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Guidelines for Mutagenicity Risk Assessment

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Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

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Note: This document represents the final guidelines. A number of editorial corrections have been made during conversion and subsequent proofreading to ensure the accuracy of this publication.

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GUIDELINES FOR MUTAGENICITY RISK ASSESSMENT
[FRL-2983-9]

AGENCY: U.S. Environmental Protection Agency (EPA).

ACTION: Final Guidelines for Mutagenicity Risk Assessment.

SUMMARY: The U.S. Environmental Protection Agency is today issuing five guidelines for assessing the health risks of environmental pollutants:

Guidelines for Carcinogen Risk Assessment
Guidelines for Estimating Exposures
Guidelines for Mutagenicity Risk Assessment
Guidelines for the Health Assessment of Suspect Developmental Toxicants
Guidelines for the Health Risk Assessment of Chemical Mixtures.

This notice contains the Guidelines for Mutagenicity Risk Assessment.

The Guidelines for Mutagenicity Risk Assessment (hereafter Guidelines) are intended to guide Agency analysis of mutagenicity data in line with the policies and procedures established in the statutes administered by EPA. These Guidelines were developed as part of an interoffice guidelines development program under the auspices of the Office of Health and Environmental Assessment (OHEA) in the Agency s Office of Research and Development. They reflect Agency consideration of public and Science Advisory Board (SAB) comments on the Proposed Guidelines for Mutagenicity Risk Assessment published November 23, 1984 (49 FR 46314).

This publication completes the first round of risk assessment guidelines development. These Guidelines will be revised, and new guidelines will be developed, as appropriate.

EFFECTIVE DATE: The Guidelines will be effective September 24, 1986.

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SUPPLEMENTARY INFORMATION: In 1983, the National Academy of Sciences (NAS) published its book entitled *Risk Assessment in the Federal Government: Managing the Process.* In that book, the NAS recommended that Federal regulatory agencies establish inference guidelines to ensure consistency and technical quality in risk assessments and to ensure that the risk assessment process was maintained as a scientific effort separate from risk management. A task force within EPA accepted that recommendation and requested that Agency scientists begin to develop such guidelines.

General

The guidelines published today are products of a two-year Agencywide effort, which has included many scientists from the larger scientific community. These guidelines set forth principles and procedures to guide EPA scientists in the conduct of Agency risk assessments, and to inform Agency decision makers and the public about these procedures. In particular, the guidelines emphasize that risk assessments will be conducted on a case-by-case basis, giving full consideration to all relevant scientific information. This case-by-case approach means that Agency experts review the scientific information on each agent and use the most scientifically appropriate interpretation to assess risk. The guidelines also stress that this information will be fully presented in Agency risk assessment documents, and that Agency scientists will identify the strengths and weaknesses of each assessment by describing uncertainties, assumptions, and limitations, as well as the scientific basis and rationale for each assessment.

Finally, the guidelines are formulated in part to bridge gaps in risk assessment methodology and data. By identifying these gaps and the importance of the missing information to the risk assessment process, EPA wishes to encourage research and analysis that will lead to new risk assessment methods and data.

Guidelines for Mutagenicity Risk Assessment

Work on the Guidelines for Mutagenicity Risk Assessment began in January 1984. Draft guidelines were developed by Agency work groups composed of expert scientists from throughout the Agency. The drafts were peer-reviewed by expert scientists in the field of genetic toxicology from universities, environmental groups, industry, labor, and other governmental agencies. They were then proposed for public comment in the Federal Register (49 FR 46314). On November 9, 1984, the Administrator directed that Agency offices use the proposed guidelines in performing risk assessments until final guidelines became available.

After the close of the public comment period, Agency staff prepared summaries of the comments, analyses of the major issues presented by the commentors, and preliminary Agency responses to those comments. These analyses were presented to review panels of the SAB on

March 4 and April 22-23, 1985, and to the Executive Committee of the SAB on April 25-26, 1985. The SAB meetings were announced in the Federal Register as follows: February 12, 1985 (50 FR 5811), and April 4, 1985 (50 FR 13420 and 13421).

