CHEMICALS, INDUSTRIAL PROCESSES AND INDUSTRIES ASSOCIATED WITH CANCER IN HUMANS

INTRODUCTION

The programme on the Evaluation of the Carcinogenic Risk of Chemicals to Humans has existed since 1971 and involves the preparation and publication of monographs that evaluate individual chemicals and, more recently, carcinogenic risks resulting from exposures to complex mixtures, since it is in this way that human populations are often exposed. Exposures occurring in the wood and leather industries and in the rubber manufacturing industry were thus the subject of recent IARC monographs. (A full list of *IARC Monographs*, both published and in press, is given in Appendix 1.) The evaluations contained in each volume of monographs are made by independent international Working Groups and provide governments and their advisers with authoritative scientific opinions on which to base preventive measures.

The criteria used for preparing draft monographs, for judging the adequacy of available data and for evaluating carcinogenic risk to humans were first established in 1971, and these criteria (with minor modifications) were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the IARC Monographs. In 1977, a further *ad hoc* Working Group revised the criteria[1], and these have appeared as the Preamble[2] to the Monographs since Volume 17.

The terms *'sufficient evidence'* and *'limited evidence'* of carcinogenicity used in those criteria refer only to the amount of evidence available and not to the potency of the carcinogenic effect nor to the mechanism involved. However, in the case of chemicals for which there is *sufficient evidence* of carcinogenicity in experimental animals, it was considered reasonable to recommend that, for practical purposes, such chemicals be regarded as if they presented a carcinogenic risk to humans. In the case of chemicals for which there is only *limited evidence* of carcinogenicity, further experimental and epidemiological research was deemed to be desirable.

The use of the expressions 'for practical purposes' and 'as if they presented a carcinogenic risk' indicates that at the present time a correlation between carcinogenicity in animals and possible human risk cannot be made on a purely scientific basis, but only pragmatically. Such a pragmatic correlation may be useful to regulatory agencies in making decisions related to the primary prevention of cancer.

An international *ad hoc* Working Group of 20 experts in cancer research met in Lyon in January 1979 to re-evaluate the epidemiological and experimental carcinogenicity data on 54 chemicals, groups of chemicals or industrial processes which had been evaluated in Volumes 1-20 of the *IARC Monographs*. Of these, 18 chemicals and industrial processes were considered to be carcinogenic for humans. A further 18 chemicals and groups of chemicals were considered to be probably carcinogenic for humans, although the data were considered inadequate to establish a causal association. To reflect different degrees of evidence within the latter group, it was subdivided: six chemicals were found to have

a higher degree of evidence and 12 chemicals a lower degree. Data on the remaining 18 chemicals were considered to be insufficient to allow an evaluation of their carcinogenicity for humans. A report summarizing the background, purpose and overall conclusions of the Working Group, and the evidence on which the evaluation for each chemical was based was published as Supplement 1 of the *IARC Monographs*[3] and as a leading article in *Cancer Research*[4].

In the first 29 volumes of the *IARC Monographs*, 585 chemicals, groups of chemicals, industrial processes and occupational exposures were evaluated or re-evaluated. Previous analyses of these evaluations indicated that for 44 of these, the working groups found that there was positive evidence of or a suspicion of an association with human cancer. For the remaining 541 exposures, epidemiological data were either unavailable or were considered to be inadequate to evaluate carcinogenicity to humans; one exception was fluorides used in drinking-water and dental preparations, for which no evidence of a carcinogenic effect was found. For 147 of the exposures, there was considered to be *sufficient evidence* of carcinogenicity in animals, and for a further 157 exposures there was *limited evidence*. The data were inadequate to evaluate the presence or absence of a carcinogenic effect for the remaining 236 exposures.

A list of all exposures for which it is currently considered that there is *sufficient evidence* of carcinogenicity in experimental animals is given in Appendix 2.

Objective

The aim of the present *ad hoc* Working Group was to update Supplement 1 of the *IARC Monographs*. All chemicals, groups of chemicals, industrial processes and occupational exposures for which some data on carcinogenicity in humans were available were re-evaluated, on the basis both of studies summarized previously in the monographs and of data published subsequently. Similar data from studies on experimental animals and from short-term tests were also summarized.

Short-term tests for the detection of potential chemical carcinogens

The induction of cancer is thought to proceed by a series of steps, some of which have been distinguished experimentally[5-9]. The first step - 'initiation' - is thought to involve damage to DNA resulting in heritable modifications in, or rearrangements of, genetic information. Proliferation of cells whose properties have been permanently altered during initiation (which may involve somatic mutation) is thought to result in the formation of clones of cells whose further progress to malignancy is dependent on a series of events - 'promotion' and 'progression' - the underlying mechanisms of which are largely unknown. Although this is a useful model, it should be kept in mind that the carcinogenic process may not always proceed by such a multi-step mechanism.

The idea that damage to DNA is a critical event in the initiation of carcinogenesis is based on a large body of data which show that many carcinogens are reactive

electrophiles *per se*, or can be readily converted to reactive electrophiles by enzymic pathways characteristic of eukaryotic metabolism[10]. A variety of DNA-carcinogen adducts, formed by reaction of electrophilic moieties with nucleophilic centres in DNA, have been identified in DNA recovered from reactions performed with carcinogens *in vitro*, or from cultured cells or intact organisms treated with carcinogens[8,11,12]. Moreover, the recognition that many classes of carcinogens (including ionizing and ultra-violet radiation and chemicals of a very wide range of structure and reactivity) are mutagenic[13] supports the idea that DNA is a critical target of carcinogenic agents. Assays for mutagenicity and allied effects, such as the induction of DNA repair, the misincorporation of nucleotide triphosphates during in-vitro nucleic acid synthesis, and various manifestations of chromosomal damage, in organisms ranging from bacteriophages to mammals, all exploit this characteristic ability of carcinogens to cause DNA damage or chromosomal anomalies either directly or indirectly. It should be noted, however, that some carcinogens may act by mechanisms that do not involve DNA damage[14] and thus would not cause such genetic effects.

