarboxylase (ODC) involved in polyamine biosynthesis, the effect of alpha-difluoromethylornithine (DFMO), an inhibitor of ODC, on tumour yield from beta radiation was tested in female ICR mice [O12]. The chronic radiation exposure consisted of three times 3 Gy ^{90}Sr/^{90}Y surface dose per week to the back. DFMO was added to the drinking water in a final concentration of 1%. It significantly delayed the time of tumour emergence from 245 days with radiation exposure only to 330 days in animals also given DFMO. The antagonistic effect of DFMO was also observed for bone tumours.

90. Monchaux et al. [M61] addressed the important question of possible synergistic contributions from diesel fumes present in mine air to radon-induced lung tumours. Three groups of 50 male Sprague-Dawley rats were exposed to radon (1,000 WLM) and/or diesel exhaust (300 hours; 22–25 ppm CO and 4–5 mg m^{-3} diesel particles), with the diesel exposure succeeding the radon exposure by one month. Contrary to the strong synergistic effect of cigarette smoke found in this system (discussed under tobacco), exhausts had only a slight, non-significant effect on the risk for thoracic tumours from radon. Diesel exhausts alone were not carcinogenic.

91. Since phosphorylation and dephosphorylation of proteins play an important role in cellular metabolism, Nakamura and Antoku [N21] studied the effect of calyculin A (CL-A), a specific inhibitor of protein phosphatase 1 and 2A isolated from the marine sponge *Discodermia calyx*, on x-ray-induced cell killing in cultured mammalian cells (BHK21). At concentrations above 2.5 nM, CL-A enhanced the radiation effect considerably. As also shown in another cell culture system with the inhibition of protein kinases [H40], agents that interfere with protein-kinase-mediated signal transduction after radiation exposure may enhance damage and represent a new class of radiosensitizers.

92. Many non-genotoxic agents clearly produce strong synergistic effects with ionizing radiation. The combined effects of this class of agent are summarized in Table A.1. Table A.3 lists more detailed effects of TPA, probably the best-studied modifier of genotoxic agents, on several endpoints. These studies are of great importance for the elucidation of mechanisms affecting expression of risk. At this stage, however, no functional analogues of potent experimental enhancers of radiation risk, such as TPA or DNA bases, are known to exist in critical concentrations in the human environment.

3. Tobacco

93. The important interaction of tobacco smoke and radiation was introduced in the main text of this Annex. Epidemiological studies of uranium miners have allowed the risks and interaction coefficients to be quantified, at least for higher radiation doses. The complex composition of tobacco smoke makes the interaction not simply a binary combination, however. Some 4,000 individual chemical components of cigarette smoke have been identified, and a number of additional unidentified components surely exist (for example, extremely reactive, short-lived compounds or those present in very low concentrations) [G1]. Identified compounds in smoke include several known carcinogens of the polycyclic aromatic hydrocarbon and nitrosamine classes.

94. The studies reviewed below refer to mainstream smoke, sidestream smoke, or environmental tobacco smoke. Mainstream smoke is defined as the smoke originating from the butt end of a cigarette; it is generated during the active puffing process. Sidestream smoke is the smoke released at the burning tip of a cigarette, whether the cigarette is being puffed or simply smoldering. Lastly, environmental tobacco smoke is a mixture of sidestream smoke and exhaled mainstream smoke. This term most accurately describes the smoke that would be found within an enclosed space with a smoker present. Tobacco smoke contains relatively small amounts of DNA-reactive carcinogens, such as nitrosamines, polycyclic aromatic hydrocarbons, and pyrolysis products, such as carbolines. Hence enhancing and promotional factors, e.g. catechols, other phenols, and terpenes, are an important component. Probably because it reduces pressure from the action of promoters, discontinuation of smoking progressively reduces the risk of cancer development with time since withdrawal [W1].

Table A.3
TPA as a modulator of transformation and cancer yield from ionizing radiation

Endpoint	*Experimental system*	*Interaction*	*Proposed mechanism*	*Outcome*	*Ref.*
Transformations in surviving cells	10T½ cell culture	x rays, TPA	Initiation, promotion	Higher linear yield Loss of threshold *(see Figure V)*	[H3]
Transformations in surviving cells	10T½ cell culture	x rays/neutrons, TPA	Initiation, promotion	Enhancement factor greater at lower exposures RBE of neutrons enhanced at higher doses	[H3]
Transformations in surviving cells	10T½ cell culture	Radiation, TPA	Two genetic steps, epigenetic modulation	Genetic effect fading with culture time TPA interferes with cell-cell interaction	[L24]
Squamous-cell carcinoma	CD-1 mice	Beta radiation, TPA; MNNG, TPA, beta radiation	Initiation, promotion, progression	High papilloma yield with TPA only Progression to carcinoma by radiation	[J1]
Skin papilloma	Mice	Beta radiation, TPA; DMBA, beta radiation	Initiation, promotion	Promotion by repetitive irradiation different from TPA	[O2]
Skin papilloma	SHR mice	Radiation (4.2 Gy) to father TPA to offspring F_1 and F_2	Genetic modification, promotion	Skin tumours elevated in TPA-treated offspring Weaker effect in female offspring	[V5]

(a) Epidemiological studies

95. In the last few years, joint analyses of original data sets [C1, L18] and meta-analyses of published results [T14] have yielded detailed assessments of risk patterns from combined exposure to high-LET alpha radiation from radon and its short-lived decay products and tobacco smoke, and have allowed investigators to test risk models. The most comprehensive and complete analysis of radon-induced health risks was published by Lubin et al. [L18]. The review contains a joint analysis of original data from 11 studies of male underground miners; 2,736 lung cancer deaths among 67,746 miners were observed in 1,151,315 person-years. A linear relationship was found for the ERR of lung cancer with the cumulative exposure to radon progeny, estimated in working level months (WLM). This coefficient (ERR/WLM) was strongly influenced by various factors. Contrary to the low-LET experience from Hiroshima and Nagasaki, ERR/WLM decreased significantly with attained age and time after cessation of exposure to radon progeny. A stronger decline of risk with time since exposure than in survivors of the atomic bombings was also found. A considerably higher lung cancer risk was initially found for exposures received at low rates as compared with high rates. Depletion of stem cells at risk in high dose rate exposures was implied. However, the epidemiological database was said to be too weak to project this indication of an inverse dose-rate effect to non-occupational settings, i.e. to typical indoor radon exposures and exposure rates [L18]. Also, a recent reassessment of the Beaverlodge cohort, which earlier on gave the strongest indication of such an effect, no longer does so. Revised exposure estimates of this study of miners with relatively low exposures now bring the modifying effects of risk with time since exposure and age at risk in line with those from other studies [H46]. The highly significant decrease in ERR with time since exposure may be explained with microdosimetric considerations. In the case of high-LET alpha radiation from radon progeny, the minimal local dose from one single alpha track averaged over a cell nucleus is already in the range of several hundred milligray, whereas one electron track yields a dose to the nucleus in the range of only 1–3 mGy. This means that even at the lowest possible nuclear dose from alpha exposure, stem cells that are hit carry a multitude of DNA lesions, which may considerably impair long-term cell survival and maintenance of proliferative capacity [B25, B27].

96. In the joint analysis by Lubin et al. [L18], data on smoking were available for 6 of the 11 cohorts, but assessments were limited by incomplete data on lifetime tobacco consumption patterns and sometimes exotic tobacco use, such as in water pipes in the Chinese study. Most studies for which smoking data could be analysed were generally not informa-

tive enough to allow deciding between an additive or a multiplicative joint relationship for radon progeny and smoking. The Chinese cohort seemed to suggest an association more consistent with additivity, while the Colorado cohort suggested a relationship more consistent with a multiplicative interaction. For all studies combined, the joint relationship of smoking and radon progeny exposures with lung cancer was stable over the different age groups and deviated quite clearly from either a purely additive or a multiplicative relationship. The most recent analyses of the BEIR VI Committee [C46], which were based on an update of these data, suggest that the joint effect is statistically closer to a multiplicative than an additive interaction. To further characterize the association, more detailed data on tobacco use would be needed. Age at onset of smoking, amount and duration of smoking, and type of tobacco were recognized as important determinants of risk. Such a refined analysis of smoking patterns is possible only in the prospective part of ongoing studies and is subject, furthermore, to potential bias in the affected individuals owing to the rapidly decreasing public acceptance of smoking. In general, the single-exposure subcohorts of lifetime non-smokers are very small in all studies. The statistical power of the conclusions on the interaction between radon and tobacco smoke is correspondingly small. Applying the two-mutation clonal expansion model of carcinogenesis of Moolgavkar et al. to data from the Colorado plateau miners shows no interaction between radon and tobacco smoke in any of the three steps [M39], but the predicted lung cancer incidence caused by radon and smoking remains more than additive and less than multiplicative, an indication of isoadditivity.

97. Microdosimetric considerations are also important in extrapolating the inverse dose-rate effect found for oncogenic endpoints caused by alpha radiation in general and for lung cancer in miners [L36]. Brenner [B40] postulated that protraction enhancement is a mechanism limited to cells receiving multiple hits over a human lifespan. Since a typical domestic exposure to radon progeny of 14 WLM yields a very small probability of multiple traversals in a cell nucleus (<1% for the most highly exposed stem cells in the tracheobronchial epithelium), dose-rate effects are probably of no relevance, and lung cancer risk per unit exposure will not increase further at low radon levels.

98. Two recent analyses by Yao et al. [Y7] and Thomas et al. ([T18] with erratum) on the radon-smoking interaction showed a considerable influence of timing of exposures. The former study found a higher lung cancer risk for exposure to radon progeny and tobacco use occurring together as compared to radon exposure preceding tobacco use. The second study on Colorado uranium miners found a significantly more-than-multiplicative effect for smoking followed by radon, whereas radon exposure followed by tobacco use produced an essentially additive effect. These findings are in conflict with earlier notions based on experimental results in rats, whereby radon is an initiator and tobacco smoke, a promoter [G20]. However the relevance of this animal system is questionable, because tobacco smoke alone does not produce lung tumours in this system.

99. Despite the remaining uncertainties, it is quite clear that the joint effect of radon progeny exposure and smoking is greater than the sum of each individual effect. The combined analysis [L18] shows that a linear exposure-response estimate for radon and lung cancer is compatible with the data and gives a relative risk that is about three times higher in non-smokers than in smokers. Assuming a 10-fold difference in the tobacco-caused lung cancer risk between smokers and non-smokers, this means that the lung cancer risk for smokers expressed in absolute terms is higher by a factor of about 3. Such a supra-additive effect, if also demonstrated to hold for present occupational and non-occupational exposure settings, would be of great importance for the regulation of smoking and radon progeny in the human environment. Until now, little quantitative evidence has come from indoor radon studies. The few case-control studies published are inconclusive [A28, P11]. Only one larger study [P11] was indicative of an indoor radon risk and its modification by tobacco that is comparable to what is predicted from miner studies. It remains doubtful whether the results from the many case-control studies under way will in the near future allow narrowing of the uncertainties that surround indoor radon risk and possible interactions with smoking. Based on inconclusive results from 1,000 computer-simulated large case-control studies assuming an ERR of 0.015 WLM^{-1}, Lubin et al. [L33] questioned the assumption that epidemiological studies, even when pooled in meta-analyses, will produce reliable estimates of risk from residential radon exposure. Errors in exposure assessment, migration, and confounding by smoking are at the root of this pessimistic assessment. At least for the second confounder, studies in Europe based on much longer mean residence times may offer better statistical power. Several large indoor case-control studies under way will narrow uncertainties in the next few years. First results from the United Kingdom [D33] and Germany [W35] are indicative of a lung cancer risk in the range of ICRP projections. However, confidence intervals are relatively large and include zero risk in most analyses.

100. Because of the limitation of the indoor radon studies, risk estimates based on miner data remain the main basis for predicting lung cancer from indoor radon exposure. A best linear estimate of the risk coefficients found in the joint analysis of Lubin et al. [L18, L35] for the indoor environment indicates that in the United States, some 10%–12%, or 10,000 cases, of the lung cancer deaths among smokers and 28%–31%, or 5,000 cases, of the lung cancer deaths among never-smokers are caused by radon progeny. About half of these 15,000 lung cancer deaths traceable to radon would then be the result of overadditivity, i.e. synergistic interactions between radon and tobacco. Based on the same risk model, Steindorf et al. [S47] predicted that about 7% of all lung cancer deaths in the western part of Germany are due to residential radon. This corresponds to 2,000 deaths per year, 1,600 in males and 400 in females. The attributable risk estimate was 4%–7% for smokers and 14%–22% for non-smokers. The most recent central estimates for the proportion of radon-attributable lung cancer deaths in the United States in 1995 was recently provided by the BEIR VI Committee [C46] in

1998, based on an updated data set of the miners studies reported by Lubin et al. [L18]. The Committee applied a sub-multiplicative relation to model the joint effect of tobacco smoking and radon. Depending on two different models (exposure-age-concentration model or exposure-age-duration model) about 14% or 9% of all lung cancer deaths among ever-smokers and 27% or 19% among never-smokers were estimated to be attributable to radon. Because of the many differences between mines and homes and the additional carcinogens such as arsenic, dust and diesel exhaust in mine air, these figures should be interpreted with caution. A population-based case-control study of incident lung cancers among women in Missouri who where lifetime non-smokers or long-term ex-smokers yielded a very low and non-significant estimate of the attributable lung cancer risk from radon in non-smokers [A4].

101. It has been questioned whether toxicants other than joint exposures to radon progeny and cigarette smoke contribute considerably to the high lung cancer risk found in miners [I5]. Heavy exposures to mine dust containing silicates, diesel exhausts, and fumes from explosives may add to or combine with the two main lung carcinogens, radon and cigarette smoke. Patients who received thorotrast continuously exhale the very short-lived ^{220}Rn derived from ^{232}Th deposits in the body and therefore provide a model for lung carcinogenesis by radon without concomitant dust exposure. Ishikawa et al. [I5] studied the lung cancer incidence in a Japanese thorotrast cohort and found 11 lung cancer cases in 359 thorotrast autopsy cases. The analysis revealed that while the proportion of small-cell lung cancer considered to be related to alpha radiation was significantly increased, the overall lung cancer incidence was not significantly higher than in controls, in spite of the high levels of ^{220}Rn in the patients' breath. The authors took this as an indication that the risk for radon-induced lung cancer is not as high as expected from risk coefficients deduced from miner studies. To substantiate this hypothesis, the build-up of ^{220}Rn decay products in the lung air space before exhalation and the resulting exposure to critical stem cells would have to be quantified.

102. Owing to the generally good linear correlation between radon progeny exposure and lung cancer in the major miner studies, few additional carcinogens in mine dust were considered in depth. Toxic metals are, however, of special concern. Results from the Chinese [X1], Canadian (Ontario), [K21] and Czech [T41] cohorts showed arsenic to be an important additional risk factor for lung cancer. Adjustment for arsenic exposure reduced the radon risk estimate in these cohorts considerably. Even in the most recent joint analysis by Lubin et al. [L18], other mine exposures were difficult to interpret, since the information was quite limited and of poor quality. In most cases these concomitant exposures to suspected carcinogens or promoters are typically highly correlated with radon progeny exposures in a given study and therefore difficult to assess independently (see also following Section B.4).

103. The mechanism of interaction between DNA lesions caused by radon progeny and those caused by chemical toxicants contained in tobacco smoke is not known. There is clear evidence that the prevalence of mutations in critical genes is dependent on the type of insult. The most common known gene mutations in lung cancer cells are found in the tumour-suppressor gene *p53*, which is thought to be crucial in the initiation of this and many other types of cancer. Several groups analysed the molecular changes in the conserved regions of the *p53* gene in lung cancer tissue and reported differences between non-smokers (survivors of the atomic bombings and unirradiated controls), Japanese smokers, and uranium miners with high radon exposures [T12, T13] (see also Annex F, "*DNA repair and mutagenesis*"). The non-smokers from Hiroshima showed mainly transition mutations (all G:C to A:T) but no G:C to T:A transversions. By contrast, the changes in 77 Japanese smokers showed a predominance of G:C to T:A transversions in which the guanine residues occur in the non-transcribed DNA [T12]. In 16 of 52 lung cancers of miners, a specific transversion AGG to ATG at codon 249 was reported [T13]. The prevalence of 31% for this mutation in miners was compared with only 1 reported case in 241 published *p53* mutations from lung cancers in the general population (mainly smokers). Such a marker might help to define a causal relationship, but even in the first study, only a minor fraction of the *p53* genes from lung cancer tissue of miners, all of whom had a unique genotoxic exposure, showed the specific change. However, later studies were not able to confirm the initial finding [B73, L53]. As was pointed out, a multitude of different primary lesions can lead to the same cellular and clinical endpoints, in this case a non-functional repressor protein and lung cancer, respectively, and highly specific molecular markers of single agents in all affected individuals are not to be expected.

104. A difficult matter of some concern for the protection of the public is the combined exposure to indoor radon progeny and environmental tobacco smoke. The presence of environmental tobacco smoke in homes has been implicated in the causation of lung cancer. In the absence of direct epidemiological information, the clearly higher-than-additive combined effects of smoking and radon progeny in mine air may lead to the application of a multiplicative model for risk assessment. While of interest in its own right, environmental tobacco smoke also influences the risk imposed by radon and its decay products through its strong influence on aerosol characteristics. The interaction between radon progeny and environmental tobacco smoke alters the exposure, intake, uptake, biokinetics, dosimetry, and radiobiology of those progeny. Crawford-Brown [C10] developed model predictions of the various influences of environmental tobacco smoke on these factors in the population of the United States and provided estimates of the resulting change in the dose from average levels of radon progeny. It was predicted that environmental tobacco smoke produces a very small, non-measurable increase in the risk of radiation-induced tracheobronchial cancer in homes with initially very high

particle concentrations for both active and never-smokers but that it significantly lowers the dose in homes with initially lower particle concentrations for both groups when generation 4 of the tracheobronchial tree is considered the target site. For generation 16, the presence of environmental tobacco smoke generally increases the lung dose from radon progeny, although the increase should be unmeasurable at high initial particle concentrations. Although the author shows that the dose-modifying effects of environmental tobacco smoke are negligible, the main problem, a potential synergism between environmental tobacco smoke and radon progeny, was not assessed.

105. A smaller but still considerable cohort may be at risk from the combined effects of low-LET radiation and tobacco smoke, namely cigarette-smoking women who underwent breast cancer radiation therapy. Ionizing radiation has already been shown to be a lung carcinogen after breast cancer radiation therapy. Neugut et al. [N4] used a case-control study to explore whether cigarette smoking and breast cancer radiation therapy have a multiplicative effect on the risk of subsequent lung cancer. Case and control women were persons registered with primary breast cancer in the Connecticut Tumour Registry who developed a second malignancy between 1986 and 1989. Cases, i.e. those diagnosed with a subsequent primary lung cancer, were compared with controls diagnosed with a subsequent non-smoking, non-radiation-related second malignancy, and age-adjusted odds ratios were calculated with logistic regression. No effects from radiation therapy were observed within 10 years of initial primary breast cancer. Among both smokers and non-smokers diagnosed with second primary cancers more than 10 years after an initial primary breast cancer, radiation therapy was associated with a threefold increased risk of lung cancer. A multiplicative effect was observed, with women exposed to both cigarette smoking and breast cancer radiation therapy having a relative risk of 32.7 (95% CI: 6.9–154) (Figure A.III). Further evidence for a direct causal relationship was the observation that the carcinogenic effect of radiation was seen only for the ipsilateral lung and not for the contralateral lung in both smokers and non-smokers. The authors concluded that breast cancer radiation therapy, as delivered before 1980, increased the risk of lung cancer after 10 years in non-smokers, and a multiplicative effect was observed in smokers. The significance of the findings is, however, strongly reduced by the fact that the study also indicates a large difference in the incidence of ipsilateral and contralateral lung tumours for smokers who had no radiation therapy (Figure A.III), resulting in concerns about unidentified bias [I10]. A similar case-control investigation was based on 61 lung cancer cases from the Connecticut Tumour Registry who had received radiation therapy for the treatment of breast cancer [I9]. The authors of this study found no indication of a strong positive association between smoking and radiotherapy in the 27 cases where information on cigarette use was available. Therefore, it is not possible at this stage to decide whether current treatment practices involving much lower radiation doses to the lung may need to be reassessed in view of the detriment (late stochastic effects) for young breast cancer patients who smoke.

