GENERAL REMARKS ON THE AGENTS CONSIDERED

This sixtieth volume of *IARC Monographs* covers a number of industrial chemicals, several of which have been reviewed by previously convened working groups. Their conclusions are listed in Table 1. Some of these chemicals have considerable commercial importance, as the building blocks of widely used polymers and copolymers. Justification for their re-evaluation is that a substantial body of new data has become available, and the Preamble has been modified (IARC, 1991, 1992a) to permit more explicit inclusion of data on aspects other than cancer in the evaluation process.

Table 1. Previous evaluations of agents considered in this volume

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans	Volume (year of publication)
	Human	Animal		
Ethylene	ND	ND	3	19 (1979)
Ethylene oxide	L	S	2A	11 (1976); 36 (1985); Suppl. 7 (1987)
Propylene	ND	ND	3	19 (1979)
Propylene oxide	I	S	2A	11 (1976); 36 (1985); Suppl. 7 (1987)
Styrene	I	L	2B[a]	19 (1979); Suppl. 7 (1987)
Styrene oxide	ND	S	2A[a]	11 (1976); 19 (1979); 36 (1985)
4-Vinylcyclohexene	ND	L	3	11 (1976); 39 (1986)
4-Vinylcyclohexene diepoxide	ND	L	3	11 (1976)
Acrylamide	ND	S	2B	39 (1986)
Methyl methacrylate	ND	I	3	19 (1979)

S, sufficient evidence; L, limited evidence; I, inadequate evidence; ND, no data available
For definitions of categories, see preamble, pp. 27–30.
[a]Other relevant data taken into account in making the overall evaluation

Several of these chemicals are characterized by unsaturated chemical bonds, which are necessary for polymerization. These bonds may also be targets for metabolic reactions in which epoxides are formed, some of which are also industrially important chemicals in their own right and are also reviewed and evaluated in this volume.

The Working Group noted the scarcity of data on occupational exposure to such industrially important chemicals as ethylene and propylene. Even though this is explainable

by the absence of regulations on occupational exposure levels in most countries, the gathering and publication of detailed exposure data are to be encouraged.

Parent compounds and electrophilic metabolites

When an alkene and its epoxide are both tested for toxicity, the epoxide is often mutagenic and carcinogenic, while the data are much less clear for the parent alkene, even when it is administered at higher levels than the epoxide. How can an epoxide be carcinogenic and its alkene not?

Pharmacokinetic differences in the way in which the alkene and its epoxide are handled appear to be important. For instance, activation of the parent compound to the epoxide can be saturated and rate-limiting, so that the tissue concentrations reached after administration of the epoxide cannot be attained after administration of the parent compound. Alternatively, rapid detoxification of the epoxide in the cell in which it is formed can result in a lower systemic burden of the epoxide than would be expected if all of it left the site of formation. Furthermore, the reactivity of the epoxide with DNA may be so weak that DNA damage severe enough to give rise to a detectable increase in tumour incidence can be attained only at the site of administration of the epoxide. Finally, detoxification processes may be saturated after administration of high doses of the reactive metabolite, whereas epoxide generated intracellularly from the parent compound may be inactivated.

The carcinogenicity of an epoxide is dependent on the rate of its reaction with critical as opposed to non-critical molecules, on the biological consequences of the adducts formed and on repair of the adducted molecule. The last may be cell type- and adduct-specific. At high levels of the epoxide, repair mechanisms can be saturated, especially at the site of administration by injection or gavage.

In view of these differences, it is not surprising that the cancer risk from exposure to a parent compound may be much lower than that from exposure to its preformed electrophilic metabolite.

Background DNA damage

Some of the adducts formed by the chemicals evaluated in this volume are the same as those found in apparently unexposed individuals, which are probably derived from endogenous and/or environmental agents and processes. How should the additional adducts formed be evaluated in terms of cancer risk?

Ethylene oxide, for instance, reacts with DNA in experimental animals to form predominantly 7-hydroxyethylguanine. This is one of a number of adducts that have recently been identified in humans and animals without specific exposure to a known DNA-reactive carcinogen. This (and other) background DNA damage might contribute to what is called 'spontaneous' mutagenesis and carcinogenesis. Such background damage can be evaluated in appropriately designed studies that take into account host and confounding factors.

Repair of many types of DNA adducts and lesions is well known. It is now becoming evident that efficient repair systems have evolved to handle many of the adducts formed by endogenous and ubiquitous DNA damaging agents. This appears to be true also for the repair of 7-hydroxyethylguanine formed by ethylene oxide. As in other biological processes,

individuals differ with respect to their repair capacities. In any population of animals and humans, DNA damage induced by a specific exposure is detectable only if it is significantly higher than that in the control population. Thus, the limit of detection of 'background' DNA adducts that are formed endogenously is no longer different from that for other genetic end-points which make a background contribution and which vary among and within individuals.

Estimates of dose

Pharmacokinetic models allow description and prediction of the concentrations of a parent alkene and its epoxide metabolite in different tissues and as a function of time. Exposure conditions can be taken into account, and species-specific susceptibility derived from quantitative differences in the rates of metabolic processes can be explained.