In a letter to the Administrator dated June 19, 1985, the Executive Committee generally concurred on all five of the guidelines, but recommended certain revisions and requested that any revised guidelines be submitted to the appropriate SAB review panel chairman for review and concurrence on behalf of the Executive Committee. As described in the responses to comments (see Part B: Response to the Public and Science Advisory Board Comments), each guidelines document was revised, where appropriate, consistent with the SAB recommendations, and revised draft guidelines were submitted to the panel chairmen. Revised draft Guidelines for Mutagenicity Risk Assessment were concurred on in a letter dated September 24, 1985. Copies of the letters are available at the Public Information Reference Unit, EPA Headquarters Library, as indicated elsewhere in this notice.

Following this Preamble are two parts: Part A contains the Guidelines and Part B the Response to the Public and Science Advisory Board Comments (a summary of the major public comments, SAB comments, and Agency responses to those comments).

The Agency is continuing to study the risk assessment issues raised in the guidelines and will revise these Guidelines in line with new information as appropriate.

References, supporting documents, and comments received on the proposed guidelines, as well as copies of the final guidelines, are available for inspection and copying at the Public Information Reference Unit (202-382-5926), EPA Headquarters Library, 401 M Street, SW, Washington, DC, between the hours of 8:00 a.m. and 4:30 p.m.

I certify that these Guidelines are not major rules as defined by Executive Order 12291, because they are nonbinding policy statements and have no direct effect on the regulated community. Therefore, they will have no effect on costs or prices, and they will have no other significant adverse effects on the economy. These Guidelines were reviewed by the Office of Management and Budget under Executive Order 12291.

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Dated: August 22, 1986

Signed by EPA Administrator
Lee M. Thomas

PART A: GUIDELINES FOR MUTAGENICITY RISK ASSESSMENT

1. INTRODUCTION

This section describes the procedures that the U.S. Environmental Protection Agency will follow in evaluating the potential genetic risk associated with human exposure to chemicals. The central purpose of the health risk assessment is to provide a judgment concerning the weight of evidence that an agent is a potential human mutagen capable of inducing transmitted genetic changes, and, if so, to provide a judgment on how great an impact this agent is likely to have on public health. Regulatory decision making involves two components: risk assessment and risk management. Risk assessment estimates the potential adverse health consequences of exposure to toxic chemicals; risk management combines the risk assessment with the directives of the enabling regulatory legislation together with socioeconomic, technical, political, and other considerations to reach a decision as to whether or how much to control future exposure to the chemicals. The issue of risk management will not be dealt with in these Guidelines.

Risk assessment is comprised of the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization (Committee on Institutional Means, 1983). Hazard identification is the qualitative risk assessment, dealing with the inherent toxicity of a chemical substance. The qualitative mutagenicity assessment answers the question of how likely an agent is to be a human mutagen. The three remaining components comprise quantitative risk assessment, which provides a numerical estimate of the public health consequences of exposure to an agent. The quantitative mutagenicity risk assessment deals with the question of how much mutational damage is likely to be produced by exposure to a given agent under particular exposure scenarios.

In a dose-response assessment, the relationship between the dose of a chemical and the probability of induction of an adverse effect is defined. The component generally entails an extrapolation from the high doses administered to experimental animals or noted in some epidemiologic studies to the low exposure levels expected from human contact with the chemical in the environment.

The exposure assessment identifies populations exposed to toxic chemicals, describes their composition and size, and presents the types, magnitudes, frequencies, and durations of exposure to the chemicals. This component is developed independently of the other components of the mutagenicity assessment and is addressed in separate Agency guidelines (U.S. EPA, 1986).

In risk characterization, the outputs of the exposure assessment and the dose-response assessment are combined to estimate quantitatively the mutation risk, which is expressed as either estimated increase of genetic disease per generation or per lifetime, or the fractional

increase in the assumed background mutation rate of humans. In each step of the assessment, the strengths and weaknesses of the major assumptions need to be presented, and the nature and magnitude of uncertainties need to be characterized.

The procedures set forth in these Guidelines will ensure consistency in the Agency's scientific risk assessments for mutagenic effects. The necessity for a consistent approach to the evaluation of mutagenic risk from chemical substances arises from the authority conferred upon the Agency by a number of statutes to regulate potential mutagens. As appropriate, these Guidelines will apply to statutes administered by the Agency, including the Federal Insecticide, Fungicide, and Rodenticide Act; the Toxic Substances Control Act; the Clean Air Act; the Federal Water Pollution Control Act; the Safe Drinking Water Act; the Resource Conservation and Recovery Act; and the Comprehensive Environmental Response, Compensation, and Liability Act. Because each statute is administered by separate offices, a consistent Agencywide approach for performing risk assessments is desirable.