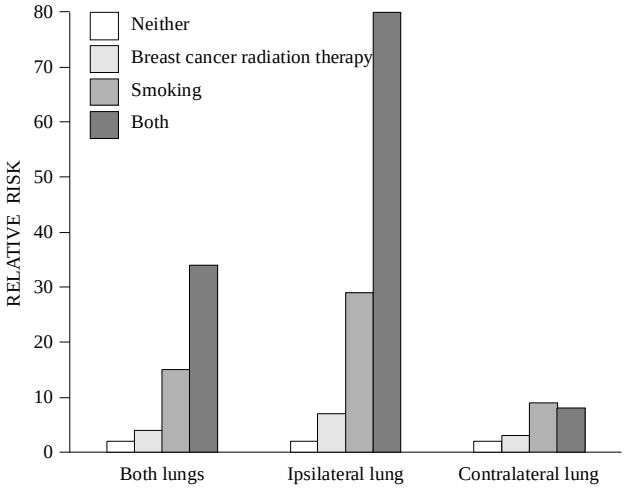

Figure A.III. Age-adjusted relative risk of lung cancer for separate or combined exposures to radiation and cigarette smoke [N4].

106. Long-term survivors of Hodgkin's disease display an increased lung cancer risk. Van Leeuwen et al. [V7] conducted a case-control study with 30 lung cancer cases from a cohort of 1,939 patients treated for Hodgkin's disease from 1966 through 1986 in the Netherlands to investigate the effects of radiation, chemotherapy, and smoking. Comparing patients who had a radiation dose of more than 9 Gy to the area where malignant growth developed with those who had less than 1 Gy, the relative risk was 9.6 (95% CI: 0.98–98, p for trend = 0.01). Patients smoking more than 10 pack-years (number of years with more than 1 pack per day) after diagnosis had a sixfold higher risk than patients with less than 1 pack-year. A multiplicative interaction was observed between the lung cancer risk from smoking and from increasing levels of radiation. On the other hand, no such trend was found with the drugs mechlorethamine or procarbazine, either in relation to the number of cycles of chemotherapy or to cumulative dose. It was suggested that Hodgkin's disease patients should be dissuaded from smoking after radiotherapy [V7].

(b) Animal studies

107. Although there are no well-suited animal model systems in which to examine potential carcinogenic interactions between environmental tobacco smoke and radiation, the issue of interactions between exposure to mainstream cigarette smoke and either radon or $^{239}PuO_2$ has been examined. Relationships between increased risk for lung cancer in animals and exposure to radon and/or radon progeny [G11] or to $^{239}PuO_2$ [C2] have recently been reviewed.

108. Studies conducted in France involved the whole-body exposure of rats to diluted mainstream cigarette smoke administered either before or after exposure to radon [C9]. Rats received high-level exposures to smoke for ten 15-minute periods four times weekly for one year. Smoke exposures given before the exposures to radon did not influence radon-induced tumour incidence, but smoke

exposures given after radon exposure increased the tumour incidence by a factor of 2–3 over rats receiving radon alone. These data indicated that cigarette smoke may have acted to promote radon-induced carcinogenesis, as reviewed in the UNSCEAR 1982 Report [U6].

109. In contrast, studies conducted on dogs exposed to the smoke from 10 cigarettes per day for 4–5 years combined with radon suggested that the incidence of lung tumours was less than that in dogs receiving radon alone [C21]. Lung tumours were produced in 8 of 20 dogs receiving radon alone, whereas tumours developed in only 2 of 20 dogs receiving both agents. The investigators speculated that increased mucus flow may have led to a reduced radiation dose to target cells in the smoke-exposed dogs; however, the small number of animals made interpretation of these results difficult.

110. Thus, despite the directly relevant epidemiological data on smoking and albeit high exposure to radon progeny, a significant problem remains, for example, for extrapolations to low exposures, in that the epidemiological and animal data related to lung cancer are in agreement for rats [C9] but in disagreement for dogs [C21]. Archer [A21] tried to explain this disagreement by advancing a hypothesis based on an additive interaction of the two agents at the level of initiation and on temporal differences of cancer expression. The hypothesis is that among cigarette smokers a given radiation exposure induces a finite number of lung cancers that have shorter latency periods as a result of the cancer-promoting activity of smoke.

111. In a study with hamsters exposed to ^{210}Po, benzo(a)pyrene was used as a substitute for tobacco smoke [L24]. As compared with animals exposed only to ionizing radiation (lung cancers incident in about 3% of the animals) or only to benzo(a)pyrene (no incident cases in over 280 treated animals), animals receiving benzo(a)-pyrene instillations after exposure to ionizing radiation were at a much higher risk (about 50%) of developing a lung tumour. It is noteworthy that the instillation of saline after radiation exposure also induced lung tumours in about 30% of the animals.

112. Douriez et al. [D30] investigated the role of cytochrome P-450 1A1 (CYP1A1) inducers on radon-induced lung cancers in rats. CYP1A1 is the member of the cytochrome P-450 gene family producing the most mutagenic activation products from polycyclic hydro-carbons. All three inducers tested (methylcholanthrene, 5,6-benzoflavone, 2,3,7,8-tetrachlorodibenzo-p-dioxin) increased the incidence of epidermoid carcinoma to 100%, independent of whether the inducer itself was converted to a powerful carcinogen or not. Depletion of retinoid acid in CYP1A1-stimulated rats is implicated as a further step leading to increased susceptibility to lung cancer. Since tobacco smoke is a powerful inducer of CYP1A1, this mechanism could account for the supra-additive effects in radiation-exposed smokers.

113. Preliminary studies on an interaction between $^{239}PuO_2$ and cigarette smoke were reported by Talbot et al. [T2]. The experiments were designed to show whether exposure to cigarette smoke for 12 months enhances the incidence of lung tumours in mice that had previously inhaled $^{239}PuO_2$. The main difference found was a reduced growth rate in both smoke- and sham-exposed mice relative to that of cage controls. After 3 months of treatment, histopathology and morphometry of lung sections found only slight smoke-induced changes. On a per-unit-area basis, these changes included a reduced proportion of alveolar space and an increased number of pulmonary alveolar macrophages that were larger than those from sham-exposed or control mice and had an increased proportion of binucleated cells. All mice in a second study were initially exposed to $^{239}PuO_2$, then subsequently divided into three treatment groups as above. Cigarette smoke exposure was shown to increase lung weight and inhibit clearance of ^{239}Pu from the lung. The authors pointed out a dosimetric problem: the group receiving $^{239}PuO_2$ and subsequently tobacco smoke would receive a higher radiation dose to the lung than those receiving $^{239}PuO_2$ alone. Although this aspect is important for elucidating the mechanisms by which synergism or antagonism occur, for radiation protection, an apparent combined effect traced to a modification of exposure/dose conversion factors by one agent would still be considered synergism or antagonism.

114. A cigarette-smoke-induced reduction in the lung clearance of inhaled $^{239}PuO_2$ was also observed in a study in rats [F15, F28]. Animals were first exposed by a whole-body inhalation mode to diluted mainstream cigarette smoke at a concentration of 100 or 250 mg m^{-3} of total particulate matter for six hours per day, five days per week. Control rats received filtered air alone. After three months, all groups of rats received a single pernasal exposure to radioactively labelled insoluble tracer particles; then the rats were returned to their respective cigarette smoke or filtered air exposure. External whole-body counting of the tracer was continued for six months, and substantial smoke-induced clearance inhibition was found. Lifetime radiation doses were 3.8 Gy, 4.4 Gy, or 6.7 Gy for the control, 100 and 250 mg m^{-3} total particulate matter groups, respectively [F28]. The results for the highest level of cigarette smoke exposure suggested that the radiation dose increased by a factor between 1.6 and 1.7 by this effect, compared with the group of rats receiving filtered air alone. It should be noted that cigarette smoking has been shown to reduce the lung clearance of relatively insoluble particles in humans as well as in animals [C5].

115. The study described above is part of a carcinogenicity experiment in which 2,170 male and female F344 rats received exposures to cigarette smoke and/or $^{239}PuO_2$ [F17]. Groups of animals were exposed for up to 30 months to filtered air or to low or high concentrations of cigarette smoke. For each of these groups, approximately one half of the rats also received a single pernasal inhalation exposure to $^{239}PuO_2$ that resulted in an initial lung burden of approximately 400 Bq. Cigarette smoke exposure did not

markedly influence survival, but it did result in decreased weight gain and a variety of lung lesions such as alveolar macrophage hyperplasia, interstitial fibrosis, chronic-active inflammation, hyperplasia of the alveolar epithelium, and bronchial mucous-cell hyperplasia. A preliminary evaluation of lung cancer in females indicated that crude lung tumour incidences were approximately 7% in rats exposed to high concentrations of smoke, 20% in rats exposed to $^{239}PuO_2$, and 74% in groups receiving both agents (Figure A.IV). Thus, the interaction was clearly synergistic. This study illustrates the manner in which a dose from one agent can be markedly affected by exposure to a second agent, leading to a clear synergism in carcinogenic response. Less certain, however, is the extent to which the interaction resulted strictly from the impaired clearance (and associated increased radiation dose) in the combined exposure groups rather than a more fundamental interaction between the radiation and cigarette smoke constituents at the molecular or cellular level. Another mechanism by which synergism could occur might relate to the localized radiation dose rather than the dose to the whole organ. For example, the synergistic interaction between smoking and radiation in this example could result from the alpha radiation dose delivered at the site of smoke-induced lung lesions, where the processes of cell hyperplasia, fibrosis, and activated phagocytes were already occurring.

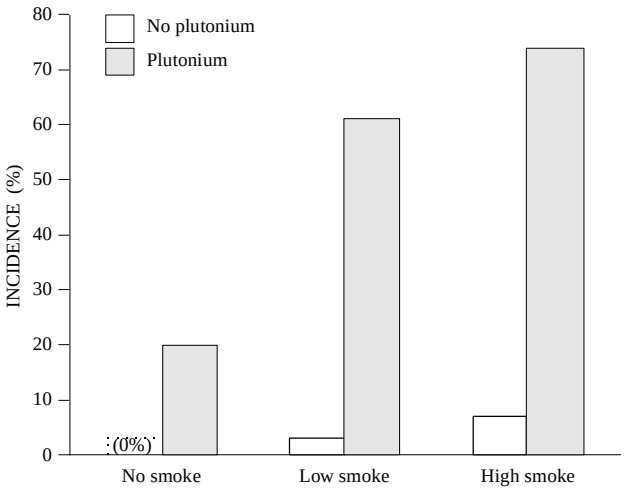

Figure A.IV. Incidence of lung tumours in female rats exposed to plutonium dioxide in combination with varying levels of cigarette smoke [F17].

116. In an early study by Cowdry et al. [C49], the carcinogenicity of ^{90}Sr beta irradiation to the skin of Swiss mice applied twice weekly and 3 tar paintings per week to distributed skin areas was studied. Surface doses were about 2 Gy per fraction and 200 Gy totally. Skin tumour incidences after 30 months were 12.3% for radiation alone, 42.9% for tar paint alone, and 61.3% for the combined treatment. It was concluded that there is no synergism between the two carcinogens in the study system. It is noteworthy that a monthly skin irradiation of several Gy surface dose did not produce any skin tumours, whereas in the control group only painted with acetone (the solvent for cigarette tar) an incidence of 6.8% was seen after 30 months.

117. In view of the many active substances contained in cigarette smoke, possible interactions are very numerous. It is outside the scope of this Annex to cover this fully, but the many reported interactions of caffeine with cigarette smoke components may merit mention, especially because caffeine at higher concentrations also modifies the effects of ionizing radiation. Rothwell [R13] found an inhibition of cigarette-smoke-induced carcinogenesis in mouse skin by caffeine. Other recent reports showed an inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by polyphenols extracted from green tea and cruciferous vegetables [C24, X2]. It is believed that these dietary compounds act as antioxidants (see also Section B.6).

(c) Cellular studies

118. To examine the interaction between radiation exposure and smoking, Piao and Hei [P8] studied the toxicity and oncogenic transforming incidence of alpha-particle irradiation with and without concurrent exposure to cigarette smoke condensate on C3H10T½ cells *in vitro*. In this system, additive modes of interaction between cigarette smoke condensate and ionizing radiation were observed for the oncogenic transforming potential of both gamma rays and alpha particles. In a recent study made possible by the development of charged-particle microbeams, it was shown that even for radon alone, induction of transformation in C3H10T½ cells *in vitro* from exactly one alpha particle was significantly lower than for a Poisson-distributed mean of one alpha particle through a cell nucleus [M66]. This implies that cells traversed by multiple alpha particles contribute most of the risk, and that a linear extrapolation from high exposures may overestimate the transforming potential of high-LET radiation in low-level exposures. If generally applicable, such results would speak against the potential of combined effects at low exposures to surpass values expected from linear dose-effect relationships and additivity.

119. The combined genotoxic effect of cigarette smoke condensate and gamma radiation was also studied in a simple eukaryotic organism [S8]. The induction of gene conversion in diploid yeast (*Saccharomyces cerevisiae*) strains was investigated following exposure to cigarette smoke condensate and gamma radiation. Cells exposed to a combination of cigarette smoke condensate and low-LET radiation showed an additive response irrespective of the order of treatments. The system also showed large differences in sensitivity depending on growth status, with log-phase cells being 2–3 times more sensitive than stationary cells. The relevance of these findings is limited by the fact that critical toxicants in tobacco smoke require activation by biotransformation, a mechanism that is highly species- and tissue-specific.

(d) Summary

120. In summing up the many results from the well-studied interaction between tobacco smoking and high levels of radon exposure, it can be stated that this important combined exposure leads to clearly overadditive effects for lung cancer in humans. Some of the more

important findings are summarized in Table A.1. The quite different dose-effect relationships for the two agents, apparently linear for radon and clearly non-linear for tobacco smoke, speaks against iso-addition and for a true synergism. However, large uncertainties remain with regard to quantifying the health effects of these important agents at prevailing levels of combined exposures in the present-day workplace and in non-occupational settings. In view of the complexities involved in the toxicological assessment of tobacco smoke, which is itself a combination of genotoxic and non-genotoxic agents, it is not possible at this time to extend inferences from mechanistic considerations to low combined exposures. Several large case-control studies under way involving non-occupational exposures may help to solve this enigma by creating better estimates and reducing the uncertainties surrounding synergistic effects from smoking and radon.

4. Metals

121. Toxic metals are important trace pollutants in the human environment. They interact in many ways with cell constituents and may produce oxidative gene damage or influence enzyme activity at low concentrations, e.g. by competing with essential metal ions [H38]. Carcinogenic transition metals are capable of causing promutagenic damage such as DNA base modifications, DNA-protein cross-links, and strand breaks [K7]. The underlying mechanism seems to involve active oxygen and other radicals arising from metal-catalysed redox reactions. Cadmium, nickel, cobalt, lead, and arsenic may also disturb DNA repair processes [H48]. Lead neurotoxicity, an example of an important non-genotoxic metal effect, is a result of intracellular regulatory dysfunction caused by this heavy metal. Lead activates calmodulin-dependent phosphodiesterase, calmodulin-sensitive potassium channels, and calmodulin-independent protein kinase C(PKC) [G31]. The latter effect already occurs at picomolar concentrations and indicates second messenger metabolism as a potential sensitive site for the disruptive action of lead. Epidemiologically proven metal lung carcinogens are arsenic, cadmium, chromium, nickel, and antimony [M65]. In the critical field of underground mining, possible metal effects have to be assessed together with high-LET radiation from radon. Arsenic in particular has been shown to be a major risk factor in combined exposures to mineral dust, radon, metals, and diesel fumes [K48, T5]. The risk-enhancing effects of iron dust seems to be limited to very high dust concentrations, leading to changes in lung function [B74]. An elevated stomach cancer risk in Ontario gold miners was statistically associated with chronium exposures but not with arsenic, mineral fibres, or diesel emissions [K50].

122. Multiple exposures to radon, arsenic, and tobacco smoke were common in several uranium mines (see also Section B.3). An assessment of 107 living tin miners who had lung cancer and an equal number of age-matched controls from tin miners without lung cancer provided no evidence for synergism between radon and arsenic or between arsenic and smoking [T5]. That there is no obvious synergism between this heavy metal and radon progeny exposure is implied by the fact that the risk of lung cancer among workers exposed to arsenic (and radon) in mining only is slightly less than for miners whose exposure came from smelting operations. In a study on gold miners with quite low radon exposures, linear regressions indicated that exposure to 1 WLM of radon decay products increases lung cancer mortality rates by 1.2%, a finding comparable to other studies, and that each year of employment in a poorly ventilated mine (before 1946) with an arsenic content of the host rock of 1% is associated with a 31% increase in lung cancer mortality rates [K21, K51]. Adding an interaction term to allow for a deviation from additivity for the combined effect of arsenic and radon decay products did not improve the fit. Noteworthy is the fact that the duration of the arsenic exposure seems to be more important than its intensity [T5].

123. The induction of radical-scavenging metallothionein by higher concentrations of heavy metals may confer protection against ionizing radiation. Single ip injection of cadmium (1 mg kg^{-1}) two hours before radiation exposure increased the yield of DNA lesions in peripheral blood lymphocytes of mice, but cadmium injection 24–48 hours in advance of ionizing radiation reduced DNA damage in lymphocytes in comparison with untreated animals [P27]. In this study the protective effect was due to reduced levels of initial DNA damage per unit dose of radiation as well as accelerated DNA repair measured in a single-cell gel assay.

124. Beryllium is another metal that has been examined for potential interactions with radiation. Although not considered to be a genotoxic metal [A10], beryllium is a known animal carcinogen and has recently been classified as a demonstrated human carcinogen [W9]. The potential for carcinogenic interactions between inhaled beryllium oxide and $^{239}PuO_2$ was examined in rats [S42]. The agents were administered alone or in combination at initial lung burdens of 1–91 µg beryllium and 0.15–6.7 kBq ^{239}Pu. Beryllium oxide exposure induced few tumours and did not markedly influence Pu-induced lung tumorigenicity, despite the fact that beryllium oxide exposure decreased the clearance of ^{239}Pu from the lung and thus served to increase the total radiation dose to the lung.

125. Another ongoing study investigates the potential carcinogenic interactions between inhaled beryllium metal and $^{239}PuO_2$ in some 5,456 male and female F344 rats [F1]. Preliminary results from the study demonstrate that inhaled beryllium metal is a potent rat lung carcinogen; over 90% of rats that survived at least 12 months after inhaling an initial lung burden of 450 µg beryllium developed benign and/or malignant lung tumours [F12]. At lower lung burdens of approximately 50 µg beryllium, some 65% of exposed rats (39 of 60 rats) developed at least one malignant lung tumour. When this level of beryllium was combined with a lung burden of 60 and 170 Bq $^{239}PuO_2$, which alone caused crude malignant lung tumour incidences of 8% (6 of 60 rats) and 7% (2 of 27 rats), respectively, crude tumour incidences ranged from 57% (16 of 28 rats) to 90% (54 of 60 rats) [F16, F28]. Thus, indications of a more-than-additive response were observed.