A number of short-term tests for carcinogens employ as endpoints well-defined genetic markers in prokaryotes and lower eukaryotes (e.g., bacteria and fungi) and in mammalian cell lines. Many of these cells do not possess or have lost, following culture, the range of enzyme systems known in intact mammals to metabolize chemically unreactive carcinogens to reactive electrophiles. It is often necessary, therefore, to provide an exogenous source of such activity in the form of a tissue extract or cell feeder-layer or whole-cell systems prepared from mammalian sources[15]. In-vitro metabolic systems may not accurately reflect the fate of a chemical subjected to the checks and balances afforded by absorption, distribution, metabolism and excretion in mammals[15], and this must be borne in mind when evaluating the results from short-term tests which employ in-vitro metabolic activation. In addition, the organization of genetic material and its repair processes in mammalian cells is highly complex and is not fully reflected in some lower biological systems.

Tests have been devised which exploit the useful attributes of microbial or cellular genetic systems without compromising the integrity of mammalian pharmacodynamics and metabolism. Such 'host-mediated' assays involve the inoculation of indicator organisms into mammals (usually rodents) which are then dosed with the test chemical. There are limitations to both the numbers and types of organisms which can be introduced and recovered from dosed animals and to the access of indicator organisms to activated metabolites. Lack of sensitivity may therefore be a problem.

A group of short-term tests use 'transformation' of cultured mammalian cells, rather than manifestation of DNA damage or chromosomal anomalies, as an indicator of carcinogenic potential. Some of the assays also employ an exogenous metabolic activation system. Cell transformation is assessed by scoring characteristic changes in cellular and colonial morphology, or changes in growth characteristics (e.g., growth of colonies in soft agar) following treatment with the test compound. In some protocols, the ability of transformed cells to produce tumours is tested by injecting the cells into appropriate animals.

Manifestations of damage to DNA and other components of the genetic apparatus can also be assayed directly by exposing animals to the test compound and assaying the effect in these animals or in their offspring. For example, the following endpoints can be scored: mutations in the fruit fly, chromosomal anomalies in bone-marrow cells and blood lymphocytes of rodents, and specific-locus mutations in rodents treated with the test agent and in their offspring.

Similar studies may be conducted on cells taken from people exposed to putative chemical carcinogens and by examining the cells for mutation and for chromosomal anomalies either directly or after short-term culture *in vitro*. Samples of sperm from such individuals may also be analysed for morphological abnormalities. Evidence of absorption of putative carcinogens may be adduced from the assay of body fluids and excreta for DNA-damaging activity, using, for example, bacterial mutation assays.

Results from several studies[16,17] of the predictive value of various short-term tests show that some chemicals of proven carcinogenicity in experimental animals are, as far as could be judged, inactive in tests that utilize DNA or chromosomal damage as endpoints. These include, for example, certain hormones, metals, minerals and tumour promoters[14], which do not appear to exert their effects through modifications of DNA that are expressed in the form of mutations or chromosomal anomalies. No well-validated short-term tests for putative promoters are yet available, although several lines of investigation are being pursued[18-21].

Uses of short-term tests

Validated short-term tests of the type described above are useful (a) for predicting potential carcinogenicity in the absence of data on animal carcinogenicity, (b) as a contribution in deciding which chemicals should be tested or retested in animals, (c) for identifying active fractions of complex mixtures containing putative carcinogens, (d) for recognizing active metabolites of known carcinogens in human or animal body fluids, (e) in helping to elucidate mechanisms of carcinogenesis and (f) as additional evidence in interpreting ambiguous data from experimental or epidemiological studies.

In view of the limitations of current knowledge about mechanisms of carcinogenesis, certain cautions should be emphasized: (1) at present, these tests should not be used by themselves to conclude whether or not an agent is carcinogenic; (2) even when positive results are obtained in one or more of these tests, it is not clear that they can be used reliably to predict the relative potencies of compounds as carcinogens in intact animals; (3) since the currently available tests do not detect all classes of agents that are active in the carcinogenic process (e.g., hormones, promoters), one must be cautious in utilizing these tests as the sole criterion for setting priorities in carcinogenesis research and in selecting compounds for animal bioassays.

The present state of knowledge does not permit the selection of a specific test(s) as the most appropriate for identifying all classes of potential carcinogens, although certain systems are more sensitive to some classes. Before the results of a particular test can be considered to be fully acceptable for predicting potential carcinogenicity, certain criteria should be met: (a) the test should have been validated with respect to known animal carcinogens and noncarcinogens, and (b) when possible, a structurally related carcinogen(s) and noncarcinogen(s) should have been tested simultaneously with the chemical in question. The results should have been confirmed in additional test systems. Confidence in positive results is increased if a mechanism of action can be deduced and if appropriate dose-response data are available. Ideally, a compound should be tested in a battery of short term tests. For optimum usefulness, data on purity must be given. For several recent reviews on the use of short-term tests see IARC[15,16], de Serres and Ashby[17], Bartsch *et al.*[22], Hollstein *et al.*[23] and Sugimura *et al.*[21].