The mutagenicity risk assessments prepared pursuant to these Guidelines will be utilized with the requirements and constraints of the applicable statutes to arrive at regulatory decisions concerning mutagenicity. The standards of the applicable statutes and regulations may dictate that additional considerations (e.g, the economic and social benefits associated with use of the chemical substance) will come into play in reaching appropriate regulatory decisions.

The Agency has not attempted to provide in the Guidelines a detailed discussion of the mechanisms of mutagenicity or of the various test systems that are currently in use to detect mutagenic potential. Background information on mutagenesis and mutagenicity test systems is available in Identifying and Estimating the Genetic Impact of Chemical Mutagens, National Academy of Sciences (NAS) Committee on Chemical Environmental Mutagens (NAS, 1962), as well as in other recent publications (Committee 1, 1983).

The Agency is concerned with the risk associated with both germ-cell mutations and somatic-cell mutations. Mutations carried in germ cells may be inherited by future generations and may contribute to genetic disease, whereas mutations occurring in somatic cells may be implicated in the etiology of several disease states, including cancer. These Guidelines, however, are concerned only with genetic damage as it relates to germ-cell mutations. The use of mutagenicity test results in the assessment of carcinogenic risk is described in the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986).

As a result of progress in the control of infectious diseases, increases in average human lifespan, and better procedures for identifying genetic disorders, a considerable heritable genetic disease burden has been recognized in the human population. It is estimated that at least 10% of all human disease is related to specific genetic abnormalities, such as abnormal composition, arrangement, or dosage of genes and chromosomes (NAS, 1962; NRC, 1972, 1977, 1980; UN,

1958, 1962, 1966, 1969, 1972, 1982). Such genetic abnormalities can lead to structural or functional health impairments. These conditions may be expressed in utero; at the time of birth; or during infancy, childhood, adolescence, or adult life; and may be chronic or acute in nature. As a result, they often have a severe impact upon the affected individuals and their families in terms of physical and mental suffering and economic losses, and upon society in general, which often becomes responsible for institutional care of severely affected individuals. Some examples of genetic disorders are Down's and Klinefelter syndromes, cystic fibrosis, hemophilia, sickle-cell anemia, and achondroplastic dwarfism. Other commonly recognized conditions that are likely to have a genetic component include hypercholesterolemia, hypertension, pyloric stenosis, glaucoma, allergies, several types of cancer, and mental retardation. These disorders are only a few of the thousands that are at least partially genetically determined (McKusick, 1983).

Estimation of the fraction of human genetic disorders that result from new mutations is difficult, although in certain specific cases insights are available (Crow and Denniston, 1981). It is clear that recurring mutation is important in determining the incidence of certain genetic disorders, such as some chromosomal aberration syndromes (e.g., Down s syndrome) and rare dominant and X-linked recessive diseases (e.g., achondroplasia and hemophilia A). For other single-factor disorders (e.g., sickle-cell anemia) and certain multifactorial disorders (e.g., pyloric stenosis), the contribution of new mutations to disease frequency is probably small. However, it is generally recognized that most newly arising mutations that are phenotypically expressed are in some ways deleterious to the organism receiving them (NAS, 1962; NRC, 1972, 1977, 1980; UN, 1958, 1962, 1966, 1969, 1972, 1982). Adverse effects may be manifested at the biochemical, cellular, or physiological levels of organization. Although mutations are the building blocks for further evolutionary change of species, it is believed that increases in the mutation rate could lead to an increased frequency of expressed genetic disorders in the first and subsequent generations.

Life in our technological society results in exposure to many natural and synthetic chemicals. Some have been shown to have mutagenic activity in mammalian and submammalian test systems, and thus may have the potential to increase genetic damage in the human population. Chemicals exhibiting mutagenic activity in various test systems have been found distributed among foods, tobacco, drugs, food additives, cosmetics, industrial compounds, pesticides, and consumer products. The extent to which exposure to natural and synthetic environmental agents may have increased the frequency of genetic disorders in the present human population and contributed to the mutational load that will be transmitted to future generations is unknown at this time. However, for the reasons cited above, it seems prudent to limit exposures to potential human mutagens.