As was the case for the cigarette smoke or beryllium oxide exposures described above, inhalation of beryllium metal was found to markedly decrease the clearance of $^{239}PuO_2$ from the lung [F1], serving to increase the radiation dose in groups receiving combined exposures and to leave in question the role of beryllium-induced increased radiation dose vs. a more fundamental interaction between the two agents at the molecular or cellular level. In addition, the investigators reported that exposure to beryllium markedly reduced the median lifespan of exposed animals, and they noted that a complete analysis of the combined carcinogenic effects of the two agents would require an analysis more sophisticated than an examination of crude tumour incidence [F15]. Specifically, the authors noted that the age-specific tumour incidence for the two agents alone and in combination should be analysed, and it was noted that this analysis is under way.

126. The potential for beryllium and x radiation administered alone and in combination to affect cell-cycle kinetics, cell killing, and induction of chromosomal aberrations was examined in mammalian cell culture (Chinese hamster ovary cells, Figure A.V) [B37]. Beryllium was administered in a soluble form ($BeSO_4$) at 0.2 or 1 mM concentrations, and x rays at levels of 1 or 2 Gy. It was found that exposure to beryllium significantly inhibited the capacity of the cells to repair DNA damage induced by x rays. The combined exposures were characterized by a multiplicative model when total chromosomal aberrations were examined hours after exposure. Both agents caused an accumulation of cells in the G_2/M stage of the cell cycle, and an analysis using varying times between exposures suggested that the multiplicative interaction observed may have been limited to cells in the S and G_2 stages of the cell cycle.

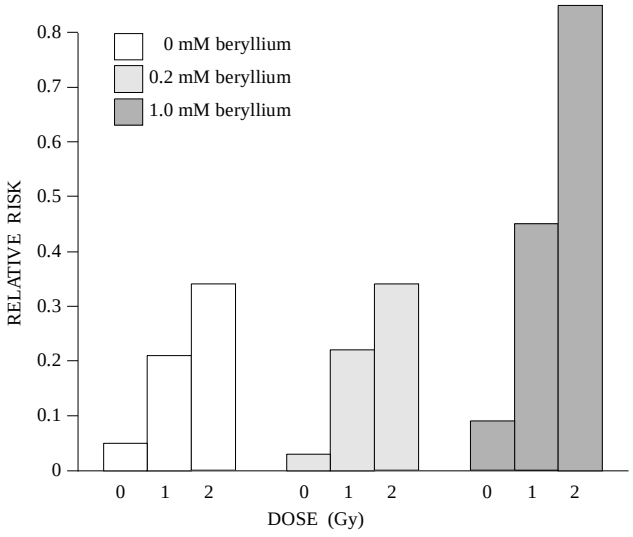

Figure A.V. Induction of chromosome aberrations in Chinese hamster ovary cells from exposures to x rays and beryllium [B37].

127. Micronucleus formation in mouse bone marrow polychromatocytes as a measure of the modulation of the mutagenic action of gamma rays by chromium and lead salts was used by Vitvitskii et al. [V12]. Chromium (VI) ions enhanced radiation effects in acute and chronic experiments. Acute exposures of lead (II) ions below 15 mg kg^{-1} body weight had an antagonistic effect, i.e. they decreased the number of gamma-ray-induced micronuclei, whereas higher doses increased it. Chronic combined action of lead (III) ions and gamma rays resulted in a lower yield of micronuclei. For an extrapolation to environmental exposures and humans, an elucidation of the underlying mechanisms, i.e. heavy metal influence on cell kinetics and/or on DNA damage and repair, will be necessary.

128. The combined effect of $^{134,137}Cs$ and lead (Pb^{2+}) at the soil concentrations found in highly contaminated habitats in the Russian Federation on the mutation rate in the plant *Arabidopsis thaliana (L:) Heynh* has been investigated [K42]. At concentrations of 220–2,500 Bq kg^{-1} and 16–320 mg kg^{-1}, respectively, both antagonistic and synergistic effects were seen. The radiation-induced mutation rate was significantly reduced in the presence of 16 mg kg^{-1} Pb^{2+}, whereas higher lead concentrations increased the rate in plants grown in soil with up to 1,000 Bq kg^{-1} radiocaesium. At the highest radiation level and 32–320 mg kg^{-1} Pb^{2+}, an apparent decrease in the mutation rate was linked to a large number of sterile seeds. In an ecological study, the combined effect of zinc or cadmium and external radiation on microbial activity in soil was determined by measuring nitrogen fixation, dentrification, and CO_2 flux [E20]. At metal concentrations in soil of 10–100 mg kg^{-1} for Zn^{2+} and 0.5–16 mg kg^{-1} for Cd^{2+}, small radiation doses ranging from 3.6 to 12 mGy led to a supra-additive effect in the inhibition of microbial activity in soddy-podzolic soil. It was further shown that the enzyme level of invertase increased in combined exposures, whereas catalase and dehydrogenase activities were lower [E19].

129. A sensitive assay in spring barley (*Hordeum vulgare L.*) leaf meristem to record effects from ionizing radiation and/or heavy metals was developed by Gerask'in et al. [G34]. The radiation-induced frequency of cells with aberrant chromosomes in the intercalary meristem allows doses to be registered in the range of a few tens of milligray [G35]. Irradiations were performed at the shoot stage and involved doses of 40, 80, and 200 mGy at a dose rate of 2 Gy h^{-1}. Lead (II) and cadmium (II) were applied as nitrates in two concentrations of 40 and 200, and 3 and 20 mg kg^{-1} of soil, respectively. The authors claimed that in this system, radiation and heavy metals alone exhibit clearly non-linear relationships, i.e. supralinearity, with a higher slope for aberrations at lower doses than at higher doses. Combined exposures show an antagonism for low doses of ionizing radiation (40 mGy) and for all lead concentrations. At doses of 80 and 200 mGy, a slightly supra-additive effect is reported. For cadmium, supra-additivity is found at low metal concentrations of 4 mg kg^{-1} for 80 and 200 mGy but not for 40 mGy. At high metal concentration, less-than-additive effects were found. Although these findings may be important for environmental assessments and potentially extendable to mechanistic studies, no direct inferences to humans are warranted at this stage.

130. Metals and ionizing radiation have been shown to produce combined effects in many biological systems (Table A.1). Because metals cause many biological effects with no or very low thresholds, possible interactions would potentially extend to very low exposures. In the case of relatively unspecific damage to DNA, such as oxidative attack, iso-addition would be predicted. As an example of a synergistic effect at high exposure levels, a threshold phenomenon, decreased lung clearance of internal radionuclide content by high metal concentrations, was found to be the cause of the combined effect. No supra-additive effects are seen in the albeit weak database on combined occupational exposure to radon and arsenic. The relative importance of different damage-inducing mechanisms of metals for combined exposures in human remains to be elucidated.

5. Mitogens and cytotoxicants

131. Although mitogenic and cytotoxic compounds are generally non-genotoxic agents and could have been included in Section B.2, they are considered here separately, principally because of their ability to stimulate cell proliferation. The combination of mitogens or differentiation-inducing agents with radiation has some potential as a cancer therapeutic strategy. Experiments in this area employ high doses, but studies intended to elucidate the mechanisms of interaction may still be relevant outside the clinic. Leith and Bliven [L15] investigated the x-ray responses of a human colon tumour cell line after exposure to the differentiation-inducing agent N-methylformamide (NMF). A human colon tumour line was exposed for three passages to varying concentrations (0–170 mM) of NMF and the change in sensitivity to ionizing radiation was examined *in vitro*. The linear-quadratic formalism of survival with two constants (alpha and beta) was used to characterize the single graded dose-survival curves. As the NMF concentration increased, the alpha parameter increased and the beta parameter decreased, yielding a concentration-dependent radiosensitization that was most marked in the low-dose region of the survival curve. Upon removal of NMF, the original radioresistance was regained within two or three cell culture doubling times.

132. Müller et al. [M11] studied the formation of micronuclei in preimplantation mouse embryos *in vitro* after combined treatment with x rays and caffeine. The exposures to caffeine were 0.1 or 2 mM and to x rays, 0.2 or 0.9 Gy. X rays as well as caffeine induced micronuclei. The dose-effect curve after irradiation was linear for the dose range measured (0–3.8 Gy). Caffeine only induced micronuclei at concentrations higher than 1 mM; between 1 mM and 7 mM, however, there was a linear increase in the number of micronuclei. A considerable enhancement of the number of radiation-induced micronuclei was observed when irradiation of the embryos was followed by treatment with caffeine. The sum of the single effects was clearly exceeded by the combination effects. An earlier study in the same laboratory [M12] was on the effects of a combination of x rays (0.2, 0.9, or 1.9 Gy) and caffeine (0.1, 1, or 2 mM) on the formation of blastocysts (96 hours post-conception), hatching of blastocysts (144 hours post-conception), and on the cell numbers of embryos at different times (48, 56, 96, and 144 hours post-conception). The embryos were irradiated in the G_2 phase of the two-cell stage (28 or 32 hours post-conception), either 1 hour after or immediately before application of caffeine. Caffeine was present during the whole incubation period (until 144 hours post-conception). Specific conditions under which caffeine markedly enhanced the radiation risk, i.e. under which the combination effect exceeded the sum of the single effects, were described. This was the case, in particular, for embryonal development, for which the risk was almost doubled, whereas the enhancement of risk was smaller for the proliferation of cells. The amount of caffeine necessary for supra-additivity, however, is so high (at least 1 mM caffeine for rather long times) that it is clearly above the range achievable *in vivo* by consumption of caffeine-containing beverages. At physiological levels, caffeine also displays antioxidant properties and inhibits carcinogenesis induced in rats and mice by various known carcinogens. Examples are the inhibition of smoke-condensate-induced carcinogenesis in mouse skin [R13] and gastric tumour promotion by NaCl in rats [N10]. Based on these and other findings, Devasagayam et al. [D1] suggest that at lower concentrations, the potency of the antioxidant action of caffeine far outweighs the deleterious effects, if any, from its inhibition of DNA repair.

133. Besides the interference of caffeine with repair processes as a consequence of its effect on cell-cycle blocks at high concentrations, this ubiquitous substance also scavenges oxygen species induced by radiation and genotoxic chemicals [K27, K28]. The chemical basis of this effect was shown to be the removal of free electrons and hydroxyl radicals by caffeine. The reaction rate constants for these two reactions were shown to be about $1.5\ 10^{10}\ M^{-1}\ s^{-1}$ and $6.9\ 10^{9}\ M^{-1}\ s^{-1}$, respectively [K27]. The former value is high enough to compete with oxygen for the scavenging of free electrons and therefore may reduce oxidative damage involving superoxide anion (O_2^-), hydroperoxyl radical (HO_2^-), and hydrogen peroxide (H_2O_2). This mode of action is backed by recent findings in barley seeds that caffeine affords protection only at high oxygen concentrations but potentiates radiation damage (albeit less damage) at low oxygen pressures [K29].

134. Both a reduction of the radiation-induced G_2/M phase arrest and the antioxidant effect of caffeine may indirectly influence apoptosis and modulate survival and expansion of cells with a modified genome. In different systems, an enhancement of the degree of DNA fragmentation by caffeine, theobromine, theophylline, and 2-aminopurine was found in murine T-lymphoma cells [P10], whereas in TKG cells, 2 mM caffeine eliminated the degradation of DNA entirely [Z4]. At this stage, it is doubtful whether these findings have any meaning for risk assessments at controlled exposure levels.

135. Caffeine, which may potentiate radiation damage at higher concentrations owing to its release of protective cell-

cycle blocks, seems also to influence the clastogenic effects of radiation and other genotoxic agents (see also Section B.5). Several studies found an inhibition of oxic radiation damage [K25, K26]. Stoilov et al. [S43] found both potentiation and protection against radiation-induced chromosomal damage in human lymphocytes. Temperature and concentration were shown to be decisive for the direction of the effect.

136. Kalmykova et al. [K3] evaluated the effectiveness of joint exposure to ^{239}Pu and tributyl phosphate on the induction of leukopenia in Wistar rats. It was shown in this system that the additive effect of the two agents delivered simultaneously was exceeded only at high doses, i.e. acute levels. With levels ranging from subacute effective to minimum effective, the effect of the combined treatment was less than projected from additivity.

137. Cattanach and Rasberry [C27] reviewed the literature on the genetic effects of combined treatments with cytotoxic chemicals and x rays. Some pretreatments clearly enhanced the yield of genetic damage. With spermatogonial cells, chemicals that kill cells can substantially modify the genetic response to subsequent radiation exposure over several days or weeks. Both enhancement and reduction in the genetic yield were found, and the modifications also depended on the type of genetic damage scored, with specific-locus-mutation response differing from that for translocations. Selective killing of rapidly dividing cells in the areas most heavily damaged by radiation was a suggested explanation [C28]. In general, such interactions based on perturbations of cell kinetics should be of little relevance for lower exposure levels.

138. Cyanate (KOCN)-induced modification of the effect of gamma radiation and benzo(a)pyrene was studied by Serebryanyi et al. [S80] in cultured CHO-AT3-2 cells. Sensitizing effect was found for radiation and benzo(a)pyrene effects such as cell viability, micronuclei induction, and mutations in the thymidinekinase and Na^+/K^+-ATPase loci. The authors suggested that repair inhibition and/or changes in the cell chromatin structure produced by KOCN is responsible for these sensitizing effects. The proposed mechanisms as well as the concentration and dose ranges used in the experiment preclude direct transfer to occupational or environmental levels.

139. In summary, many studies assessing deviations from additivity in combined exposures between mitogens/cytotoxicants and ionizing radiation are found in the literature (Table A.1). In most cases, the high exposure levels applied and the biological endpoints studied do not allow the transfer of results to humans. However, any endogenous or dietary levels of agents influencing stem-cell population size or kinetics will have the potential to modulate response to radiation.

6. Antioxidants, vitamins, and other dietary factors

140. The genetic effects of combined treatments of radioprotecting agents and x rays were reviewed by Cattanach and Rasberry [C27]. Chemicals such as cysteamine, mexamine, and glutathione given in advance of radiation were not always protective but gave contradictory results, with significant protection of specific germ-cell stages being restricted to different dose ranges. This might be attributable to the different radiation sensitivities and cell-cycle kinetics of the germ-cell stages tested. Some pretreatments clearly enhanced the yield of genetic damage.

141. Dietary caloric intake and type of food are important variables affecting the rate of spontaneous DNA damage, as was discovered recently in humans [D11, S37, S38]. These findings are supported by similar findings of reduced oxidative damage to mitochondrial and nuclear DNA in food-restricted rats and mice [C12]. It is known from experiments with rats that caloric restriction of food is correlated with a lower incidence of cancer, an increased lifespan, and less free-radical damage to lipids, proteins, and DNA [W4, Y3, Y5, Y14]. Dietary fat is associated with increased breast cancer risk. In a study involving 21 women at high risk, the level of the oxidized thymine (5-hydroxymethyluracil) per 10^4 thymine was 9.3±1.9 in the nucleated peripheral blood cells of women consuming 57 g of dietary fat per day compared with 3±0.6 for women consuming 32 g per day [D11, F3].

142. Diet can also modify the effectiveness of chemical carcinogens, sometimes by a large factor. Some of the underlying mechanisms have been identified. Rats with a deficiency of riboflavin in their diet become highly sensitive to liver tumour formation when treated with 4-dimethylaminoazobenzene, because reduced levels of a flavin adenine dinucleotide-dependent azo dye reductase increase the effective dosage of the carcinogen [C25]. On the other hand, a protein-free diet prevents liver toxicity of dimethylnitrosamine in rats, and a fat-restricted diet decreases tumour induction in mammary glands of rats. Silverman et al. [S32] studied the effect of dietary fat on mammary cancer induction in Sprague-Dawley rats given 3.5 Gy whole-body x-irradiation at 50 days of age. Rats on a high-fat diet (20% lard) from 30 days of age had more tumours than rats on a low-fat diet (5% lard) and a higher multiplicity of carcinomas per rat. Rats on the low-fat diet exhibited longer median tumour latency periods than did those on the high-fat diet. Spontaneous breast cancer incidence in humans is also influenced by the level and type of fat intake. Potential mechanisms in dietary-fat-dependent mammary tumorigenesis were reviewed by Welsch [W5]. Yoshida et al. [Y14] reported that caloric restriction significantly reduced the incidence of x-ray-induced myeloid leukemia in C3H mice. Again, in this system, caloric restriction either before or after irradiation also significantly prolonged the lifespan of the animals.

143. In some instances, the degree of tumour formation depends on the amount of food provided during the promoting phase and not on the nutritional status at time of exposure. Polyunsaturated oils are potent promoters, probably also for humans [W14]. It is now generally accepted that restricted food intake, particularly during development phases, reduces the incidence of neoplasms

and increases longevity. An epigenetic effect, namely a general decrease in cell duplication rates, especially in endocrine-sensitive organs, is at the root of this finding [C26].

144. Several vitamins and many food constituents display radical scavenging activities and antioxidant properties. There is considerable scientific and economic interest in the still unresolved question whether diets enriched in vitamins, antioxidants, carotinoids, and selenium reduce the risk of cancer [W13]. Vitamin A and retinoic acid derivatives are considered important micronutrients involved in the modulation of cancer risk in humans. Vitamin A seems to affect the incidence of lung cancer in smokers and tobacco chewers positively. Hence, clinical trials in Finland and the United States randomized the use among smokers of artificial beta-carotene (precursor of vitamin A) and, in the Finnish study, the use of artificial alpha-tocopherol (vitamin E) [H7, O14]. Surprisingly, these two studies found significant increases in lung cancer risk related to beta-carotene use. Whether this finding is due to the dietary form of the provitamin remains to be elucidated. Human cervix and bladder cancer are somewhat more frequent in individuals with low vitamin intake [S45]. These beneficial effects are thought to arise from differentiation of epithelial tissues and from improved cell-cell communication. Vitamins E and K are benzo- and naphthoquinones and therefore potential antioxidants. Reduction of tumour induction by the former in animal systems was shown only at levels much higher than are found in the human organism.

145. Selenium also reduces tumour risk in animal systems. Its salts are indicated as a co-factor for glutathione peroxidase. Vitamins C, E, and K, the latter two in the lipid phase and its boundary, prevent the formation of nitrosamines and nitrosamides and seem to be important in the protection of the gastro-intestinal linings, the liver, and the respiratory tract [M31]. Although any molecule with antioxidant and radical scavenger activity is also a potential radioprotector, the extreme speed of the interaction of reactive species formed by radiation with DNA would require high concentrations to make a difference. For combined effects, the available information indicates that micronutrients are important. The sizeable influence of vitamin A, vitamin E, selenium, and 3-aminobenzamide as radioprotectors in the C3H10T½ transformation assay is shown in Figure A.VI [H11].