Data on haemoglobin adducts are also available for a number of chemicals evaluated in this volume, in both humans and experimental animals. The concentration of haemoglobin adducts formed by a given compound/metabolite may be proportional to the concentration of that agent integrated over the lifetime of erythrocytes during the period preceding blood sampling and may be proportional to the formation of DNA adducts in various tissues. Chemicals with different reactivities bind to different sites in haemoglobin. Haemoglobin binding may take place via another route than DNA binding. While both acrylamide itself and glycidamide, its metabolite, bind to haemoglobin, the main binding to DNA is through glycidamide. Thus, comparison of protein binding, as indicative of binding potency, by different compounds should be carried out with caution.

Combination of data on haemoglobin binding with pharmacokinetic models and historical information on exposure may allow estimation of levels of past human exposures to a few chemicals. Such quantitative information may improve epidemiological analyses, in particular with respect to exposure assessment.

Covalent binding indices

The efficiency of DNA binding can be exemplified by indices such as the 'covalent binding index' (Lutz, 1979). This is the concentration of the adduct in DNA (e.g. adducts per milligram) per dose and body weight. The interpretation of covalent binding indices is complex, and they should therefore be used with caution. The data are typically calculated from a single dose and single time-point. The rate of adduct formation (and often even the identity of the adduct) and its disappearance have not been addressed in many of the studies from which indices were calculated. Single time-point experiments fail to take into consideration differential repair of adducts at various sites of DNA. It is well known that the main adduct seen at the beginning of an experiment may not be the main one seen later (Swenberg *et al.*, 1990). Ideally, a DNA binding indicator should reflect steady-state levels, mimicking chronic human exposure.

Design of studies of genetic effects in humans

Many of the studies of human populations that involve biological markers of exposure or chromosomal or genetic damage need to be considered not only in terms of the biological

mechanisms involved but also for their epidemiological and statistical features, including how and which subjects are selected and their number and personal characteristics. Potential confounding factors, such as cigarette smoking, diet, alcohol and caffeine use, medications and medical history, should be taken into account in subject selection and data analysis. The more powerful the effect of a confounding factor on the end-point, the greater the need to measure it accurately.

Many of the cytogenetic studies reviewed in this volume are smaller than most of the epidemiological studies. Having too few subjects reduces the statistical power to detect an effect of a given magnitude and can make the results of a study appear to be negative when the study is really uninformative.

In studies to compare groups of people for biological markers of effect, such as cytogenetic outcomes, it may be necessary to consider whether the unit of statistical analysis is the person or cells. Comparisons of groups of people allow assessment of confounding and effect-modifying factors, which may not be possible in analyses based only on cells. The fact that changes in an individual's cells are not independent of each other must be taken into account in the statistical analysis. In particular, when small groups of people are studied, the variability within an individual can overshadow variability between people, which is generally the object of studying human populations.

One factor that may strongly affect the risk of an effect is genetic susceptibility. When only a small proportion of a study population is susceptible to a genotoxic agent, differences between groups can be masked. This factor may be of particular importance when it involves a genetically conditioned host response, such as metabolic activation, or the capacity to repair genetic lesions.

Cancer clusters

For several of the chemicals evaluated in this volume, the earliest epidemiological studies were carried out because an apparently high incidence of cancer had been noted in a work force (a case cluster). If subsequent studies included the index cases which prompted the investigation in their analyses, minimal weight should be attached to any risk estimate for cancer found, because case clusters frequently occur by chance, and analyses that include clusters can be expected to show excess cancer. This comment does not detract, however, from the value of case clusters as an initial clue to cancer hazard.

Exposure assessment in studies of humans

Detailed attention must be paid to exposure assessment. Too often, assessment is purely dichotomous and qualitative. In studies in which exposure–response relationships are evaluated, the method of exposure characterization should take into account whether exposures are continuous or intermittent, constant at the same level or fluctuate. The exposure measurement must also correspond to the biological attributes of the outcome and is thus relevant to the time-frame of the biological events being measured. Subjects in epidemiological studies are often misclassified with regard to exposure, so that any effect of exposure will tend to be obscured. When important exposure misclassification occurs and is nondifferential (i.e. does not differ systematically between subjects with different biological outcomes), not much weight can be given to findings that indicate no effect.

Several of the epidemiological studies summarized in the monographs were designed to assess the risk for cancer in relation to quantitative indices of exposure. Various indices are examined, including cumulative exposure (expressed as parts per million-years or -days), duration of exposure (expressed in years), average intensity of exposure (expressed in parts per million and calculated as cumulative exposure divided by duration of exposure) and maximal exposure (expressed in parts per million). The results obtained are not always consistent. For example, in a European study of the glass-reinforced plastics industry (Kogevinas *et al.*, 1994), a positive relationship was found for mortality from lymphatic and haematopoietic cancer with estimated average intensity of exposure to styrene, but not with cumulative exposure.