1.1. CONCEPTS RELATING TO HERITABLE MUTAGENIC RISK

These Guidelines are concerned with chemical substances or mixtures of substances that can induce alterations in the genome of either somatic or germinal cells. The mutagenicity of physical agents (e.g., radiation) is not addressed here. There are several mutagenic endpoints of concern to the Agency. These include point mutations (i.e., submicroscopic changes in the base sequence of DNA) and structural or numerical chromosome aberrations. Structural aberrations include deficiencies, duplications, insertions, inversions, and translocations, whereas numerical aberrations are gains or losses of whole chromosomes (e.g., trisomy, monosomy) or sets of chromosomes (haploidy, polyploidy).

Certain mutagens, such as alkylating agents, can directly induce alterations in the DNA. Mutagenic effects may also come about through mechanisms other than chemical alterations of DNA. Among these are interference with normal DNA synthesis (as caused by some metal mutagens), interference with DNA repair, abnormal DNA methylation, abnormal nuclear division processes, or lesions in non-DNA targets (e.g., protamine, tubulin).

Evidence that an agent induces heritable mutations in human beings could be derived from epidemiologic data indicating a strong association between chemical exposure and heritable effects. It is difficult to obtain such data because any specific mutation is a rare event, and only a small fraction of the estimated thousands of human genes and conditions are currently useful as markers in estimating mutation rates. Human genetic variability, small numbers of offspring per individual, and long generation times further complicate such studies. In addition, only disorders caused by dominant mutations, some sex-linked recessive mutations, and certain chromosome aberrations can be detected in the first generation after their occurrence. Conditions caused by autosomal recessive disorders (which appear to occur more frequently than dominant disorders) or by polygenic traits may go unrecognized for many generations. Therefore, in the absence of human epidemiological data, it is appropriate to rely on data from experimental animal systems as long as the limitations of using surrogate and model systems are clearly stated.

Despite species differences in metabolism, DNA repair, and other physiological processes affecting chemical mutagenesis, the virtual universality of DNA as the genetic material and of the genetic code provides a rationale for using various nonhuman test systems to predict the intrinsic mutagenicity of test chemicals. Additional support for the use of nonhuman systems is provided by the observation that chemicals causing genetic effects in one species or test system frequently cause similar effects in other species or systems. Evidence also exists that chemicals can induce genetic damage in somatic cells of exposed humans. For example, high doses of mutagenic chemotherapeutic agents have been shown to cause chromosomal abnormalities (Musilova et al., 1979), sister chromatic exchange (Musilova et al., 1979), and, quite probably, point mutations in human lymphocytes exposed in vivo (Strauss and Albertini, 1979). While these results are not in

germ cells, they do indicate that it is possible to induce mutagenic events in human cells in vivo. Furthermore, a wide variety of different types of mutations have been observed in humans, including numerical chromosome aberrations, translocations, base-pair substitutions, and frameshift mutations. Although the cause of these mutations is uncertain, it is clear from these observations that human germ-cell DNA is subject to the same types of mutational events that are observed in other species and test systems.

Certain test systems offer notable advantages: cost; anatomical, histological, and/or metabolic similarities to humans; suitability for handling large numbers of test organisms; a large database; or a basis for characterizing genetic events.

1.2. TEST SYSTEMS

Many test systems can contribute information about the mutagenic potential of a test compound with respect to various genetic endpoints. These tests have recently been evaluated through the EPA Gene-Tox Programs, and the results of Phase I have been published.[1] The Agency's Office of Pesticides and Toxic Substances has published various testing guidelines for the detection of mutagenic effects (U.S. EPA, 1982, 1983).