146. Borek et al. [B24] studied the anticarcinogenic action of selenium and vitamin E. The single and combined effects of these chemicals were examined on cell transformations induced in C3H10T½ cells by x rays and benzo(a)pyrene and on the levels of cellular scavenging and peroxide destruction. Incubation of C3H10T½ cells with 2.5 µM Na_2SeO_3 (selenium) or with 7 µM alpha-tocopherol succinate (vitamin E) 24 hours prior to exposure to x rays or the chemical carcinogens resulted in an inhibition of transformation by each of the antioxidants with an additive-inhibitory action when the two nutrients were combined. Cellular pretreatment with selenium resulted in increased levels of cellular glutathione peroxidase, catalase, and non-protein thiols (glutathione) and in an enhanced destruction of peroxide. Cells pretreated with vitamin E did not show these biochemical effects, and the combined pretreatment with vitamin E and selenium did not augment the effect of selenium on these parameters. These results support the notion that free-radical-mediated events play a role in radiation and chemically induced transformation. They indicate that selenium and vitamin E act alone and in additive fashion as radioprotecting and chemopreventing agents. The results further suggest that selenium confers protection in part by inducing or activating cellular free-radical scavenging systems and by enhancing peroxide breakdown, while vitamin E appears to confer its protection through another, complementary mechanism.

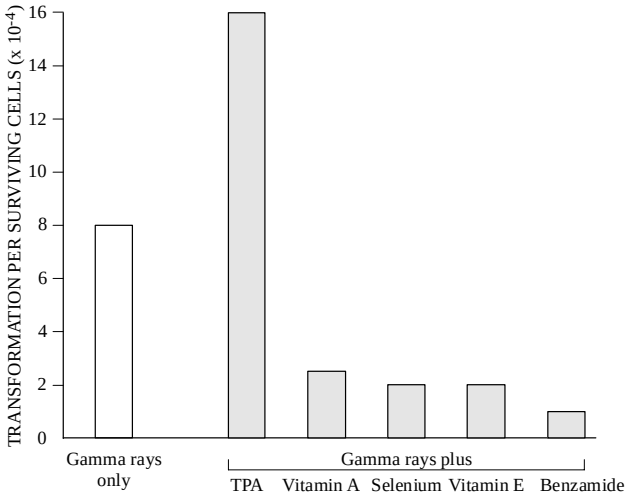

Figure A.VI. Effect of chemical and dietary factors on response of C3H10T½ cells in combined exposures with gamma rays giving a dose of 4 Gy [H11].

147. The importance of sulfhydryl groups as an antagonist of or protector against radiation-induced radical attack on DNA is well known from molecular and *in vivo* studies [M4, M6, M7, V4]. In view of the fast and localized action of ionizing radiation, these substances have to be small enough to reach the target to be protective and to be present there in considerable concentrations for noticeable effects. Even for small water-soluble substances displaying sulfhydryl groups, such as cysteamines, this is difficult to achieve in humans. Therefore the use of sulfhydryl radioprotectors is limited by their toxicity and the short period during which they are active [M4]. At environmental levels, no protective effect is to be envisaged. However, for lipophilic substances such as vitamins A, E, and K or for coenzymes with high-affinity binding to active centres, local concentrations in specific compartments might become high enough for a protective effect, even with low dietary intakes. In addition to these directly acting protectors, immunomodulators such as endotoxins and bacterial or yeast polysaccharides are known to protect against the deleterious effects of radiation [M4]. Their mode of action, stimulation of the reticulo-endothelial system, is probably irrelevant for stochastic effects.

148. Ethanol consumed in alcoholic beverages is known to increase the incidence of several cancers of the oral cavity and the oesophagus, especially in combination with active cigarette smoking [I8]. No data from human studies on ethanol and ionizing radiation are available, but the irritating effect of higher concentrations of ethanol makes this form a potential tumour promoter. Mechanistic studies also suggest that ethanol modifies the biochemical activation in the oral cavity and the oesophagus of some tobacco-specific carcinogens [S44], a mechanism of no direct relevance to radiation. Acetaldehyde is a toxic reactive metabolite of ethanol in tissue where biotransformation occurs. Very important in view of population health are behavioural changes and a tendency to malnutrition in alcohol addicts, which may increase their susceptibility to toxicants in the environment or at the workplace. The many direct and indirect effects of alcohol consumption on radiation-induced changes at the cellular, organ, and behavioral levels were discussed in depth in a review by Ushakov et al. [U18] assessing experiences in human populations affected by the Chernobyl accident.

149. Iodine as a constituent of thyroxine, the hormone of the thyroid gland, is often deficient in inland areas, where geological factors and the absence of seafood produce a diet low in iodine. Since its fission yield, relative volatility, and half-life make ^{131}I one of the critical fission products that may be present in environmental exposures, the potential increase of thyroid dose per unit uptake in humans with iodine-deficient diets is a major concern in radiation protection. It is still not known whether the higher stimulation of the gland in iodine deficiency by endogenous hormones will also alter the radiosensitivity of the stem cells and the risk coefficient for thyroid carcinoma. The wealth of data, mainly from therapeutic procedures in nuclear medicine, showing little to no carcinogenic potential for ^{131}I, even at high exposures [U2], is somehow contradicted by recent results showing large increases in thyroid carcinoma in children affected by the Chernobyl accident. Initial measurements of iodine in urine from Belarus indicated that areas most heavily affected by iodine deposition are also deficient in a dietary supply of iodine and are endemic goitre areas.

150. The modifying influence of sodium chloride (NaCl), miso (Japanese soybean paste), and ethanol on the development of intestinal metaplasia after x-ray exposure was examined by Watanabe et al. [W29] in CD(SD):Crj rats. Intestinal metaplasia in the glandular stomach is considered a precursor lesion for differentiated gastric adenocarcinoma. Five-week-old rats were treated with two doses of 10 Gy from x rays to the gastric region at a three-day interval. After exposure, the rats were given NaCl (1% or 10% in diet), miso (10% in diet), or ethanol (10% in drinking water) for 12 months. The number of alkaline phosphatase-positive foci of intestinal metaplasia in rats given a 1% NaCl diet after x rays was significantly elevated compared with that in rats given x rays alone or x rays with a 10% NaCl diet. In the pyloric gland mucosae, total numbers of metaplastic foci in rats given x rays and 1% NaCl diet were much higher than other combined-treatment groups. The incidence of atypical hyperplasia was less than 6% in all treatment groups, and no promoting effect on gastric tumorigenesis was observed. These results demonstrated that the occurrence of intestinal metaplasia induced by x rays can be significantly modified by basic and common food constituents, but this is not associated with any influence on gastric neoplasia.

151. A potentially important interaction was investigated by Montour et al. [M24], who studied the modification of radiation carcinogenesis by marihuana (tetrahydrocannabinol, delta(9)-tetrahydro-cannabinolic acid). Male, female, and ovariectomized female Sprague-Dawley rats were irradiated with doses of 1.5, 3, or 4 Gy, respectively, from ^{60}Co gamma rays at between 40 and 50 days of age. The animals were injected three times weekly with either marihuana extract or with alcohol-emulphor carrier. Mean survival time in males was significantly shorter in the 4 Gy plus marihuana group compared with the three other groups, whose mean survival times did not differ. Throughout the 546-day period in which the male rats were observed, the total number of tumours other than fibrosarcomas was significantly greater following radiation and marihuana administration (22) than following irradiation alone (6). Fifteen of the tumours originated in breast or endocrine tissues. No differences were seen in the unirradiated groups. In the females, which were observed for 635 days, the total number of breast tumours was significantly higher in the combined treatment group (38) compared with the group treated with radiation alone (22). This was entirely due to a marked difference in the adenocarcinoma incidence, which was 21 (radiation plus marihuana) compared with 4 (radiation alone). The number of adenofibromas was similar in the two groups. In the unirradiated female groups, the breast adenocarcinoma incidence was 8 in the marihuana group and 2 in the control group. Ovariectomy resulted in a lower breast tumour incidence in all groups. Non-breast tumours were more frequent in the ovariectomized-irradiated groups. Radiation plus marihuana produced more non-breast tumours (25) than radiation alone (17) in the ovariectomized females.

152. Dietary factors are proven modifiers of risk from diverse agents at levels found in human populations and probably also influence the production and repair of endogenously arising lesions. Absence or deficiency of important coenzymes and nutrients on one side and high levels of directly or indirectly acting mitogens on the other interfere with molecular, cellular, and tissue responses to ionizing radiation. In view of the many mechanisms involved, the full spectrum of interactions from antagonisms to synergisms must be expected (see also Table A.1). A reduction in the radiation risk may occur in situations where growth stimuli are reduced owing to nutritional deficiency or where the number of stem cells at risk are reduced. Synergisms are to be expected where reduced levels of radical scavengers or coenzymes needed for repair increase the yield of primary damage from

ionizing radiation or impair the speed and accuracy of cellular responses to damage. In general, the health risks not only from ionizing radiation but also those from most other deleterious agents in the human environment will be affected by deviations from an optimum diet.

C. RADIATION AND BIOLOGICAL AGENTS, MISCELLANEOUS

1. Hormones

153. Many hormones are potent growth stimulators. Considerable evidence is available for the modulation of cancer risk by hormones. Animal experiments have shown that increased levels of thyroid-stimulating hormone (TSH) can enhance tumour growth and increase the risk of cancer [D31]. Thyroid stimulating hormone is increased during puberty and pregnancy as a result of increased levels of female sex hormones [H37, P25]. There is epidemiological evidence suggesting that the development of thyroid cancer after high-dose radiation exposure in females can be potentiated by subsequent child bearing. Marshall Islanders who were exposed to radioactive fallout from a nuclear weapons test in 1954 received high thyroid doses from radioiodines. Women who later became pregnant were at higher risk of thyroid cancer than exposed women who remained nulliparous [C43]. The numbers, however, were small.

154. The same effect was found in a population-based case-control study in Connecticut in the United States involving 159 subjects with thyroid cancer and 285 controls [R11]; 12% of the cases but only 4% of the controls reported prior radiotherapy to the head and neck. Among women, this risk appeared to be potentiated by subsequent live births (RR = 2.7). The risk for ever parous alone was, however, higher (1.6) than for prior radiotherapy (1.1). Another case-control study, carried out in Washington in the United States, linked a 16.5-fold increased risk of thyroid cancer to prior radiotherapy of the head and neck among 282 females and 394 controls [M17]. Overall, 20.2% of the cases but only 1.5% of the controls reported earlier radiotherapy (RR = 16.5; 95% CI: 8.1–33.5). In this study, pregnancy following radiotherapy was associated with only a small additional risk (RR = 1.3), which was far from statistically significant (95% CI: 0.1–15.7) [M9]. Combined with similar findings from Sweden [W30], these studies suggest that TSH-mediated tissue proliferation in adolescence and pregnancy may be a risk factor in radiation-induced thyroid cancer.

155. The long-term use of tamoxifen, a synthetic anti-oestrogen that has been shown to reduce mortality from breast cancer and the occurrence of contralateral breast cancer, increases the risk for endometrial cancer. In a case-control study of woman treated for breast cancer, Sasco et al. [S26] showed that tamoxifen or radiation castration (which included high doses to the uterus as well as the ovaries) considerably increased the risk for subsequent endometrial cancer. The odds ratios for tamoxifen use for more than five years and radiotherapeutic castration were 3.5 and 7.7, respectively. Women who had undergone combined treatment had an odds ratio of only 7.1. Since the study was based on small numbers (43 cases and 177 controls), the power is not sufficient to postulate an antagonism, but there is enough evidence to reject an enhancement of risk between the two carcinogenic factors.

156. One well-studied interaction is that between radiation and the natural hormone oestradiol-17 beta (E_2) in mammary carcinogenesis. In a publication by Broerse et al. [B32], the combined effects of irradiation and E_2 administration on the mammary gland in different rat strains were investigated. Three rat strains, Sprague-Dawley, Wistar WAG/Rij, and Brown Norway, with different susceptibilities to the induction of mammary cancer, were irradiated with x rays and mono-energetic neutrons; increased hormone levels were obtained by subcutaneous implantation of pellets with E_2. Mean plasma levels were 100–300 pg ml^{-1} plasma, while normal levels in these rat strains were about 50 pg ml^{-1}. The latency period for the hormone-treated animals was shown to be considerably shorter than for animals with normal endocrinological levels. Administration of the hormone alone also appreciably increased the proportion of rats with malignant tumours. At the high levels of hormones applied in the study, there was little indication that radiation and hormones produced any supra-additive effect, but the single-agent effect levels in this study might have been too high to properly assess this other effect. The effect of hormone administration and irradiation on mammary tumorigenesis was the same for hormone administration one week prior to or 12 weeks after irradiation. The RBE values for induction of mammary carcinomas after irradiation with 0.5 MeV neutrons have a maximum value of 20 and are not strongly dependent on hormone levels.

157. The carcinogenic and co-carcinogenic effects of radiation on rat mammary carcinogenesis and mouse T-cell lymphomagenesis were studied by Yokoro et al. [Y6]. For both experimental models, the study clearly showed the importance of the promotion stage and of the physiological condition of target cells at the time of initiation. In rat mammary carcinogenesis, prolactin was shown to be a powerful promoter regardless of the initiating agent. The authors also suggested that an enhancer like prolactin might be useful in detecting the carcinogenicity of small doses of carcinogens; for example, a high RBE of 2.0 MeV fission spectrum neutrons was demonstrated by the application of prolactin to radiation-initiated mammary carcinogenesis in rats. Because cellular reactions are somewhat different for different LET values, it remains to be proven that the sensitizing effect of a hormone is independent of radiation quality.

158. Shellabarger et al. [S24] investigated the influence of the interval between neutron irradiation and diethylstilbestrol (DES) on mammary carcinogenesis in female ACI rats. Both radiation and DES are carcinogens for the

mammary gland of ACI female rats and act in a synergistic fashion, particularly with regard to the number of mammary adenocarcinomas per rat when DES is given at about the same time as radiation. DES, in the form of a compressed pellet containing a mixture of cholesterol and DES, formulated to average 1.25 mg of DES per 100 g body weight, was given to groups of approximately 28 rats at 2 days before or 50, 100, or 200 days after 0.064 Gy from 0.43 MeV neutron irradiation. Every time DES was given to irradiated rats, it was also given to non-irradiated rats. When the total number of mammary adenocarcinomas 375 days after administration of DES was analysed as a percentage of 24 sites per rat at risk, DES and radiation always produced a response that was larger than the sum of the responses of DES alone and radiation alone. The supra-additive interaction between radiation and DES did not decline as the time interval between irradiation and DES was lengthened, which suggests that neutron-initiated mammary carcinogenesis is not subject to repair, since DES promotion continues to be effective for long times.

159. Irradiation of pregnant Wistar rats at days 7, 14, and 20 of pregnancy, followed by DES treatment after nursing for one year, showed a strong correlation of mammary gland tumours with the hormonal status of the gland during radiation exposure [I11]. Irradiation alone (2.6 Gy) resulted in a 23% incidence of mammary gland tumours. The additional implantation of a DES pellet (releasing about 1 mg d^{-1}) increased this value to 35% and 93% for radiation exposure at days 14 and 20, respectively. The data suggest that the initiation of tumorigenesis by gamma rays is critically dependent on the developmental status of the gland at exposure. Since no group with DES exposure only was included, no direct assessment of the combined effects is possible from this study. When the radiation exposure was delayed to day 21 of lactation or day 5 post-weaning, combined treatment with gamma rays and DES resulted in an incidence of mammary gland tumours of 94% and 73%, respectively. Since the value from combined treatment in virgin animals was only 24%, it is suggested that the differentiation state of the radiation-exposed tissue is more relevant than the hormonal and proliferative state of the cell populations at risk [S40]. Rats with weaning experience receiving only a gamma dose at day 21 of lactation or DES had tumour incidences of 35% and 27%. When compared with the combined effect of 94%, a synergistic effect has to be postulated.

160. The effect of age and oestrogen treatment on radiation-induced mammary tumours in rats was analysed by Bartstra et al. [B71, B72]. The excess normalized risk of mammary carcinoma was 0.9 for 1 Gy and 2.2 for 2 Gy in the age groups 8, 12, 16, 22, and 36 weeks, with no significant differences between the age groups. However, irradiation at 64 weeks yielded fewer carcinomas than in the controls, the excess normalized risk being 0.7 and −0.3 for 1 and 2 Gy, respectively. After oestradiol-17 beta2 treatment, the excess normalized risk for carcinomas was 7.7 for both 1 and 2 Gy in the age groups 8, 10, 12, and 15 weeks, with no significant differences between the age groups. However, in the age groups 22, 36, and 64 weeks, the excess normalized risk decreased with increasing age at exposure. Irradiation at 64 weeks yielded fewer carcinomas than in controls, with an excess normalized risk of −0.6 for both 1 and 2 Gy. The excess normalized risk was 10–80 in oestrogen-treated controls compared with untreated animals. The findings indicated that administration of oestrogen increased the radiation sensitivity of the mammary gland in young animals considerably. Administration of oestrogen influenced the shape of the dose-response curve for radiation-induced mammary cancer in young rats. In untreated animals there was a linear dose-effect relationship, whereas in oestrogen-treated ones the relationship could only be described by a quadratic function. In older rats, radiation dose-effect relationships in oestrogen-treated and non-treated animals were best fitted by linear relationships. The reduced risk of radiation exposure at mid-life was observed in oestrogen-treated and control rats.

161. The influence of androgens in the development of radiation-induced thyroid tumours in male Long-Evans rats was investigated by Hofmann et al. [H12]. When eight-week-old male rats were treated with radiation (1.5 MBq $Na^{131}I$), thyroid follicular adenomas and carcinomas were observed at 24 months of age with a high incidence, 94%. Castration of males prior to irradiation significantly reduced this tumour incidence to 60%. When testosterone was replaced in castrated, irradiated male rats, differentially increased incidences of thyroid tumours occurred, depending on the time interval for hormone replacement. Immediate (age 2–6 months) or early (age 6–12 months) testosterone replacement at approximate physiological levels led to thyroid follicular tumour incidences of 100% and 82%, respectively, whereas intermediate (12–18 months) or late (18–24 months) testosterone treatment led to only 70% and 73% incidences, respectively. Continuous testosterone replacement (2–24 months) in castrated, irradiated male rats raised the thyroid tumour incidence to 100%. Only the two 100% values are significantly different from the value of 60% in castrated irradiated animals not receiving testosterone replacement. Since elevated TSH is a reported requisite for the development of radiation-associated thyroid tumours, the effects of testosterone on serum thyroid-stimulating hormone levels were examined. Mean serum thyroid-stimulating hormone values in all irradiated animal groups were significantly elevated and well above those in age-matched, non-irradiated animals at 6, 12, 18, and 24 months. Serum thyroid-stimulating hormone levels were higher in continuous testosterone-replaced irradiated castrates than in intact, irradiated males but lower in irradiated castrates without testosterone treatment. Interval testosterone replacement in castrated male rats was generally associated with increased serum thyroid-stimulating hormone levels during the treatment interval and with lowered thyroid-stimulating hormone levels after discontinuation of testosterone treatment, particularly in irradiated rats. However, when irradiated, castrated males received late (age 18–24 months) testosterone replacement, there was no elevation of thyroid-stimulating hormone at

the end of the treatment interval. Thus an indirect effect of testosterone via early stimulation of thyroid-stimulating hormone may be at least partly responsible for the high incidence of radiation-induced thyroid tumours in male rats.

162. Watanabe et al. [W28] examined the influence of sex hormones on the induction of intestinal metaplasia by x rays in five-week-old Crj:CD(SD) rats of both sexes. At the age of four weeks, the animals were gonadectomized and given testosterone or DES in the form of subcutaneous implants containing 0.25–2.5 mg hormone. One week later, they were irradiated with x rays to give two doses of 10 Gy to the gastric region at a three-day interval, for a total of 20 Gy. Six months after radiation exposure, the incidence of intestinal metaplasia with alkaline phosphatase (ALP) positive foci in males was significantly higher than in females, in orchidectomized males, or orchidectomized plus DES-treated rats. The incidence of intestinal metaplasia with ALP-positive foci in normal females appeared lower than in ovariectomized females and was increased by treatment with testosterone or decreased by DES. Numbers of foci of intestinal metaplasia with Paneth cells and total numbers appeared to increase in males treated with DES. These results suggest a promoting role for testosterone in the development of radiation-induced ALP positive lesions and also indicate considerable differences among intestinal metaplasia subtypes in their response to hormone stimulation.