It is not clear which index of exposure is most relevant for the chemicals under review. The answer to this question will depend on the pharmacokinetics and postulated mechanisms of action of the substances and also on the nature of any relevant repair mechanisms. On the one hand, for example, if a carcinogenic effect depends on metabolic activation and that activation becomes saturated above a certain exposure level, it is possible that no increase in risk occurs once the saturation threshold has been exceeded. The risk from long-term, lower intensity exposure might then be higher than that from the same cumulative exposure experienced over a very short time. On the other hand, if repair mechanisms become saturated above a critical exposure level, risk may be elevated only when exposures exceed the relevant threshold.

In the absence of clear indications about the most appropriate exposure index, the Working Group set most store by findings for cumulative exposure, especially when allowance was made for a latent interval between exposure and the development of risk. It is recognized, however, that with better information about pharmacokinetics and biological mechanisms, it may be necessary to revise this view in the future.

Genetic changes in carcinogenesis

The development in our understanding of the importance of mutational change, at the gene and chromosome level, in the genesis of human cancers has reinforced the conclusion that cancers emerge as a consequence of alterations in the structure and/or expression of a number of genes that are normally involved in controlling the processes of cellular proliferation, differentiation and programmed cell death (apoptosis). Both gene mutations and cancers arise 'spontaneously', but their prevalence is increased in humans exposed to mutagenic and carcinogenic agents.

Many of the agents that are known to induce cancers in humans also induce cancers in animals and DNA damage and mutations in somatic cells of exposed humans and in various mammalian and nonmammalian cells *in vitro*. For example, human populations exposed to ionizing and certain types of nonionizing radiation have increased levels of DNA damage and chromosomal and gene mutations in their somatic cells and increased frequencies of cancers over those in controls. In the case of skin cancers resulting from exposure to solar radiation, the nature of the changes resulting in mutation of specific oncogenes in the emerging tumours reflects the specific nature of the DNA lesions (pyrimidine dimers) induced by ultraviolet light (IARC, 1992b). Similarly, oncogenic mutations in lung carcinomas of miners exposed to radon (Vähäkangas *et al.*, 1992) reflect changes (deletions

of nucleotide sequences) associated with ionizing radiation-induced lesions in DNA. Liver tumours in humans exposed to aflatoxins also reflect specific mutations in oncogenes induced by this agent, and similar associations are found in liver and skin tumours of experimental animals exposed to other chemical mutagens (IARC, 1993). Many of the mutations induced in exposed individuals by radiation or chemicals may be irrelevant in an oncogenic sense, but what is relevant is that a *proportion* of any induced *additional* mutations will have important oncogenic consequences (Bos & van Kreijl, 1992).

The association between mutation and human cancer has been especially highlighted by:

(i) the much increased cancer incidence in individuals who inherit single gene mutations that result in increased genomic instability and/or inefficient DNA repair. Such inherited defects predispose these individuals to various cancers, e.g. of skin (xeroderma pigmentosum), of lymphocytes (ataxia telangiectasia) or of colon (hereditary nonpolyposis coli).

(ii) studies on other familial cancer predispositions, in which the inheritance of a specific mutation (usually deletion of a chromosomal segment containing a tumour suppressor gene or a mutation that inactivates that gene) results in a considerable elevation in the frequencies of specific cancers, e.g. of the breast, ovary and colon; and

(iii) the identification of specific genes—oncogenes, tumour suppressor and apoptotic genes—whose activation, inactivation and loss by gene mutation, chromosomal deletion or translocation (fusion genes) are essential steps in the development of cancers, whether they arise as a consequence of an evident inherent predisposition, are sporadic or are associated with exposure to a known environmental carcinogen.

Much of the initial information on genes involved in human cancers came from studies on experimental animals and on mammalian and other cells *in vitro*; moreover, the chromosomal mutations and the genes involved are common (if not identical) across a wide range of species. Information on agents that produce these changes in experimental animals and other organisms *in vitro* and *in vivo* clearly must be of relevance to the carcinogenic potential of these agents to induce cancers in humans.

Those agents that induce mutations in the germinal cells of rodents are considered to be especially relevant in terms of their carcinogenic potential. Few agents—about 30—many of which are known carcinogens, have thus far been documented as germ-cell mutagens in animals, perhaps because germinal cells represent a distal target with a high capacity for exclusion of genetic damage. Those agents capable of inducing heritable genetic effects in germinal cells may also be expected to do so in somatic cells, thereby increasing the burden of viable genetic lesions and predisposing to carcinogenesis.

The importance of epidemiological evidence for cancer in humans cannot be overstated. Nevertheless, epidemiological evidence requires relatively large populations, unless the risks are very high, whereas small groups may be exposed to potential carcinogens. The advances in our understanding of the processes involved in carcinogenesis indicate the importance of attaching increased relevance to data obtained from studies on genetic and related effects of exposure to an agent. Data from such studies were therefore considered by the Working Group in arriving at their evaluations; the instances in which such data influenced the final evaluation of an agent are described in the text relating to that agent.

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