Test systems for detecting point mutations include those in bacteria, eukaryotic microorganisms, higher plants, insects, mammalian somatic cells in culture, and germinal cells of intact mammals. Data from heritable, mammalian germ-cell tests provide the best experimental evidence that a chemical is a potential human germ-cell mutagen because these tests require that mutations occur in germinal cells and that they are transmitted to the next generation. To date, the most extensively used test for the induction of heritable mutation is the mouse specific-locus test, which measures the induction of recessive mutations at seven loci concerned with coat color and ear morphology. While this test has a large database compared to other germ-cell assays, it is difficult to extrapolate results to humans because recessive mutations may occur more frequently than dominants, and the impact of recessive mutations is not seen for many generations. Information on frequencies of induced mutations resulting in health disorders in the first generation may be obtained from mouse systems designed to detect skeletal abnormalities, cataracts, or general morphological abnormalities. However, these assays have been used to a relatively limited extent, and there is a need for additional studies with known, chemical germ-cell mutagens to further characterize the test systems. Because large numbers of offspring must usually be generated in the systems described above, it is not expected that many chemicals will

[1]A complete reference of all Gene-Tox publications is available from the TSCA Industry Assistance Office (TS-794), Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC 20460.

be tested using these systems. To obtain data on a large number of environmental chemicals, it will be necessary to rely on other tests to identify and characterize hazards from gene mutations.

Test systems for detecting structural chromosome aberrations have been developed in a variety of organisms including higher plants, insects, fish, birds, and several mammalian species. Many of these assays can be performed in vitro or in vivo, and in either germ or somatic cells. Procedures available for detecting structural chromosome aberrations in mammalian germ cells include measurement of heritable translocations or dominant lethality, as well as direct cytogenetic analyses of germ cells and early embryos in rodents.

Some chemicals may cause numerical chromosome changes (i.e., aneuploidy) as their sole mutagenic effect. These agents may not be detected as mutagens if evaluated only in tests for DNA damage, gene mutations, or chromosome breakage and rearrangement. Therefore, it is important to consider tests for changes in chromosome number in the total assessment of mutagenic hazards. Although tests for the detection of variation in the chromosome number are still at an early stage of development, systems exist in such diverse organisms as fungi, *Drosophila*, mammalian cells in culture, and intact mammals (e.g., mouse X-chromosome loss assay). Aneuploidy can arise from disturbances in a number of events affecting the meiotic process (Parker and Williamson, 1974; Grell, 1979). Although the mechanisms by which nondisjunction occurs are not well understood, mitotic structures other than DNA may be the target molecules for at least some mechanisms of induced nondisjunction.

Other endpoints that provide information bearing on the mutagenicity of a chemical can be detected by a variety of test systems. Such tests measure DNA damage in eukaryotic or prokaryotic cells, unscheduled DNA synthesis in mammalian somatic and germ cells, mitotic recombination and gene conversion in yeast, and sister-chromatid exchange in mammalian somatic and germ cells. Results in these assays are useful because the induction of these endpoints often correlates positively with the potential of a chemical to induce mutations.

In general, for all three endpoints (i.e., point mutations and numerical and structural aberrations), the Agency will place greater weight on tests conducted in germ cells than in somatic cells, on tests performed in vivo rather than in vitro, in eukaryotes rather than prokaryotes, and in mammalian species rather than in submammalian species. Formal numerical weighting systems have been developed (Russell et al., 1984); however, the Agency has concluded that these do not readily accommodate such variables as dose range, route of exposure, and magnitude of response.

The Agency anticipates that from time to time somatic cell data from chemically exposed human beings will be available (e.g., cytogenetic markers in peripheral lymphocytes). When possible, the Agency will use such data in conjunction with somatic and germ-cell comparisons from in vivo mammalian experimental systems as a component in performing risk assessments.

The test systems mentioned previously are not the only ones that will provide evidence of mutagenicity or related DNA effects. These systems are enumerated merely to demonstrate the breadth of the available techniques for characterizing mutagenic hazards, and to indicate the types of data that the Agency will consider in its evaluation of mutagenic potential of a chemical agent. Most systems possess certain limitations that must be taken into account. The selection and performance of appropriate tests for evaluating the risks associated with human exposure to any suspected mutagen will depend on sound scientific judgment and experience, and may necessitate consultation with geneticists familiar with the sensitivity and experimental design of the test system in question. In view of the rapid advances in test methodology, the Agency expects that both the number and quality of the tools for assessing genetic risk to human beings will increase with time. The Agency will closely monitor developments in mutagenicity evaluation and will refine its risk assessment scheme as better test systems become available.