163. Rat prostate tumours after androgen ablation by castration showed an increase, from 0.4% to 1.0%, of the apoptotic index as determined by the TUNEL assay. The apoptotic index did not vary significantly over time after castration. Irradiation of intact rats to 7 Gy resulted in an apoptotic response of 2.3%. When castration was initiated three days prior to irradiation, peak levels of 10.1% for the apoptotic response were recorded. Androgen restoration with testosterone implants restored the intact animal response [J10].

164. In conclusion, it can be said that many hormones are powerful regulators of cell proliferation and programmed cell death in specific tissues and organs. The resulting influence on radiation risk per unit dose is well proven (see also Table A.1). An important part of differences in risks linked to gender or age may be traced to hormones acting as endogenous growth factors.

2. Viruses, bacteria, and genetic sequences

165. Viruses, bacteria, and microbial genetic sequences have been shown to play an important role in the pathogenesis of animal tumours. Human malignancies such as Burkitt lymphoma and T-cell leukaemia are caused by the Epstein-Barr virus [L12] and the retrovirus HTLV-1 [D4], respectively, and a variety of carcinomas, including cervical, skin, anal, and others, by papilloma viruses [H13]. Hepatitis type B and C virus, the bacterium Heliobacter pylori, some parasites such as Opistorchis viverrini and Schistosoma haematobium are proven or putative causes of hepatoma, gastric cancer, cholangiocarcinoma and urinary bladder cancer, respectively [M64]. One mechanism of interaction might be the inhibition of DNA repair by viral proteins. The HBV protein HBx was shown to interact with cellular DNA repair capacity in a p53-independent manner after ultraviolet C irradiation [G37]. Interaction of cancer viruses with radiation may also occur by mutation or translocation of dormant viral sequences. In a multi-stage process, virally infected organisms may also be much more susceptible to radiation-induced cancer if a virus is causing or facilitating one of the genetic transformations leading to the outbreak of malignancy. Astier-Gin et al. [A25] investigated the role of retroviruses in murine radioleukaemogenesis in C57B1 mice. The protocol associated the injection of a non-pathogenic retrovirus (T1223/B virus) and a dose from x rays (2 × 1.75 Gy), which alone was non-leukaemogenic in this system. Thymic lymphomas induced by the combined effect of virus and irradiation or irradiation alone were analysed for MuLV proviral organization and RNA expression with the Southern or Northern blotting techniques, respectively. The active involvement of the retrovirus was shown by the detection of a recombinant provirus in the chromosomal DNA of every tumour induced by the combined treatment with virus and radiation. No specific site in the genome was found for provirus integration and no relationship was observed between viral RNA expression and tumour induction. Trisomy 15 was observed in all metaphases irrespective of the protocol of tumour induction. The G-banding technique revealed an extra band in several thymic lymphomas induced by irradiation and T1223/B virus injection. This complex pattern of viral behaviour may pose great obstacles for diagnosis and for the elucidation of risk from combined exposures.

3. Miscellaneous factors

166. Many other sometimes poorly defined biological materials have also been shown to influence the response of organisms to ionizing radiation. For example, the modulating effect of microbial substances on survival after acute radiation doses in mammals (mice, rats, dogs, sheep, and monkeys) was studied by Andrushenko et al. [A12]. The highest protection was found for some vaccines containing inactivated bacteria and given before the radiation exposure. Polysaccharides, lipopolysaccharides, and protein-lipopolysaccharide complexes were also able to increase the radioresistance. The mechanisms involved in the modulation of the status and the number of stem cells of the immune system remains to be elucidated. Such effects might also be of importance at low exposure levels, e.g. for malignancies of the haematopoietic system.

167. To test the hypothesis that low-dose radiation, such as is used for diagnosis, may act as a co-carcinogen in inflammatory bowel disease, Weinerman et al. [W2] induced inflammation with DMH in a mouse system to study potential sensitization towards the radiation exposure (see Section B.1 for genotoxic action of DMH). Four

groups of BALB/c mice (a control, DMH, DMH plus low-dose radiation, and low-dose radiation) were studied. No protective or carcinogenic effects of the radiation in combination with DMH were found compared with DMH alone. This type of negative experimental finding is directly important for radiation protection of the patient, in that individuals with inflammatory bowel disease undergo many diagnostic x-ray examinations throughout life.

168. A strong effect was, however, found from the interaction of ionizing radiation with surgical procedures on the stomach. Griem et al. [G15] followed patients with peptic ulcer who had received radiotherapy to control excessive gastric acid secretions, a method used between 1937 and 1965 (mean dose to the stomach = 14.8 Gy). The mortality study involved 3,609 patients; 1,831 were treated with radiation and 1,778 were treated by other means. Compared with the general population, patients treated with or without radiation were at significantly increased risk of dying of cancer and non-malignant diseases of the digestive system. Radiotherapy was linked to significantly elevated relative risk for all cancers combined (RR = 1.53; 95% CI: 1.3–1.8). Radiotherapy and surgery together increased the rate of stomach cancer (RR = 10) above the sum of individual effects. There is no specific information on co-carcinogenic mechanisms in the post-surgical reaction of stomach tissue or on tumour location.

169. The influence of pre-immunization with a rectal extract on radiation-induced carcinoma of the rectum was studied by Terada et al. [T37] in 4–7-week-old A/HeJ mice. The animals received 40 Gy (20 Gy per week from x rays) in the pelvic region with or without two prior injections of rectal extract from adult animals of the same strain emulsified with complete Freud's adjuvant. After eight months, rectal adenocarcinomas were observed in significantly higher numbers in pre-immunized mice compared with non-immunized animals (62% vs. 18%). The results indicate that local immunological reactions sensitize to the carcinogenic action of ionizing radiation.

170. Finally, the effect of psychosocial factors such as fear, anguish, and chronic stress on the health status of individuals and populations, both in psychosomatic expressions and in the subjective perception of radiation-exposed persons, is clearly an important problem during and after accidents and cases of environmental contamination, such as seen in areas affected by the Chernobyl accident [I13]. However, a review of these aspects is beyond the scope of this Annex and involves professional disciplines outside the realm of UNSCEAR. Despite the attention given by the media to the potential deleterious effects of ionizing radiation in combination with conventional industrial pollutants in such instances, little scientific information is available on specific exposure situations. Some potentially important modifying factors are discussed in connection with dietary factors in Section B.6.

D. COMBINED MODALITIES IN RADIATION THERAPY

171. A large number of chemotherapeutic drugs are used in clinical cancer therapy in combination with radiation. The main ones in use or about to be used are described in this Section, with emphasis on the mechanisms of interaction between the drugs and radiation to reveal possible mechanisms of interaction between chemical agents and radiation under environmental and normal occupational settings. The main findings relating to modes of action and combined effects are summarized in Table 6. However, it should be clearly noted here that the final goal of tumour-therapy-related studies is tumour control and therefore cell death (apopotosis, necrosis) or cell inactivation (loss of proliferative capacity, differentiation, senescence). These effects are mostly deterministic and often mechanistically different from the stochastic radiation effects that are of concern in radiation protection. Therefore, highly sigmoidal dose-effect relationships and considerable threshold doses are found for the contribution of many of these agents to the interaction with radiation. Several groups of agents are also covered in the preceding sections, e.g. alkylating agents under the heading "genotoxic chemicals".

1. Alkylating agents, nitrosoureas, and platinum coordination complexes

172. Alkylating agents were among the first compounds found to be useful in cancer chemotherapy, and because of their variety and relative tumouricidal selectivity, they remain important components of many modern chemotherapeutic regimens. Although the alkylating agents are a diverse series of chemical compounds, they all have the common property of displaying a positively charged, electrophilic alkyl group capable of attacking negatively charged, electron-rich nucleophilic sites on most biologic molecules, thereby adding alkyl groups to oxygen, nitrogen, phosphorus, or sulphur atoms. Their chemotherapeutic usefulness derives from their ability to form a variety of DNA adducts that sufficiently alter DNA structure or function, or both, so as to have a cytotoxic effect [L37]. Many of the pharmacologically useful agents undergo a complex activation process.

173. The most common site of DNA alkylation is the N-7 position of guanine. Alterations at this position are relatively silent in their effect on DNA function, because these adducts do not interfere with the base-pairing scheme. In contrast, adducts at the N-3 position of cytosine, the O-6 position of guanine, and the O-4 position of thymidine interfere with the Watson-Crick base-pairing scheme and are therefore likely to interfere with fidelity of replication and transcription, leading to mutagenicity and cytotoxicity. In addition to direct interference with replication and transcription, the formation of DNA adducts leads to a variety of structural lesions, including ring openings, base deletions, and strand scissions [B41, F20, H20]. Many of the DNA adducts and lesions are further acted on by repair enzymes that can restore the integrity of the DNA, or if the repair process is only partially

completed, they can cause additional DNA damage, such as the creation of apurinic or apyrimidinic sites or DNA strand breaks. Bifunctional alkylating agents with the capacity to generate two electrophilic groups and to form two adducts are capable of forming DNA-interstrand and DNA-protein cross-links that interfere directly with DNA replication, repair, and transcription [L38].

174. Alkylating agents are cell-cycle-dependent but not cell-cycle-specific. They exert their cytotoxic effects on cells throughout the cell cycle but have quantitatively greater activity against rapidly proliferating cells, possibly because these cells have less time to repair damage before entering the vulnerable S phase of the cell cycle [T19]. Cells in which cross-links occur accumulate and die in the G_2 phase of the cell cycle. Persistent DNA strand breaks may result in lethal chromosomal damage in the mitotic phase of the cell cycle.

(a) Nitrogen mustards (mechlorethamine, melphalan, chlorambucil, cyclophosphamide)

175. Nitrogen mustard, originally studied for its potential as a vesicant in chemical warfare, is a highly reactive analogue of sulphur mustard and was the first alkylating agent introduced into clinical therapy [G22]. Exposure to these alkylating agents results in the formation of simple DNA adducts, DNA-interstrand cross-links, and DNA-protein cross-links [E12].

176. Experimental investigations of interactions between derivatives of nitrogen mustard and radiation *in vitro* showed that these interactions are additive, independent of sequence of treatment with the two agents, and not markedly influenced by the interval between treatments [D14, H21]. An isobolic analysis confirms the additivity, although when radiation precedes the mustard by 4 hours, the effect is on the borderline of supra-additivity, indicating that the two agents may share a common mechanism of cell killing [D14]. Neither radiosensitization nor interference with sublethal damage repair has been implicated in these interactions. Hetzel et al. [H21], examining the effects of combined treatment on V79 cell spheroids, put forth the interesting proposal that the enhancement seen with the nitrogen mustard derivative chlorambucil in combination with irradiation may be related to its ability to alter the internal oxygen profile in spheroids, resulting in partial reoxygenation.

177. The main use of nitrogen mustards was in the treatment of lymphomas, breast and ovarian cancer, and cancers of the central nervous system. A prospective randomized study examined whether MOPP (nitrogen mustard, vincristine, procarbacine prednisone) therapy alone is superior to combined modality treatment of extended field radiation and MOPP in patients with Hodgkin's disease [O13]. No significant differences were noted between the combined modality therapy and therapy with MOPP alone. However, overall toxicity was different. Viral and fungal infections occurred more frequently in the combined modality. In an overview by Cuzick et al. [C44] of post-operative radiation therapy of breast cancer, no difference was seen in mortality over the first 10 years between patients treated with radiation therapy. After 10 years, however, there was a lower survival associated with radiation therapy. In recent years, chemotherapy has been favoured for breast cancer treatment. However, the use of post-operative radiation therapy needs to be reconsidered in patients who receive adjuvant chemotherapy and in whom drug resistance develops, leading to failure of chemotherapy. By decreasing the local tumour burden, adjuvant radiation therapy may decrease the probability of drug resistance and increase the probability of cure in those patients [H42].

(b) Nitrosoureas

178. The chloroethylnitrosoureas, including 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and methyl-CCNU (Me-CCNU), are highly lipophilic and chemically reactive compounds that are clinically active against a variety of tumours (reviewed in [L39]). Chemical decomposition of these agents in aqueous solution yields two reactive intermediates, a chloroethyldiazohydroxide and an isocyanate group [C30, M40]. The latter react with amine groups in a carbamoylation reaction. The isocyanates are believed to deplete glutathione, inhibit DNA repair, and alter maturation of RNA. The chloroethyldiazohydroxide undergoes further decomposition to yield reactive chloroethyl carbonium ions that form a variety of adducts with all four DNA bases and the phosphate groups of DNA. Of major importance in the antitumour effects of nitrosoureas is the formation of DNA interstrand cross-links, as demonstrated by the close correlation between cross-link formation and cytotoxicity [E13, K30, L40]. Alkylation seems to be the more important feature of direct nitrosourea action.

179. Additive or greater-than-additive responses have been recorded in *in vitro* and animal studies, with the greatest enhancement associated with the presence of the drug in some experiments before irradiation and in others after irradiation. Deen and Williams [D15] provided an isobolic analysis of the effects of combined BCNU-radiation treatment of 9L rat brain tumour that suggested some concentration dependence of these interactions. At two levels of BCNU (1 and 7.5 mg ml^{-1}) all data points fell within the additivity envelope, indicating similar mechanisms of action for the drug and radiation, but at other levels (3 and 5 mg ml^{-1}) supra-additivity was noted, suggesting that alternative mechanisms might be operating. CCNU resulted in less interaction than did BCNU [D16], a finding confirmed by the study of Kann et al. [K31] on L1210 cells. In experiments comparing the radiosensitizing effects of four nitrosoureas, the compound without alkylating activity, 1,3-bicyclohexyl-1-nitrosourea (BCyNU), was the most effective sensitizer [K31]. BCyNU was reported to selectively inhibit glutathione reductase activity [M41]. Kann et al. [K31] concluded that because the agent without alkylating activity was the most potent radiation synergist, alkylation was not involved in the enhancing effect, which

may relate instead to differential repair-inhibiting activity. However, even if the enhancement of the cytotoxic effects of ionizing radiation could be ascribed to repair inhibition, details of the mechanisms of cell killing are still not clear, because inhibition of DNA repair by the nitrosoureas was not complete and permanent [K32]. Rather, their effect was to slow the rate of strand rejoining, prolonging the period when numerous unjoined breaks are present, and lethality was considered a consequence of this prolongation.

180. Controlled clinical trials have demonstrated the efficacy of nitrosoureas combined with irradiation as adjuvant therapy for glioblastoma and anaplastic astrocytoma (reviewed in [L39]).

(c) Platinum coordination complexes

181. Cisplatin and its analogues are an important group of agents now in use for cancer therapy [R15]. Cisplatin (cis-diaminodichloroplatinum (II)) can bind to all DNA bases, but in intact DNA, there appears to be preferential binding to the N7 positions of guanine and adenine [B42, M42, P12]. Cisplatin binds to RNA more extensively than to DNA, and to DNA more than to protein [P13]. In the reaction of cisplatin with DNA or other macromolecules, the two chloride ligands can react with two different sites to produce cross-links [E14, E15, F21, F22]. Studies of the effects of platinum DNA binding on the three-dimensional structure of the DNA double helix revealed that the platinum lesions cause bending of the DNA double helix, suggesting that the stereochemistry of the platinum molecule is maintained and that DNA is modified in its three-dimensional conformation [R14]. The cytotoxicity of cisplatin against cells in culture has been found to be related directly to total platinum binding to DNA and to interstrand and intrastrand cross-links. Intrastrand guanine-guanine cross-linkage inhibits DNA replication [G23, P14]. Diaminocyclobutane-dicarboxylatoplatinum (II), carboplatin, and other cisplatin analogues appear to have subcellular mechanisms of action similar to cisplatin. They form lesions with DNA that are recognized by antibodies reacting with cisplatin-DNA lesions [P15].

182. More than two decades ago, Zak and Drobnik [Z6] reported an apparent interaction between cisplatin and ionizing radiation after whole-body irradiation of mice. Since then, cisplatin has been reported to enhance the cytotoxicity of radiation in a number of studies in both cell culture and tumour-bearing animals (reviewed in [B26, B43, C31, D17, D18, D19, H22, H23]). Isobolic analysis provides some evidence that this interaction can be supra-additive [D20]. A pronounced inhibition of repair of both radiation-induced potentially lethal damage and sublethal damage by platinum drugs has been demonstrated in several cell lines [B44, C32, D18, D21, O3, Y8]. The survival curves of cells exposed to platinum compounds have either a reduction or no shoulder, and this effect is interpreted as evidence for the inhibition of sublethal damage repair by platinum because of the role of sublethal repair in the formation of the shoulder of the radiation survival curve. The enhanced killing of irradiated cells by platinum compounds may be due to an enhanced production of DNA double-strand breaks. Repair of DNA-platinum adducts results in a gap that, in association with radiation-induced DNA single-strand breaks (rejoining of which is retarded by platinum compounds), produces new DNA double-strand breaks [Y9, Y10].

183. DNA-protein cross-links and the binding of high-mobility-group proteins to DNA-platinum lesions seem to play a role in the radiosensitizing mechanism of cisplatin at moderate doses in hypoxic cells [K33, S50, S51, W16]. However, comparable *in vivo* experiments with RIF-1 tumours in mice failed to show the preferential radio-sensitization of hypoxic cells at low radiation doses by cisplatin [S52]. Herman et al. [H24] provided evidence that intracellular pH is an important variable in the action of cisplatin as a radiosensitizer of hypoxic cells using murine fibrosarcoma cells *in vitro*. Radiosensitization of cancer cells *in vitro* and as spheroids was observed when platinum drugs were delivered before and during irradiation. In addition, enhanced cell killing was demonstrated when these drugs were added immediately after irradiation (reviewed in [B26, H22, H23, S53]).

184. A number of animal *in vivo* studies have reported sequence-dependent positive interactions between the two modalities. Increased lifespan was reported when cisplatin was administered before whole abdominal irradiation of Krebs II ascitic carcinoma-bearing mice compared with cisplatin after irradiation [J8]. Supra-additivity was reported when cisplatin was given before x rays in SCCVII [T20, Y11] and RIF-1 [L41] or simultaneously in SCCVII and RIF-1 carcinoma-bearing mice [K34].

185. The platinum coordination complexes are the most important group of agents now in use for cancer treatment. They are curative in combination therapy for testicular cancer and ovarian cancer and play a central role in the treatment of lung [A29, K52, S54, S84, T21], head and neck [A27, B45, C33, C34, H25, O4, S55], brain [S56, S57], and bladder cancers [C35].

2. Antimetabolites

(a) Antifolates

186. Despite the clinical importance of antifolates in cancer therapy, there are only a limited number of reports of experimental data relating to interactions of methotrexate and radiation *in vitro*. Early studies of Berry [B47, B48] suggested that methotrexate might be useful as a radiosensitizer, with the greatest enhancement occurring with a cytotoxic drug concentration or in hypoxic cells. Enhancement was influenced by the proliferation status of the cells, and although stationary-phase cells showed an enhanced response to radiation, this was accompanied by a decreased response to methotrexate, which cancelled any gain [B49]. The synergistic effects between methotrexate and radiation can be explained by impaired DNA repair

owing to depleted intracellular pyrimidine and purine pools [A19]. Methotrexate cytotoxicity may also result from drug-induced single- and double-strand breakage of DNA [B46]. These breaks appear come from the methotrexate-induced depletion of intracellular nucleotide pools, with impairment of the ability to repair DNA damage. Synergistic effects were observed only when drug and radiation were given at the same time. Radioprotective effects of methotrexate were observed when it was administered hours before radiation treatment (see paragraph 119).

187. Effects of intracerebral injections of methotrexate, whole-brain radiation, or a combination of both were analysed on intracerebrally implanted RT-9 gliosarcoma in male CD-Fisher rats. Methotrexate alone and radiation alone each prolonged survival moderately. Combined methotrexate and radiation caused a significant prolongation of survival in all animals [W17].

188. In acute lymphoblastic leukaemia, current treatment is divided into four phases: remission induction by chemotherapy; central nervous system preventive therapy by radiation or combined modality treatment (radiation plus methotrexate); consolidation; and maintenance with chemotherapy. However major adverse effects of central nervous system preventive therapy have been documented, including CT-detected brain abnormalities, impaired intellectual and psychomotor function, and neuroendocrine dysfunction. These adverse effects have been attributed mainly to radiation therapy [R16, S58]. Several approaches have been tested to decrease adverse effects, including reduction of cranial irradiation from 24 to 18 Gy in regimens using cranial radiation plus intrathecal chemotherapy with methotrexate or the use of triple intrathecal chemotherapy with methotrexate alone or with methotrexate, cytarabine, and hydrocortisone (reviewed in [P28]).

(b) Pyrimidine analogues and precursors

189. Deoxyuridine analogues that increase radiosensitivity include 5-bromodeoxyuridine (BrUdR), 5-fluoro-2'-deoxyuridine (FUdR), and 5-iododeoxyuridine (IUdR). In these compounds, a halogen atom replaces the hydrogen at the 5 position on the pyrimidine ring of deoxyuridine. Because the van der Waals radii of bromine (1.95 Å) and iodine (2.15 Å) resemble closely the methyl group of deoxythymidine (2.00 Å), BrUdR and IUdR are more accurately referred to as thymidine analogues. FUdR is considered a uridine analogue because the van der Waals radius of the fluorine atom (1.35 Å) most closely resembles hydrogen (1.20 Å) [S59]. The biological effects of FUdR are significantly different from those of BrUdR/IUdR and will be discussed separately.

190. 5-Fluorouracil (5-FU) and FUdR are the fluoropyrimidines of greatest clinical interest. The fluoropyrimidines require intracellular activation to exert their cytotoxic effects. They are converted by multiple alternative biochemical pathways to one of several active cytotoxic forms. Incorporation of 5-FU into DNA inhibits DNA replication and alters DNA stability by producing DNA single-strand breaks and DNA fragmentation [C36]. Fluoropyrimidines may also induce DNA strand breaks without being directly incorporated into DNA, possibly through the inhibition of DNA repair as a result of dTTP (deoxy-thymidine-triphosphate) depletion [Y12].

191. The synthesis and antitumour activity of 5-FU was initially described by Heidelberger et al. in 1957 [H26]. Complete tumour regression was observed in mice bearing sarcoma tumours after 5-FU and radiation, not observed after each treatment alone [H27]. In mice with a transplanted leukaemic cell line, 5-FU and radiation interacted synergistically when the drug was given before and after radiation, with the effects being most noticeable in the latter situation [V8]. Squamous-cell carcinoma responses in mice from combined exposures were dependent on total drug doses; however, the response was independent of the schedule of drug administration and consistent only with an additive effect [W18].

192. *In vitro* studies of Nakajima et al. [N15] with mouse L cells showed an enhanced effect of combined treatment of 5-FU and radiation on cell survival. The results suggest that maximum enhancement occurred when drug-treated cells were irradiated in the S phase and also confirmed the importance of post-irradiation drug treatment. Enhancement was dependent on drug concentration, increasing with increased dosage, and on treatment duration. The prolonged temporal requirement and the cytotoxic dose of 5-FU for the induction of sensitization following x-ray exposure implicates incorporation of 5-FU into RNA as an important mechanism involved in the combined effect.

193. A series of *in vitro* combined treatments using ionizing radiation and 5-FU on the human adenocarcinoma cell lines HeLa and HT-29 were performed by Byfield et al. [B50]. Based on these experiments they concluded that (a) sensitization occurred only with post-irradiation drug treatment, with prior exposure to 5-FU being strictly additive; (b) enhanced cell killing could not be explained by drug-induced additional acute damage or inhibition of sublethal damage repair; (c) the effect is maximized if cells are exposed to 5-FU for prolonged periods following irradiation; and (d) the concentration of 5-FU required for these effects is associated with dose-limiting toxicity in clinical studies.

194. Attempts to define more clearly the mechanism of interaction of 5-FU have used the derivative FUdR, which may limit the complex effects of 5-FU. Radiosensitization by FUdR in human colon cancer cells (HT-29) was critically dependent on the timing of exposure, being most marked when irradiation occurred 8–12 hours after exposure to a clinically achievable drug concentration, with no effect resulting when the cells were irradiated first [B51]. FUdR impaired sublethal damage repair in a dose-dependent manner but had no effect on the induction of double-strand breaks [H28]. Sensitization correlated with thymidylate synthase inhibition [B51] and depletion of

dTTP pools [H28] and was blocked by co-incubation with thymidine [B51]. These findings strongly suggest that FUdR acts by inhibiting thymidylate synthase.

195. In more recent studies by Miller and Kinsella [M43], a 2-hour exposure to low doses of FUdR resulted in extended thymidylate synthase inhibition after the drug was removed (up to 30 hours after treatment). Although the enzyme was nearly completely inhibited (>90%), an increase in radiosensitivity of cells was not evident until 16 hours after removal of the drug. Therefore, no direct correlation between thymidylate synthase inhibition and radiosensitization was observed. Parallel analysis of cell-cycle kinetics showed that cells accumulated during the early S phase after drug exposure and the rise and fall of radiosensitivity of the entire cell population over time followed the change of proportion of cells in early S phase [M44], a relatively radiosensitive phase of the cell cycle [T22]. These data suggest that radiosensitization by FUdR is in part caused by alterations in cell kinetics and a redistribution of cells through the cell cycle.

196. Concomitant radiotherapy with 5-FU has been evaluated in patients with cancers of the oesophagus, rectum, anus, bladder, and advanced laryngeal tumours (reviewed in [K35, M45, O5]). A recent consensus conference at the National Institute of Health reviewed the data from clinical trials and has recommended combined post-operative 5-FU and radiotherapy as the most effective management of patients with stage II or III surgically resected rectal cancer [N16]. In general, 5-FU and radiation in combined modality treatment is superior to radiation alone in the treatment of intestinal tumours.

197. Differential sensitization of tumours with bromated or iodinated pyrimidines, including BrUdR and IUdR, has been observed. These analogues influence only proliferating cells and may therefore preferentially sensitize rapidly growing tumours surrounded by more slowly proliferating normal tissue. BrUdR and IUdR are readily incorporated into the DNA of mammalian cells. The incorporation follows the thymidine salvage pathway. The extent of thymidine replacement in DNA, however, is not simply a function of competition within the salvage pathway, because the preferred pathway for thymidine incorporation is through the *de novo* synthesis of pyrimidine nucleotides.

198. Steric hindrance resulting from analogue incorporation into DNA appears minimal. In contrast, the physicochemical properties of altered DNA are influenced by thymidine replacement. Incorporation of BrUdR increases the forces that bind the strands of DNA together [P16]. This may alter DNA transcription and replication. The affinity of chromosomal proteins for BrUdR- and IUdR-substituted DNA is increased. This increased affinity has been associated with the repression or induction of cellular proteins, receptors, and growth factors (reviewed in [M45]). BrUdR and IUdR cause a dose-dependent delay of cells in the S and G_2 phases of the cell cycle, as demonstrated in human ileal and spleen cells *in vitro* [P17].

199. The physicochemical properties of IUdR- and BrUdR-containing DNA have been implicated in its increased sensitivity to radiation. The large, highly electronegative halogen atoms greatly increase the cross-sectional area available for trapping radiation-produced electrons. In addition, migration of absorbed energy to a halogenated base has been demonstrated [F23, L43]. Highly reactive uracilyl radicals may result from these reactions.

200. Erikson and Szybalski [E16, E17] reported radiosensitization of human cells exposed to BrUdR and IUdR. These studies revealed that the incorporation of halogenated pyrimidine radiosensitizes the cell through a direct effect on DNA [S59]. It was demonstrated that BrUdR resulted in greater thymidine replacement than did IUdR. However, IUdR was a more effective sensitizer to x rays, even at lower levels of incorporation [E18, M46, M47]. The distribution between the two DNA strands was not a critical factor in radiosensitization. Sensitization was also shown to be independent of the presence of oxygen [H29].

201. Recent analysis of radiosensitization by IUdR and BrUdR in two exponentially growing human colon cancer cell lines (HCT116 and HT29) using the linear-quadratic model revealed that an increase in the initial slope of the cell survival curve is the predominant mode of radiosensitization [M46, M47]. This suggests that the radiosensitizing effect may be the result of an increase in the amount of initial DNA damage. However, other recent *in vitro* studies with plateau-phase cells (CHO cells) suggest that IUdR and BrUdR are, in fact, potentially lethal damage repair inhibitors [F27, W19]. These different proposed mechanisms of radiosensitization of BrUdR and IUdR in exponentially growing and plateau-phase cells are not inconsistent and may reflect a bimodal mechanism.

202. Significant systemic toxicity was noted in animals [B52, G24] and humans, suggesting minimal tumour selectivity for these analogues. For clinical investigations in humans, therefore, tumours were selected that were surrounded by practically non-proliferating normal tissue (brain, bone, and muscle), thereby limiting the incorporation of BrUdR and IUdR into normal cells within the irradiated volume (reviewed in [M44]).

203. Gemcitabine (2',2'-difluorodeoxycytidine) is a new antimetabolite. It is a pyrimidine analogue and appears to prevent the addition of other nucleotides by DNA polymerase (masked chain termination) and to impair DNA repair. Gemcitabine has been shown to be a potent radiosensitizer in a variety of tumour cell lines, including HT-29 colorectal carcinoma, pancreatic cancer, breast, non-small-cell lung and head and neck cancer cell lines. It was most effective when administered prior to radiation. For most cell lines, sensitization was evident at non-cytotoxic concentrations of gemcitabine. For most cell lines, the primary radiosensitizing effect seems to be associated with depletion of endogeneous nucleotide pools [L54, S83, S85]. Radiosensitization by gemcitabine was observed in mice bearing tumours *in vivo* [M70]. In

clinical trials gemcitabine seems to be a powerful radiation enhancer in the treatment of non-small-cell lung cancer [H51, V13].

(c) Hydroxyurea

204. Hydroxyurea, a relatively simple compound, is a representative of a group of compounds that have as their primary site of action the enzyme ribonucleoside diphosphate reductase. This enzyme, which catalyses the reactive conversion of ribonucleotides to deoxyribonucleotides, is a crucial and rate-limiting step in the biosynthesis of DNA. The mechanism of cytotoxicity from hydroxyurea is related to direct inhibition of DNA synthesis and repair. Hydroxyurea causes cells to arrest at the G_1/S phase transition [S61]. It selectively kills cells synthesizing DNA at concentrations that have no effect on cells in other stages of the cell cycle [S62].

205. Additive or greater-than-additive responses have generally been reported for combined treatment with hydroxyurea and ionizing radiation *in vitro*. Phillips and Tolmach [P7], using synchronized HeLa cells, reported that enhancement occurred only when the drug was present post-irradiation. They demonstrated that hydroxyurea inhibits potentially lethal damage repair. In synchronized V79 cells, hydroxyurea treatment was necessary before and after irradiation to be effective as a radiosensitizer [S61]. Sensitizing by hydroxyurea resulted from its inhibitory action at the G_1/S-phase transition or its lethal action during the S phase. Kimler and Leeper [K36] showed that the enhancement of radiation-induced lethality observed when hydroxyurea was present after irradiation was specific for G_1 and S phase cells, but that the drug did not interfere with recovery from radiation-induced division delay in the G_2 phase. Non-cytotoxic doses of hydroxyurea significantly increased the early S-phase population in a human bladder cancer cell line (647V) [K37]. Exposure to these non-toxic concentrations of hydroxyurea before irradiation resulted in radiosensitization. In the human cervix carcinoma cell line Caski, the radiosensitizing effect of hydroxyurea was mainly due to a significantly longer G_2 block, indicating effects on DNA repair [K2].

206. Hydroxyurea has been shown to inhibit the repair of radiation-induced single-strand breaks in HeLa cells. The time course for repair of radiation-induced, single-strand DNA breaks is partially inhibited by exposure to hydroxyurea before and after irradiation [F24]. The effects of hydroxyurea on DNA repair after UV irradiation have also been studied, and depletion of the triphosphate pools (except dTTP) appears to be responsible for the observed alterations in DNA repair and enhanced cytotoxicity [C38].

207. Piver et al. [P18] showed a significant reduction in the radiation dose needed to control mammary tumours in mice when hydroxyurea was given with fractionated radiation exposure. However, a similar study on implanted squamous cells of cervix carcinoma in nude mice showed no radiosensitizing effect [X3].

208. Clinical trials of hydroxyurea radiosensitization have involved patients with head and neck malignancies [R17, S63] and primary brain tumours [L27]. The most convincing trials, suggesting radiosensitization and improved local control with hydroxyurea, involved patients with uterine cervical carcinoma [P19, P20, S64]. Although these studies suggested improved results, none of the trials considered cell cycle times of the tumour and normal tissue or hydroxyurea concentrations in the relevant tissues [S65].

(d) Other antimetabolites

209. Other antimetabolites of clinical relevance are arabinose nucleosides, including cytarabine (cytosine arabinoside, ara-C); ara-C, a cytidine analogue, has important clinical activity against acute myelocytic leukaemia. It is an inhibitor of DNA synthesis and kills cells selectively during the S phase of the cell cycle. Synergism between cytarabine and a number of antitumour agents, including alkylating agents, platinum coordination complexes, and topoisomerase II inhibitors, has been observed *in vitro* and in animal models. ara-C enhances the activity of these compounds by inhibiting the repair of strand breaks associated with these agents.

210. Another class of antimetabolites is the purine analogues, including 6-mercaptopurine and 6-thioguanine, which act as guanine analogues, and adenosine analogues, including arabinofuranosyladenine (9-ß-arabinofuranosyladenine, ara-A). All these compounds have antileukaemic activity and are used in combination chemotherapy. Their activity is directed against DNA replication and repair.

211. The potential of the thymidylate synthase inhibitor tomudex to interact with ionizing radiation was assessed by Teicher et al. [T38]. Tomudex (1 µM) decreased the shoulder of the radiation survival curve in both oxygenated and hypoxic HT-29 cells (human colon carcinoma) and SCC-25 cells (squamous-cell carcinoma of the head and neck), respectively. The effect was more significant in oxygenated cells. In tumour-bearing animals, tomudex in combination with radiation showed an additive to supra-additive effect on tumour control. The interaction effect was dependent on the fractionation schedule of drug and radiation. In each assay, the results obtained with tomudex were equal to or exceeded the results of comparable experiments with 5-fluorouracil.

3. Antitumour antibiotics

212. Anthracyclines such as doxorubicin, daunomycin, epirubicin, and idarubicin cause a range of biochemical effects in tumour cells. The antitumour activity and toxicity are the result of free-radical formation and/or triggering of topoisomerase-II-dependent DNA fragmentation. The enzyme is prevented from finishing its cycle with the religation of the broken strands. In addition, the alteration of the DNA helical structure that occurs on DNA intercalation by anthracyclines may trigger enhanced topoisomerase II activity. The net result is that addition of

anthracyclines to tumour cells dramatically increases protein-associated breaks. There is strong evidence that the topoisomerase II mechanism is the means by which doxorubicin and other anthracyclines kill leukaemia and lymphoma cells.

213. As a second mechanism, anthracyclines are able to form oxygen radicals. Evidence suggests a role for anthracycline-induced radical formation by virtue of its killing of ovary, breast, and colon tumour cells. Much of this evidence depends on the key roles that gluthathione and gluthathione peroxidase play in detoxifying hydrogen peroxide and organic peroxides. Doxorubicin is an inhibitor of mitochondrial and cell respiration and reduces oxygen consumption by cells in the outer layers of the tumour. This may lead to improved oxygenation and radiosensitivity of hypoxic areas of the tumour [D23]. On the other hand, aclarubicin, an anthracycline differing in its sugar moiety from doxorubicin, was shown to exert its enhancement effect on x-ray-induced cell killing in HeLa cells only when given after radiation exposure (5 μg ml^{-1} for one hour) [M63]. The authors hypothesized that this potentiation, which is visible through 10 cell divisions, is due to the interaction between radiation and drug damage, a mechanism probably relevant only for very high acute exposures.

214. Bleomycin is another important antitumour antibiotic. Its action has been associated primarily with its ability to produce single- and double-strand breaks in DNA. The sequence of events leading to DNA breakage begins with the metabolic activation of bleomycin. The activated agent binds to DNA as the result of intercalation. Highly toxic oxygen intermediates, such as the superoxide or hydroxyl radicals, are then formed that attack DNA. There is indirect evidence that the same processes required to repair ionizing radiation damage also are used in bleomycin repair [C39]. The lesions caused by bleomycin include chromosome breaks and deletions, very similar to the action of ionizing radiation. Bleomycin is therefore called a radiomimetic drug. There is, however, some base sequence specificity for the site of DNA cleavage. Bleomycin binds preferentially to the DNA strand opposing the sequences GpT and GpC to attack and cleave the strand at the 3' side of G [P21, S66]. A primary point of attack in non-mitotic cells is considered to be the link regions of DNA between nucleosomes [K38].

215. The effects of the interaction between bleomycin and ionizing radiation on cell survival have been reported to range from additive to greater than additive [B26, H30, T28, T29]. These effects are schedule-dependent, with maximum interaction occurring when there is only a short time interval between administration of the two agents or when they are administered simultaneously, possibly reducing the extent of repair of any induced damage or similar lesions by these two agents. Although both ionizing radiation and bleomycin induce G_2 arrest, their damage is independent and purely additive [K39]. Thus, in contrast to the sometimes greater-than-additive effects observed for cell lethality, bleomycin and radiation do not interact in the induction of cell-cycle blocks.

216. Bleomycin and radiation have been combined frequently in the treatment of head and neck cancer. There are several randomized clinical trials (reviewed in [S67]), some of which showed a benefit in response rate and/or survival; others, however, including the largest trials, did not reveal any benefit from the use of bleomycin and radiotherapy.

217. Mitomycin C is a bioreductive alkylating agent that is inactive in its original form but is activated to an alkylating species by reduction of the quinone and subsequent loss of the methoxy group. Recent studies indicate that bifunctional alkylation by mitomycin C occurs preferentially in a reducing environment [T23]. In an aerobic environment, the reduction of mitomycin C initiates a chain of electron transfers that leads ultimately to the formation of toxic hydroxyl and superoxide radicals [B54].

218. Mitomycin C significantly reduced the radiation-resistant subpopulation of KHT carcinomas growing intramuscularly in C3H/HeJ mice when administered 24 hours before radiation. Isobolic analysis indicated that this treatment combination led to supra-additive cell killing in the tumour [S68]. Combined treatment with mitomycin C and radiation of C3H mouse mammary carcinoma *in vivo* showed that the drug significantly enhanced the radiation-induced growth delay when administered before radiation [G25]. Isobolic analysis revealed that pre-irradiation treatment with mitomycin C resulted in a supra-additive response, whereas post-irradiation treatment resulted in only an additive response. The enhancement appeared to be related to both a direct radiosensitization and a pronounced cytotoxic effect of the drug against radioresistant hypoxic cells.