2. QUALITATIVE ASSESSMENT (HAZARD IDENTIFICATION)

The assessment of potential human germ-cell mutagenic risk is a multistep process. The first step is an analysis of the evidence bearing on a chemical s ability to induce mutagenic events, while the second step involves an analysis of the chemical s ability to produce these events in the mammalian gonad. All relevant information is then integrated into a weight-of-evidence scheme that presents the strength of the information bearing on the chemical's potential ability to produce mutations in human germ cells. For chemicals demonstrating this potential, one may decide to proceed with an evaluation of the quantitative consequences of mutation following expected human exposure.

For hazard identification, it is clearly desirable to have data from mammalian germ-cell tests, such as the mouse specific-locus test for point mutations and the heritable translocation or germ-cell cytogenetic tests for structural chromosome aberrations. It is recognized, however, that in most instances such data will not be available, and alternative means of evaluation will be required. In such cases the Agency will evaluate the evidence bearing on the agent's mutagenic activity and the agent s ability to interact with or affect the mammalian gonadal target. When evidence exists that an agent possesses both these attributes, it is reasonable to deduce that the agent is a potential human germ-cell mutagen.

While mammalian germ-cell assays are performed mostly on male animals, a chemical cannot be considered to be a nonmutagen for mammalian germ cells unless it is shown to be negative in both sexes. Furthermore, because most mammalian germ-cell assays are performed in mice, it is noteworthy that the data from ionizing radiation suggest that the female mouse immature oocyte may not be an appropriate surrogate for the same stage in the human female in mutagenicity testing. However, mutagenicity data on the maturing and mature oocyte of the mouse may provide a useful model for human risk assessment.

2.1. MUTAGENIC ACTIVITY

In evaluating chemicals for mutagenic activity, a number of factors will be considered: (1) genetic endpoints (e.g., gene mutations, structural or numerical chromosomal aberrations) detected by the test systems, (2) sensitivity and predictive value of the test systems for various classes of chemical compounds, (3) number of different test systems used for detecting each genetic endpoint, (4) consistency of the results obtained in different test systems and different species, (5) aspects of the dose-response relationship, and (6) whether the tests are conducted in accordance with appropriate test protocols agreed upon by experts in the field.

2.2. CHEMICAL INTERACTIONS IN THE MAMMALIAN GONAD

Evidence for chemical interaction in the mammalian gonad spans a range of different types of findings. Each chemical under consideration needs to be extensively reviewed because this type of evidence may be part of testing exclusive of mutagenicity per se (e.g., reproduction, metabolism, and mechanistic investigations). Although it is not possible to classify clearly each type of information that may be available on a chemical, two possible groups are illustrated.

1. *Sufficient evidence* of chemical interaction is given by the demonstration that an agent interacts with germ-cell DNA or other chromatin constituents, or that it induces such endpoints as unscheduled DNA synthesis, sister-chromatid exchange, or chromosomal aberrations in germinal cells.
2. *Suggestive evidence* will include the finding of adverse gonadal effects such as sperm abnormalities following acute, subchronic, or chronic toxicity testing, or findings of adverse reproductive effects such as decreased fertility, which are consistent with the chemical's interaction with germ cells.

2.3. WEIGHT-OF-EVIDENCE DETERMINATION

The evidence for a chemical s ability to produce mutations and to interact with the germinal target is integrated into a weight-of-evidence judgment that the agent may pose a hazard as a potential human germ-cell rnutagen. All information bearing on the subject, whether indicative of potential concern or not, must be evaluated. Whatever evidence may exist from humans must also be factored into the assessment.

All germ-cell stages are important in evaluating chemicals because some chemicals have been shown to be positive in postgonial stages but not in gonia (Russell et al., 1984). When human exposures occur, effects on postgonial stages should be weighted by the relative sensitivity and the duration of the stages. Chemicals may show positive effects for some endpoints and in some test systems, but negative responses in others. Each review must take into account the limitations in the testing and in the types of responses that may exist.

To provide guidance as to the categorization of the weight of evidence, a classification scheme is presented to illustrate, in a simplified sense, the strength of the information bearing on the potential for human germ-cell mutagenicity. It is not possible to illustrate all potential combinations of evidence, and considerable judgment must be exercised in reaching conclusions. In addition, certain responses in tests that do not measure direct mutagenic end points (e.g., SCE induction in mammalian germ cells) may provide a basis for raising the weight of evidence from one category to another. The categories are presented in decreasing order of strength of evidence.

1. Positive data derived from human germ-cell mutagenicity studies, when available, will constitute the highest level of evidence for human mutagenicity.
2. Valid positive results from studies on heritable mutational events (of any kind) in mammalian germ cells.
3. Valid positive results from mammalian germ-cell chromosome aberration studies that do not include an intergeneration test.
4. Sufficient evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity test results from two assay systems, at least one of which is mammalian (in vitro or in vivo). The positive results may both be for gene mutations or both for chromosome aberrations; if one is for gene mutations and the other for chromosome aberrations, both must be from mammalian systems.
5. Suggestive evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity evidence from two assay systems as described under 4, above. Alternatively, positive mutagenicity evidence of less strength than defined under 4, above, when combined with sufficient evidence for a chemical's interaction with mammalian germ cells.
6. Positive mutagenicity test results of less strength than defined under 4, combined with suggestive evidence for a chemical's interaction with mammalian germ cells.
7. Although definitive proof of nonmutagenicity is not possible, a chemical could be classified operationally as a nonmutagen for human germ cells if it gives valid negative test results for all endpoints of concern.
8. Inadequate evidence bearing on either mutagenicity or chemical interaction with mammalian germ cells.

3. QUANTITATIVE ASSESSMENT

The preceding section addressed primarily the processes of hazard identification, i.e., the determination of whether a substance is a potential germ-cell mutagen. Often, no further data will be available, and judgments will need to be based mainly on qualitative criteria. Quantitative risk assessment is a two-step process: determination of the heritable effect per unit of exposure (dose-response) and the relationship between mutation rate and disease incidence. The procedures that are presently accepted for the estimation of an increase in disease resulting from increased mutation have been described (NAS, 1962; NRC, 1972, 1977, 1980; UN, 1958, 1962, 1966, 1969, 1972, 1982). Dose-response information is combined with anticipated levels and patterns of human exposure in order to derive a quantitative assessment (risk characterization).

3.1. DOSE RESPONSE

Dose-response assessments can presently be performed only with data from in vivo, heritable mammalian germ-cell tests, until such time as other approaches can be demonstrated to have equivalent predictability. The morphological specific locus and biochemical specific locus assays can provide data on the frequencies of recessive mutations induced by different chemical exposure levels, and similar data can be obtained for heritable chromosomal damage using the heritable translocation test. Data on the frequencies of induced mutations resulting in health disorders in the first generation may be obtained from mouse systems designed to detect skeletal abnormalities, cataracts, or general morphological abnormalities. Assays that directly detect heritable health effects in the first generation may provide the best basis for predicting human health risks that result from mutagen exposure. The experimental data on induced mutation frequency are usually obtained at exposure levels much higher than those that will be experienced by human beings. An assessment of human risk is obtained by extrapolating the induced mutation frequency or the observed phenotypic effect downward to the approximate level of anticipated human exposure. In performing these extrapolations, the Agency will place greater weight on data derived from exposures and exposure rates that most closely simulate those experienced by the human population under study.

The Agency will strive to use the most appropriate extrapolation models for risk analysis and will be guided by the available data and mechanistic considerations in this selection. However, it is anticipated that for tests involving germ cells of whole mammals, few dose points will be available to define dose-response functions. The Agency is aware that for at least one chemical that has been tested for mutations in mammalian germ cells, there exist departures from linearity at low exposure and exposure rates in a fashion similar to that seen for ionizing

radiation that has a low linear energy transfer (Russell et al., 1982). The Agency will consider all relevant models for gene and chromosomal mutations in performing low-dose extrapolations and will choose the most appropriate model. This choice will be consistent both with the experimental data available and with current knowledge of relevant mutational mechanisms.

An experimental approach for quantitative assessment of genetic risk, which may have utility in the future, uses molecular dosimetry data from intact mammals in conjunction with mutagenicity and dosimetry data from other validated test systems (Lee, 1979). The intact mammal is used primarily for relating the exposure level for a given route of administration of a chemical to germ-cell dose, i.e., the level of mutagen-DNA interactions. This information is then used in conjunction with results obtained from mutagenicity test systems in which the relationship between the induction of mutations and chemical interactions with DNA can be derived. With mutagen-DNA interactions as the common denominator, a relationship can be constructed between mammalian exposure and the induced mutation frequency. The amount of DNA binding induced by a particular chemical agent may often be determined at levels of anticipated human exposure.