219. A randomized trial using mitomycin C with radiotherapy for head and neck cancer showed a disease-free survival benefit [W21]. However, a high incidence of pulmonary complications was reported. Mitomycin C is included in many multimodality therapy regimens for gastrointestinal tumours in combination with 5-FU and radiation. For cancers of the anal region, chemotherapy with 5-FU and mitomycin C plus irradiation have been widely accepted as the conventional treatment for most patients, and surgery may not be required in many cases (reviewed by [S14]).

220. Actinomycin D (dactinomycin) binds to DNA by intercalation. The intercalation depends on a specific interaction between the polypeptide chains of the antibiotic and deoxyguanosine and blocks the ability of DNA to act as a template for RNA and DNA synthesis [R17]. The predominant effect is selective inhibition of DNA-dependent RNA synthesis. In addition to these effects, actinomycin D causes single-strand breaks in a manner similar to doxorubicin.

221. Experimental investigations of interactions between actinomycin D and radiation were reviewed by Hill and Bellamy [B26, H30]. The overall conclusion of this review was that irrespective of the sequence employed, the two agents are at least additive and that a shorter rather than a longer interval between the two agents is the most beneficial. Because actinomycin D generally leads to a decreased D_q and a decrease in split-dose recovery, it inhibits sublethal damage repair but does not effect potentially lethal damage repair in exponentially growing cells. This increased radiation damage expression, resulting from residual non-repaired single- and double-strand breaks in DNA, has been proposed as a possible mechanism for interaction between actinomycin D and ionizing radiation.

222. Actinomycin D is effective in the treatment of Wilms' tumour, Ewing's sarcoma, embryonal rhabdomyosarcoma, and gestational choriocarcinoma. It enhances radiation effects in clinical therapy when both are given simultaneously. When given after radiation therapy, actinomycin D, like doxorubicin, can recall the irradiation volumes by erythema of the skin or by producing pulmonary reactions [D32]. It is not known whether this is due to interaction between the damage done by radiation and that by the drug or whether it represents only additivity of the effects. The recall effect can be observed even after a period of several months between radiation and drug treatments.

4. Microtubule poisons

223. Many antineoplastic agents currently in use are biosynthetic products and were initially isolated from plants [D24]. In this Section, the mode of action alone and in combination with radiation of the vinca alkaloids, epipodophyllotoxins, and taxanes is reviewed.

224. Vinca alkaloids are present naturally in minute quantities in the common periwinkle plant, *Catharanthus roseus*. Vincristine (VCR), vinblastine (VBL), desacetyl vinblastine (vindesine), and vinorelbine are in clinical use. Vinca alkaloids exert their cytotoxic effects by binding to a specific site on tubulin and preventing polymerization of tubulin dimers, disrupting the formation of microtubules [M55]. The binding occurs at sites that are distinct from binding sites of other antimicrotubule agents, such as colchicine, podophyllotoxin, and paclitaxel [B55].

225. The effect of single doses of VCR on mice spermatogonia was investigated by Hansen and Sorensen [H31], and the influence of these drugs on the radiation response of murine spermatogonial stem cells was examined. VCR significantly reduced the survival in the differentiated spermatogonia and to a lesser extent in the stem cells. VCR radiosensitized spermatogonial stem cells, with the effect being most prominent when it was administered after irradiation. Grau et al. [G26] evaluated the interaction between VCR and x rays in a murine C3H mammary carcinoma and its surrounding skin. VCR caused a temporary blockage of cells in the mitotic phase. The tumour control studies, however, showed a lack of correlation between the VCR-induced accumulation of cells in the G_2/M cell-cycle phase and enhancement of tumour radiation response. Nevertheless, pre-irradiation VCR caused radiosensitization in both tumours and skin, whereas post-irradiation VCR mostly resulted in responses equal to radiation only.

226. The effect of combining VBL and ionizing radiation on tumour response was investigated in CDF1 mice bearing the MO4 mouse fibrosarcoma [V9]. Different treatment schedules for the combination of VBL and radiation all resulted in additive tumour responses. The maximum percentage of tumour cells that could be accumulated in mitosis by a single intravenous bolus of VBL was around 13%. The results show that this will probably be insufficient for significant radiation enhancement.

227. Paclitaxel (commercial name Taxol), another microtubule poison, was first isolated from the Pacific yew, *Taxus brevifolia*. Paclitaxel promotes microtubule assembly *in vitro* and stabilizes microtubules in mouse fibroblast cells exposed to the drug [S69, S70]. It binds preferentially to microtubules rather than to tubulin dimers [P22]. Although the binding site for paclitaxel on microtubules is distinct from the binding site for exchangeable guanosine triphosphate (GTP) and for colchicine, podophyllotoxin, and VBL, the specific binding site for paclitaxel on microtubules has not been identified. Unlike other antimicrotubule agents such as colchicine and the vinca alkaloids, which induce microtubule disassembly, paclitaxel shifts the equilibrium towards microtubule assembly and stabilizes microtubules. Distinct morphological effects suggest that paclitaxel adversely affects critical microtubule functions during interphase and mitosis. Paclitaxel belongs to the group of taxanes, microtubuli stabilizing agents containing a taxane ring. Microtubuli stabilizing agents without taxane ring are called taxoids.

228. Choy et al. [C40] evaluated the possible radiosensitizing effects of paclitaxel on the human leukaemic cell line (HL-60). When HL-60 cells were treated with paclitaxel, up to 70% of them were blocked in the G_2/M phase. Isobolic analysis of the data revealed that the combined effects of ionizing radiation and paclitaxel fell within the range between additivity and synergism. Reasoning that paclitaxel could function as a cell-cycle-selective radiosensitizer, Tishler et al. [T24, T25] examined the consequences of combined drug/radiation exposures on the radioresistant human grade 3 astrocytoma cell line, G18, under oxic conditions. Survival curve analysis showed a dramatic interaction between paclitaxel and ionizing radiation, with the degree of enhanced cell killing dependent on paclitaxel concentration and on the fraction of cells in the G_2 or M phases of the cell cycle.

229. Three human ovarian cancer cell lines were used to examine the radiosensitizing effects of paclitaxel: BG-1, SKOV-3, and OVCAR-3 [S71]. Paclitaxel was found to have a significant radiosensitizing effect on all cell lines. Proliferating cells were more sensitive to paclitaxel, radiation, and the combination than confluent cells.

Treatment of proliferating cells with paclitaxel 48 hours prior to irradiation had a greater radiosensitizing effect than treatment 24 hours prior to irradiation.

230. Liebmann et al. [L44] examined the radiosensitizing effects of paclitaxel in four cell lines: MCF-7, A549, OVG-1, and V79. All cell lines developed a G_2/M block after paclitaxel exposure. Paclitaxel acted as a radiosensitizer in human breast cancer cells (MCF-7), in human ovary adenocarcinoma cells (OVG-1), and in Chinese hamster lung fibroblast cells (V79). However, paclitaxel was unable to enhance the radiation sensitivity of human lung adenocarcinoma cells (A549). Paclitaxel increased the linear component of the radiation survival curves in all cell lines. The quadratic component was unaffected by paclitaxel in the rodent cell line. The cells that were sensitized to radiation by paclitaxel had a relatively small baseline linear component, while A549 cells had a large linear component. Asynchronous and synchronous cells from carcinomas of the human uterine cervix were irradiated alone and after paclitaxel treatment [G27, T39]. Irradiating paclitaxel-treated cells resulted in a strictly additive response, like the response in lung adenocarcinoma cells and in contrast to the earlier supra-additive results with astrocytoma cells, breast cancer cells, and ovarian cancer cells. Paclitaxel affected the cervical carcinoma cells at stages of the cell cycle other than G_2/M. This may explain the failure to observe paclitaxel radiosensitization with these cells, and it may indicate that paclitaxel has a multiplicity of actions, with differences in effectiveness likely between cells of different origins. Similar cell-line-specific results on the cell-cycle specificity of the combined paclitaxel radiation effects were reported for other tumour cell lines. In non-synchronized and synchronized human fibroblasts, however, the combined effect was additive to even subadditive [G36]. Subadditive effects on cell survival between radiation and paclitaxel were reported for the human laryngeal squamous-cell carcinoma cell line SCC-20 [I15].

231. Besides having cell-cycle effects, paclitaxel is able to induce apoptosis by a p53-independent mechanism. On a molecular level, paclitaxel effects primarily involve phosphorylation of the product of the bcl-2 gene downstream of p53 [M68].

232. Hei et al. [H32, H33] assessed the potential oncogenic effects of paclitaxel either alone or in combination with gamma irradiation in C3H10T½ cells. In contrast to human cells *in vitro*, the mitotic block induced by paclitaxel in 10T½ cells was only partial. While paclitaxel was ineffective in transformant induction, it enhanced the oncogenic transforming potential of gamma rays in a supra-additive manner.

233. *In vivo* experiments with animal tumours showed that enhanced tumour radiosensitivity after paclitaxel treatment was attributable to two distinct mechanisms. Paclitaxel was able to enhance the radioresponse of apoptosis-sensitive and -resistant tumours but not the normal tissue radioresponse, thus providing true therapeutic gain. Tumour reoxygenation and antiangiogenic properties occurring as a result of paclitaxel-induced apoptosis in apoptosis-sensitive tumours and mitotic arrest after paclitaxel treatment in apoptosis-resistant tumours are two distinct radiosensitizing mechanisms of paclitaxel [M67]. In mice bearing spontaneous mammary carcinoma, paclitaxel and radiation interacted in a supra-additive manner in controlling tumour growth. However, no supra-additive response has been observed in normal tissue, indicating a favourable therapeutic gain [C48].

234. Antitumour activity of paclitaxel has been observed in advanced ovarian cancer and metastatic breast cancer. The initial activity reported in refractory ovarian cancer has now been confirmed in three subsequent studies with response rates ranging from 21% to 40% (reviewed in [Y13]). Significant activity (56%–62%) has also been observed in metastatic breast cancer [H34]. Docetaxel, a paclitaxel derivative, has been shown to be 100-fold more potent than paclitaxel in bcl-2 phosphorylation and apoptotic cell death [H50]. The radiosensitizing activity of docetaxel has been reported in clinical trials with head and neck cancer [S79] and non-small-cell lung cancer [G38, O20].

5. Topoisomerase poisons

235. Epipodophyllotoxins from extracts of the mandrake plant (*Podophyllum pelatum*) have been used for medical purposes for centuries as cathartics or as treatment for parasites or venereal warts. Podophyllotoxin, an antimitotic agent that binds to a site on tubulin distinct from that occupied by the vinca alkaloids or paclitaxel, was identified as the main constituent possessing cytostatic activity. These early tubulin-binding podophyllotoxins possessed a prohibitively high degree of clinical toxicity. For example, a considerable risk of pneumonitis was observed following irinotecan and radiotherapy for lung cancer [Y15]. However, two glycosidic derivatives of podophyllotoxin, etoposide (VP-16) and teniposide (VM-26), have very significant clinical activity against a wide variety of neoplasms. Their main target is DNA topoisomerase II.

236. Epipodophyllotoxins were found to arrest cells in the late S or early G_2 phase of the cell cycle rather than the G_2/M border that would have been expected of an antimicrotubule agent [K40]. It was noted that these agents had no effect on microtubule assembly at concentrations that were highly cytotoxic [L45]. It was subsequently found that these drugs produced DNA strand breaks in intact cells but that these effects were not seen when the epipodophyllotoxins were incubated *in vitro* with purified DNA, suggesting that direct chemical cleavage in DNA was not occurring [W20]. The epipodophyllotoxins exert their cytotoxic effects by interfering with the scission-reunion reaction of the enzyme DNA topoisomerase II [Y10]. The enzyme binds to DNA covalently and forms single-strand, protein-associated breaks. On a molar basis, teniposide is approximately 10 times more effective than etoposide at inducing DNA strand breaks [L46]. In addition, the epipodophyllotoxins inhibit the catalytic or "strand-passing" activity of topoisomerase II that permits the enzyme to catenate DNA circles and disentangle topologically constrained DNA.

237. Isobolic analysis of the combined modality treatment of etoposide and radiation on asynchronous growing V79 fibroblasts showed that considerable potentiation occurs upon concomitant radiation/drug exposure [G28]. Synergistic cell killing was observed as radiation was applied before or concomitantly with etoposide. Rapidly repairable radiation-induced DNA damage was fixed into lethal lesions by etoposide, giving rise to supra-additive interaction under concomitant radiation/drug exposure. The shoulder of the radiation survival curve was eliminated. A second interaction mechanism was that cells arrested in the G_2 phase of the cell cycle by irradiation were hypersensitive to the cytotoxic effects of the drug. Recently, Goswami et al. [G29] reported that the synthesis of topoisomerase II is suppressed as cells accumulate in G_2 following irradiation. Ng et al. [N17] investigated the ability of etoposide to potentiate the x-irradiation response and to inhibit the repair of potentially lethal damage and sublethal damage in confluent cultures of a radioresistant (Sk-Mel-3) and a radiosensitive (HT-144) human melanoma cell line. In both cell lines, etoposide inhibited sublethal damage repair; however, in contrast to camptothecin, a topoisomerase I inhibitor, it also inhibited potentially lethal damage repair in HT-144 cells but not in the radioresistant cell line Sk-Mel-3.

238. Non-cytotoxic concentrations of etoposide (1.7 mM) caused little or no effect in V79 cells when combined with radiation [S72]. Even at highly toxic doses of etoposide, human bladder carcinoma cells were not radiosensitized by the drug [M48]. Etoposide and teniposide have demonstrated highly significant clinical activity against a wide variety of neoplasms, including non-Hodgkin's lymphomas, germ-cell malignancies, leukaemias, and small-cell lung carcinoma [B56, O6, W22].

239. Camptothecin, a heterocyclic alkaloid, and its analogues are inhibitors of topoisomerase I and possess antitumour activity. Camptothecin was first isolated from the stem wood of *Camptotheca acuminata*, a tree native to northern China. Characterization of the molecular structure of camptothecin critical for antitumour activity has led to the development of the camptothecin analogue topotecan and others with greater solubility and improved therapeutic indices in preclinical models.

240. DNA topoisomerase I is the unique target for camptothecin [S73]. Topoisomerase I transiently breaks a single strand of DNA, thereby reducing torsional strain and unwinding DNA ahead of the replication fork. Human DNA topoisomerase I binds to its nucleic acid substrate non-covalently. The bound enzyme then creates a transient break in one strand and concomitantly binds covalently to the 3'-phosphoryl end of the broken DNA strand. Topoisomerase I then allows passage of the unbroken DNA strand through the break site and religates the cleaved DNA. Camptothecin blocks the topoisomerase I in the form that is covalently bound to DNA [C41]. Camptothecin-induced DNA strand breaks have been detected frequently at replication forks close to growth points. The cytotoxicity of camptothecin, a highly S-phase-specific agent, may be explained by the collision of drug-stabilized topoisomerase I-DNA complexes with moving replication forks, leading to replication arrest and conversion of topoisomerase-I-bound transient DNA strand breaks into persistent breaks [H35]. A direct stereospecific interaction between camptothecin and DNA topoisomerase is essential for the radiosensitizing effect of the inhibitor [C47].

241. Exposure to camptothecin under conditions of low-dose-rate irradiation (1 Gy h^{-1}) induced the accumulation of cells in the S phase in V79 and HeLa cells. Isobolic analysis of survival data consistently showed supra-additivity of cell killing in both cell lines upon concomitant exposure to camptothecin and low-dose-rate irradiation. Cytokinetic cooperation appears to be the main determinant of cell survival in treatments associating camptothecin and radiation in growing cells. Non-cytotoxic concentrations of camptothecin produced a reproducible effect at x-ray doses of up to 2 Gy; however, like cells treated with etoposide at non-toxic concentrations, the radiation survival curves for drug-treated and untreated V79 cells were comparable at higher radiation doses [S72]. X-irradiation of camptothecin-treated SV40 transformed normal (MRC5CVI) and ataxia-telangiectasia (AT5BIVA) fibroblast cells resulted in additive prolongation of S-phase delay in MRC5CVI cultures and additive effects for cell killing in both cell lines [F11]. Hypersensitivity of AT5BIVA to camptothecin was not attributable to elevated levels of complex trapping.

242. HT-29 human colon adenocarcinoma cells growing in spheroids were more resistant to both SN-38, a metabolite of a derivative of camptothecin (irinotecan: CPT-11), and radiation than HT-29 monolayers. SN-38 at a subtoxic concentration (2.5 μg ml^{-1}) increased the lethal effects of radiation on spheroids in a supra-additive manner but only acted additively on monolayers. The mechanism of radiosensitization of SN-38 is due to the inhibition of potentially lethal damage repair in spheroids [O18]. In both small-cell lung cancer and small-cell/large-cell lung carcinoma xenografts, combination treatment with SN-38 and radiation resulted in a significant tumour regression compared with the use of SN-38 or radiation alone [T40].

243. Gamma-ray irradiation of AS-30D rat hepatoma cells followed by a 2-hour exposure to camptothecin *in vitro* was found to act additively at low radiation doses and synergistically at higher radiation doses, as shown by isobolic analysis [R18]. Treatment of established ascites tumours in rats with either camptothecin or ^{131}I-labelled monoclonal antibody RH1, which specifically localizes in hepatoma ascites, prolonged rat survival but was ineffective at curing animals of tumours. In contrast, combined therapy consisting of camptothecin followed by the injection of ^{131}I-labelled monoclonal antibody RH1 cured 86% of animals. These results suggest that topoisomerase I inhibitors may be useful for increasing the efficacy of radioimmunoconjugates for the treatment of cancer.

244. Subtoxic concentrations of topotecan potentiated radiation-induced killing of exponentially growing Chinese hamster ovary or P388 murine leukaemia cultured cells [M49]. Survival curve shoulders were reduced; the slopes of the exponential portions of the curves were slightly decreased. Potentiation of radiation-induced cell killing by topotecan was absolutely dependent on the presence of the topoisomerase I inhibitor during the first few minutes after irradiation. A dose-dependent reduction in cell survival was obtained with a 4-hour exposure of topotecan following irradiation of human carcinoma cells in culture and murine fibrosarcoma in mice [K22]. No enhancement of cell killing was seen when cells were treated with the drug before irradiation. *In vivo* tumour studies showed a significant radiosensitizing effect of topotecan that was dependent on both drug dose and time sequence (before irradiation). There was no enhanced skin reaction following the combined treatments [K22].

6. Bioreductive drugs

245. The oxygenation status of clonogenic cells in solid tumours is believed to be one of the main factors adversely affecting tumour response in radiotherapy. In totally hypoxic cells, the radiation dose must be raised by a factor as great as 3 to achieve the effects obtained in fully oxic cells. The presence of 2%–3% of such resistant cells may double the total radiation dose required for eradication of all tumour cells [G30, T26]. It appears that solid tumours can contain two distinct classes of hypoxic cells: chronically and transiently hypoxic cells [C42, T26]. However, in clinical radiotherapy, treatment is usually sufficiently protracted to allow a significant re-oxygenation.

246. Results of clinical studies on the use of hyperbaric oxygen in combination with radiotherapy to increase oxygenation of hypoxic tumour cells have been conflicting. Nine randomized trials have been reported, of which only three gave statistically significant positive results for the use of hyperbaric oxygen, particularly in tumours of the head and neck region and advanced carcinoma of the cervix [D25, D26, F13, W23]. A second approach towards increased delivery of oxygen to tumours involved the use of erythrocyte transfusions. Retrospective studies of cancer patients with anaemia showed some indications for a negative correlation between anaemia and the outcome of radiotherapy [B57, D27, D28]. Use of the perfluorochemical oxygen-carrying emulsion Fluosol-DA and 100% oxygen as an adjunct to radiotherapy is a third approach to increased oxygen delivery. Clinical trials in the treatment of head and neck cancer showed a benefit of this combined modality treatment [L42, R19].

247. Clinical trials with hypoxic cell radiosensitizers rely on a different approach [R2]. Drugs that replace oxygen in chemical reactions that lead to radiation-induced DNA damage are used as adjuncts to radiotherapy. These drugs sensitize hypoxic tumour cells to radiation but do not sensitize normal tissue, which is already maximally sensitized by oxygen. Hypoxia-directed drugs would have limited use as single agents, because they would not destroy the normally oxygenated tumour cells; however, they could be extremely valuable in combination with radiotherapy or drugs that selectively kill aerobic cells. Optimal use of hypoxia-directed drugs would therefore require the development of regimens in which concomitant therapies with agents attacking each cell population were combined effectively to eradicate all the different cell populations within the tumour. Drugs that are selectively toxic to hypoxic cells should be relatively non-toxic to healthy normal tissue, which is generally well perfused and well oxygenated.

248. Bioreductive drugs are activated by metabolic reduction in tumour cells to form highly effective cytotoxins. Tumour selectivity exploits the presence of hypoxia in tumours, since oxygen can reverse the activating step, thereby greatly reducing drug activity in most normal tissues. Selectivity can also depend on the level of expression in tumour cells of the particular reductase for which the drug can act as a substrate. These include DT-diaphorase, various P450 isozymes, cytochrome P450 reductase, xanthine oxidase, and doubtless other enzymes as well.

(a) Quinone alkylating agents

249. Quinone alkylating agents, as well as various nitro compounds and the benzotriazine di-N-oxides, have the ability to undergo metabolic reduction in such a way as to selectively kill hypoxic cells. When quinones are reduced under normal aerobic conditions, the cell is placed in oxidative stress due to a process known as redox cycling [P23, T27]. Although oxidative stress due to cycling is considered important in the toxicity of quinones and other redox labile agents to normal oxic cells, this pathway is in fact less damaging than the highly toxic metabolites that predominate in hypoxic cells. This is partly because of the protective enzymes that detoxify superoxide, that is, superoxide dismutase and catalase. Another pathway that protects oxic cells from the toxic action of quinones is direct reduction by DT-diaphorase. Unlike other reductases, DT-diaphorase catalyses a concerted two-electron reduction step, which is therefore not reversible by oxygen. Radiosensitizing effects of EO9, an analogue of mitomycin C, and porfiromycin, another quinone alkylating agent, were reported in experimental animal tumour models [A26, R20].

(b) Nitroimidazoles

250. Nitroimidazoles are reduced intracellularly, but in the absence of adequate supplies of oxygen they undergo further reduction to more reactive products [E9]. The formation of these products is initiated by an enzyme-mediated single-electron reduction of the nitro group to a free radical that is an anion at neutral pH. The reduction pathway can proceed in successive steps past the nitro-radical anion (one electron addition), the nitroso (two electrons), and the hydroxylamine (four electrons) to

terminate at the relatively inactive amine derivative (six electrons). In aerobic conditions the predominant reaction is redox cycling through radical anion analogous to quinone bioreduction, and oxidative stress may result from this pathway. The precise molecular nature of the covalent reaction products that predominate under hypoxia have not been identified, but these products almost certainly derive from the nitroso- or hydroxylamine reduction level or their ring cleavage products such as glyoxal [W24].

251. The first compound tested in clinical trials was the 5-nitroimidazole, metronidazole [D29, U16, U17]. It was selected because of its known activity both *in vitro* [C37, F25] and in experimental murine tumours [B58, R21, S74]. Misonidazole was the first in a series of 2-nitroimidazole compounds to be used in the clinic. Because the 2-nitroimidazole compounds are more electron-affinitive than metronidazole, they are more efficient as hypoxic cell sensitizers. Misonidazole was shown to be more efficient as a radiosensitizer in experimental tumour systems than metronidazole [F26]. Clinical experience with misonidazole as a radiosensitizer showed some benefit of the drug in some head and neck cancer and pharyngeal cancer studies [D12, D22, F14, O7]. However, clinical use is limited because it induces cumulative peripheral neuropathy.

252. Neurotoxicity is linked to the lipophilic properties of the compound [B59, B60, B61]. The less lipophilic misonidazole analogue SR 2508 (etanidazole), with radiosensitizing activity comparable to that of misonidazole, was subsequently used. Adding etanidazole to conventional radiotherapy was beneficial for patients who had squamous-cell carcinoma of the head and neck without regional lymph node metastasis [L34]. Nimorazole, a weakly basic 5-nitroimidazole with an electron affinity lower than that of the 2-nitroimidazoles, was evaluated in a randomized trial in patients with squamous-cell carcinoma of the larynx and pharynx [O8]. Results demonstrated a statistically significant improvement in locoregional control. Nimorazole was much less toxic than etanidazole, and the toxicity was reversible.

253. RSU 1069 is the leading compound of dual-function hypoxic cell radiosensitizers. It is a 2-nitroimidazole containing a monofunctional, alkylating aziridine ring. RSU 1069 has radiosensitizing properties and can be up to 100 times more toxic to hypoxic cells than to aerobic cells [S75]. The increased differential toxicity compared with that of other simple nitroimidazoles is due to the alkylating function in the molecule [W25]. Following bioreduction, therefore, the drug is converted into a bifunctional agent that can cause both DNA strand breaks and cross-links [J5, O9, S76, W26]. In mice injected with RSU 1069, aerobic cells exhibited large numbers of DNA single-strand breaks, while toxic DNA interstrand cross-links were produced only in hypoxic cells. Cells from bone marrow and spleen showed extensive numbers of DNA single-strand breaks but minimal cross-linking compared with tumours [O10]. However, clinical testing revealed severe gastrointestinal toxicity at doses below those needed for therapeutic benefit [H17].

254. A series of pro-drugs (e.g. RB 6145) have been developed that release RSU 1069 spontaneously under physiological conditions [J6]. *In vitro* and *in vivo* animal data showed that the hypoxic cell specificity and cytotoxic activity are retained but that at the same time the acute toxicity is reduced in animal models [A23, C4, C11, C44, S77, S78]. The efficacy of the combined treatment of SCCVII transplantable tumours is significantly higher than that of treatment with radiation alone.

(c) Benzotriazine di-N-oxides

255. Brown and collaborators [M50, Z7, Z8] introduced the benzotriazine di-N-oxide tirapazamine (SR 4233) and analogues into the field of bioreductive drugs. Like the nitro compounds and quinones, the benzotriazine di-N-oxides are reduced to one-electron reduced free radicals [B62, K10, L30, Z9]. Tirapazamine is highly efficient in killing hypoxic cells *in vitro* and *in vivo* [B53, K9, Z7, Z8]. Unlike the toxicity of other bioreductive drugs studied, the toxicity of SR 4233 does not level off at normal oxygen concentrations but continues to decrease as the oxygen concentration increases.

256. The drug appears to induce DNA strand breaks by means of an oxidative damage to pyrimidines [E8]. Analysis of DNA and chromosomal breaks after hypoxic exposure to SR 4233 suggests that DNA double-strand breaks are the primary lesion causing cell death [B28]. More DNA single-strand breaks and a greater heterogeneity in DNA damage were observed in tumour cells than in spleen and marrow cells of mice exposed to tirapazamine, consistent with the presence of hypoxic cells and the greater bioreductive capacity of tumours [O10].

257. SR 4233 is also extremely active when used in combination with fractionated radiation schedules [B62, E8, Z9]. This enhancement is seen when SR 4233 is given before and after irradiation [Z10]. In two animal tumour models (KHT and SCCVII), SR 4233 with radiation produced a significantly greater enhancement than did nicotinamide with carbogen, a combination that has been shown to improve tumour oxygenation. In RIF-1 tumour, which has the lowest hypoxic fraction of the three, the response was comparable for the two modalities [D8]. SR 4233 was able to enhance the tumour growth delay produced by radioimmunotherapy in severe combined immunodeficient phenotype mice with human cutaneous T-cell lymphoma xenografts [W27]. In a study by Lartigau and Guichard [L29], the pO_2 dependence of the survival of three human cell lines (HRT 18, Na11+, and MEWO) exposed to tirapazamine (SR 4233) alone or combined with ionizing radiation, was studied *in vitro*. There was a marked increase in cell killing when tirapazamine was combined with radiation, compared with either tirapazamine or radiation given alone.

Glossary

Absolute risk — Excess risk attributed to an agent and usually expressed as the numeric difference between exposed and unexposed populations (e.g. five cancer deaths over a lifetime per 100 people, each irradiated with 1 Sv).

Additivity — Effect of a combined exposure equaling the sum of the effects from single-agent exposures.

Absorbed dose — *Chemicals* The amount of an applied dose of chemical absorbed into the body or into organs and tissues of interest.

Radiation The average energy imparted to matter in an element of volume by ionizing radiation divided by the mass of that element of volume. The SI unit for absorbed dose is joule per kilogramme ($J\ kg^{-1}$) and its special name is gray (Gy).

Alkylating agents — compounds that transfer an alkyl group to DNA.

Antagonism — *General* A combined effect of two or more interacting agents that is smaller than the addition of the single-agent effects with known dose-effect relationships.

Chemical antagonism or inactivation Chemical reaction between two compounds to produce a less toxic product. Example: toxic metal and chelator.

Dispositional antagonism Alteration of absorption, biotransformation, distribution, or excretion of one agent in such a way that the time-concentration product in the target organ is diminished. Example: prevention of absorption with charcoal.

Functional antagonism Two agents balance each other by producing opposite effects on the same function. Example: drug with vasodepressing side effect and vasopressor.

Receptor antagonism Competitive binding to the same receptor producing a smaller effect. Examples: oxygen in carbon monoxide poisoning, ethanol in methanol poisoning.

Antioxidants — substances preventing oxidation.

Biochemical effect monitoring — Monitoring of biochemical and molecular effects, i.e. changes in sequence, structure, and/or function of biologically relevant molecules caused by an exposure to an agent or a mixture of agents. Biochemical effect monitoring determines tissue dose or biologically effective dose. Examples are direct measurement of DNA adducts and strand breaks. Biochemical effect monitoring takes into account individual differences such as genetic background and deficiencies in DNA repair. A disadvantage is the difficulty of directly monitoring changes in target cell populations. Most analyses are therefore done on surrogate tissue such as blood cells.

Biological indicator — Measurable biological effect that is clearly, specifically, and quantifiably related to an exposure.

Biological effective dose — Biological effect in cells or tissues at risk with direct relevance to the initiation or progression of a disease; see also *Biochemical effect monitoring.*

Biological monitoring — Continuous or repeated monitoring of potentially toxic agents or their metabolites in cells, tissues, body fluids, or excretions (internal dose). Biological monitoring takes into account individual differences in absorption or bioaccumulation of agents in question. It has the advantage of being comparatively easy to monitor.

Biomonitoring — monitoring the environment or a population with biological markers.

Carcinogen — An agent, chemical, physical, or biological, that can act on living tissue in such a way as to cause a malignant neoplasm.

Solitary or complete The agent does not need additional action of further exogenous cancer risk factors to cause a neoplasm.

Indirect or precarcinogen The agent has to be transformed to its active molecular form (ultimate carcinogen) in the metabolism.

Co-factor	A substance or agent that acts with another substance to bring about certain effects; e.g. coenzyme, a low-molecular entity needed for enzymatic activity of the apoenzyme.
Combined effect	The joint effects of two or more agents on the level of molecules, cells, organs, and organisms in the production of a biological effect.
Concentration additivity	Combined effect is predicted by addition of concentrations of different agents on a normalized concentration-effect graph; valid in the case of isoaddition. In this case, a combined effect can arise even when all single-agent concentrations are below their threshold for the endpoint under study (see also effect additivity).
Confounder	A variable that can cause or prevent the outcome of interest, is not an intermediate variable, and is not associated with the factor under investigation. Such a variable must be controlled in order to obtain an undistorted estimate of the effect of the study factor(s) on risk.
Confounding	A situation in which the effects of two processes are not separated. The distortion of the apparent effect of an exposure on risk brought about by the association with other factors that can influence the outcome. Distortion of a measure of the effect because of the association of exposure with other factor(s) that influence the outcome under study (WHO).
Dependent action	Action of two and more agents, in which the effect of a second agent depends on the effect of a first agent. Dependent action leads to combined effects different from heteroadditivity.
Deterministic effect	Effect on sufficient proportion of cells to disrupt tissue or organ function. The probability of causing observable damage will be zero at small doses but will increase steeply to unity above a threshold. Above the threshold, the severity of damage will also increase with dose. Examples include cataracts, skin erythema, and stem-cell depression in bone marrow or the small intestine.
Dose	*Radiation* See *Absorbed dose radiation*. *Chemicals* The amount of a chemical administered to an organism. See also *Absorbed dose chemicals*.
Dose-response relationship	The relationship between the magnitude of exposure to a chemical, biological, or physical agent (dose) and the magnitude or frequency and/or severity of associated adverse effects (response).
Effect additivity	Combined effect is predicted by adding the effects of different agents; valid in the case of heteroaddition. In this case, the combined effect is zero as long as all single-agent concentrations are below their threshold.
Environmental monitoring	Quantitative determination of a potentially detrimental agent in the environment (external dose).
Epigen, epigenetic	Changes in an organism brought about by alterations in the expression of genetic information without any change in the genome itself; the genotype is unaffected by such a change but the phenotype is altered.
Exposure	Concentration, amount, or intensity of a particular physical or chemical or environmental agent that reaches the target population, organism, organ, tissue, or cell, usually expressed in numerical terms of substance concentration, duration, and frequency (for chemical agents or microorganisms) or intensity (for physical agents such as radiation). In the radiation field, exposure may also denote the electrical charge of ions caused by x or gamma rays per unit mass of air; however the term is used in its more general sense as described here.
External dose	*Radiation* Dose from an external radiation source; obtained from being within a radiation field. *Chemicals* Concentration of an agent in an exposure medium, i.e. air or water; see also *Environmental monitoring*.
Genotoxicity	Ability to cause damage to genetic material. Such damage may be mutagenic and/or carcinogenic.

Hazard	Set of inherent properties of a substance, mixture of substances, or a process involving substances that, under production, usage, or disposal conditions, make it capable of having adverse effects on organisms or the environment, depending on the degree of exposure; in other words, a source of danger.
Heteroadditivity	Additive effect from two independently acting agents with different modes of action and therefore different dose-effect relationships. See also *Effect additivity*.
Initiator	In the multi-stage model of carcinogenesis, initiators are defined by their ability to induce persistent changes (probably due to genotoxic effects) in the cell (initiation). If there is subsequent promotion, these changes may result in tumour formation.
Independent action	Action of two and more agents in which the effect of one agent is independent of the effect of the other agent. Independent action leads to combined effects defined as heteroadditive.
Interaction	Combined, mutual effects between agents on a molecular and/or cellular level within a short time.
Internal dose	*Radiation* Dose from radioactive material deposited in the body. *Chemicals* (a) Amount of a chemical recently absorbed; measured, e.g. as metal concentration in blood; (b) amount of chemical stored in one or several body compartments or in the whole body (body burden); used mainly for cumulative toxicants; (c) in the case of ideal biological monitoring, amount of active chemical species bound to the critical sites of action (target dose; e.g. carbon monoxide binding to haemoglobin).
Isoadditivity	Additive effect from two similarly acting agents or from two increments of the same agent on an upward bent dose-effect relationship. See also *Concentration additivity*. On a descriptive level without detailed information about dose-effect relationships, isoadditivity is sometimes indistinguishable from supra-additivity or synergism.
Mitogens	substances with a mitogenic effect on cells.
Multiplicative response	Effect of two agents for which the single-agent response coefficients or relative risks have to be multiplied to describe the combined response.
Mutagen	A substance that can induce heritable changes (mutations) of the genotype in a cell as a consequence of alteration or loss of genes or chromosomes (or parts thereof).
Mutation	A hereditary change in genetic material. A mutation can be a change in a single base (point mutation) or a single gene or it can involve larger chromosomal rearrangements such as deletions and translocations.
Non-genotoxic effect	Effect of an agent at the cellular, organ, or organism level without direct effects on the genome such as DNA damage.
Non-stochastic effect	see *Deterministic effect*.
No Observed Adverse Effect Level (NOAEL)	The greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure. Alterations of morphology, functional capacity, growth, development, or lifespan of the target can be detected at this level but may be judged not to be adverse.
No Observed Effect Level (NOEL)	The greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.
Potentiation	Synergism.
Precursor	Substance from which another, usually more biologically active, substance is formed.

Progression	Increase in autonomous growth and malignancy; used in particular to describe the transition from benign to malignant tumours and the progression of malignancy. There are probably numerous stages of progression during neoplastic development. The process of progression features in the general model of carcinogenesis as well as in the multi-stage model.
Promoter	Risk factors of cancer that are capable of triggering preferential multiplication of a cell changed by initiation. Often, following initiation, a long-term action on the target tissue is necessary. Promoters often cause enzyme induction, hyperplasia, and/or tissue damage. The essential primary effects are considered to be reversible. As a rule, promoters do not bind covalently to cell components and do not exert an immediate genotoxic action.
Relative risk (RR)	Ratio between the cancer cases in the exposed population to the number of cases in the unexposed population. A relative risk of 1.5 indicates a 50% increase in cancer due to the agent under consideration. Excess relative risk (ERR) is RR – 1.
Sensitizer	An agent or substance that is capable of causing a state of abnormal responsiveness in an individual. In most cases, initial exposure results in a normal response, but repeated exposures lead to progressively strong and abnormal responses.
Stochastic effect	Effect of an agent on a cell of a random or statistical nature in which the cell is modified rather than killed. If this cell is able to transmit the modification to later cell generations, any resulting effect, of which there may be many different kinds and severity, are expressed in the progeny of the exposed cell. The probability of such a transmittable effect resulting from an exposure to a genotoxic agent increases with increments of dose, at least for doses well below the threshold for deterministic effects. The severity of the damage is not affected by the dose. When the modified cell is a germ cell, the stochastic effect is called a hereditary effect.
Subadditivity	Less than additive; effect of a combined exposure being less than the sum of effects from single-agent exposures.
Supra-additivity	More than additive; effect of a combined exposure exceeding the sum of the effects from single-agent exposures.
Synergism	A combined effect of two or more interacting agents that is greater than the addition of the single-agent effects with known dose-effect relationships.
Target (biological)	Any organism, organ, tissue, or cell that is subject to the action of a pollutant or other chemical, physical, or biological agent.
Threshold dose	The minimum dose that will produce a biological effect. Dose below which no effects occur (“true”, mechanistically derived threshold) or are measurable (apparent threshold). For a given agent there can be multiple threshold doses, in essence one for each definable effect.
Tissue dose	Local dose in an organ or a functional or structural entity of an organ. See also *Absorbed dose* and *Internal dose-chemicals*.
Topoisomerase	ubiquitous enzymes that alter DNA configuration or topology.
Toxicity	Capacity of an agent to cause injury to a living organism. Toxicity can only be defined in quantitative terms with reference to the quantity of substance administered or absorbed, the way in which this quantity is administered (e.g. inhalation, ingestion, or injection) and distributed in time (e.g. single or repeated doses), the type and severity of injury, and the time needed to produce the injury.

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