For some mutagenic events, DNA may not necessarily be the critical target. Interaction of chemicals with other macromolecules, such as tubulin, which is involved in the separation of chromosomes during nuclear division, can lead to chromosomal nondisjunction. At present, general approaches are not available for dose-response assessments for these types of mutations. Ongoing research should provide the means to make future assessments on chemicals causing aneuploidy.

3.2. EXPOSURE ASSESSMENT

The exposure assessment identifies populations exposed to toxic chemicals; describes their composition and size; and presents the types, magnitudes, frequencies, and durations of exposure to the chemicals. This component is developed independently of the other components of the mutagenicity assessment (U.S. EPA, 1986).

3.3. RISK CHARACTERIZATION

In performing mutagenicity risk assessments, it is important to consider each genetic endpoint individually. For example, although certain chemical substances that interact with DNA may cause both point and chromosomal mutations, it is expected that the ratio of these events may differ among chemicals and between doses for a given chemical. Furthermore, transmissible chromosomal aberrations are recoverable with higher frequencies from meiotic and postmeiotic germ-cell stages, which have a brief lifespan, than in spermatogonial stem cells, which can accumulate genetic damage throughout the reproductive life of an individual. For

these reasons, when data are available, the Agency, to the best extent possible will assess risks associated with all genetic endpoints.

Any risk assessment should clearly delineate the strengths and weaknesses of the data, the assumptions made, the uncertainties in the methodology, and the rationale used in reaching the conclusions, e.g., similar or different routes of exposure and metabolic differences between humans and test animals. When possible, quantitative risk assessments should be expressed in terms of the estimated increase of genetic disease per generation, or the fractional increase in the assumed background spontaneous mutation rate of humans (NRC, 1972, 1977, 1980). Examples of quantitative risk estimates have been published (NRC, 1972, 1977, 1980; UN, 1958, 1962, 1966, 1969, 1972, 1982; Ehling and Neuhauser, 1979); these examples may be of use in performing quantitative risk assessments for mutagens.

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PART B: RESPONSE TO PUBLIC AND SCIENCE ADVISORY BOARD COMMENTS

This section summarizes some of the issues raised in public and Science Advisory Board (SAB) comments on the Proposed Guidelines for Mutagenicity Risk Assessment published on November 23, 1984 (49 FR 46314). Unlike the other guidelines published on the same date, the Proposed Guidelines for Mutagenicity Risk Assessment contained a detailed section dealing with public comments received in response to the original proposal of 1980 (45 FR 74984). Several of the comments received in response to the proposed guidelines of 1984 were similar to those received in response to the proposed guidelines of 1980. Those comments are not addressed here because the position of the Agency on those issues has been presented in the responses included with the 1984 proposed guidelines (49 FR 46315-46316). A total of 44 comments were received in response to the proposed guidelines of 1984: 21 from manufacturers of regulated products, 10 from associations, 9 from government agencies, 2 from educational institutions, 1 from an individual, and 1 from a private consulting firm. The proposed guidelines and the public comments received were transmitted to the Agency's SAB prior to its public review of the proposed guidelines held April 22-23, 1985. The majority of the comments were favorable and expressed the opinion that the proposed guidelines accurately represent the existing state of knowledge in the field of mutagenesis. Several commentors offered suggestions for further clarification of particular issues, and many of the suggestions have been incorporated.

The two areas that received the most substantive comments were the sections concerning Weight-of-Evidence Determination and Dose Response. The comments on the proposed weight-of-evidence scheme ranged from suggestions for the elimination of a formal scheme to the expansion of the scheme to cover more potential data configurations. The SAB recommended an eight-level rank ordering scheme to define levels of evidence relating to human germ-cell mutagenicity. The Agency has incorporated this scheme into the Guidelines. Some commentors and the SAB suggested that the molecular dosimetry approach to dose-response data be presented as a concept that may be useful in the future rather than being available for use now. The Agency agrees that the database at the present time is too sparse to recommend a general application of this approach to a wide range of chemical classes, and the Guidelines have been changed to reflect this. It should be noted, however, that the Agency strongly supports the development of molecular dosimetry methodologies as they relate to both an understanding of dose-response relationships and to methods for studying human exposure. A number of comments suggesting clarifications and editorial changes have been incorporated and the references have been expanded.