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Proposed Guidelines for Mutagenicity Risk Assessment; Request for Comments

ENVIRONMENTAL PROTECTION AGENCY

[FRL-2706-8]

Proposed Guidelines for Mutagenicity Risk Assessment

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed Guidelines for Mutagenicity Risk Assessment and Request for Comments.

SUMMARY: The U.S. Environmental Protection Agency is proposing Guidelines for Mutagenicity Risk Assessment (Guidelines). These Guidelines are proposed for use within the policy and procedural framework provided by the various statutes that EPA administers to guide Agency analysis of mutagenicity data. We solicit public comment and will take public comment into account in revising these Guidelines. These Guidelines will be reviewed by the Science Advisory Board in meetings now tentatively scheduled for April 1985.

These proposed Guidelines were developed as part of a broad guidelines development program under the auspices of the Office of Health and Environmental Assessment (OHEA), located in the Agency's Office of Research and Development. Consonant with the role of OHEA's Reproductive Effects Assessment Group (REAG) as the Agency's senior health committee for mutagenicity assessment, the Guidelines were developed by an Agency-wide working group chaired by the REAG.

DATES: Comments must be postmarked by January 22, 1985.

ADDRESSES: Comments may be mailed or delivered to: Dr. David Jacobson-Kram, Reproductive Effects Assessment Group (RD-689), Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, 401 M Street SW., Washington, DC 20460.

FOR FURTHER INFORMATION CONTACT: Dr. David Jacobson-Kram, Telephone: 202-382-7338.

SUPPLEMENTARY INFORMATION: Public comments received as a result of the proposed guidelines for Mutagenicity Risk Assessment, which was published in the Federal Register (45(221):74984-74988) on November 13, 1980, have been addressed. The guidelines published here reflect the suggestions that were provided during that initial comment period. A new draft of these Guidelines, taking into account the earlier public comments, was recently sent for review to approximately 14 scientists in the field of chemical mutagenesis within government, universities in the United States, and the private sector. Comments received from these reviews, generally favorable, were also taken into account in developing the Guidelines proposed here.

References and supporting documents used in the preparation of these Guidelines as well as comments received 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.

Dated: November 9, 1984.

William D. Ruckelshaus,

Administrator.

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I. Introduction

On November 13, 1980, the U.S. Environmental Protection Agency (EPA) published purposed guidelines for Mutagenicity Risk Assessment (*1*) and solicited comments on those guidelines. The proposed guidelines of 1980 described the procedures that the Agency would follow to evaluate the genetic risks associated with the exposure of humans to chemical mutagens. These procedures incorporated a weight-of-evidence approach that considered the quality and adequacy of all the available data on a chemical substance in order to make qualitative, and, where possible, quantitative evaluations of mutagenic potential. The Agency stated that mutagenicity risk assessments prepared pursuant to the proposed guidelines would be utilized within the requirements and constraints of the applicable statutes that the Agency administers to arrive at regulatory decisions concerning mutagenicity.

The current proposed Guidelines address the comments received in response to the Agency's proposed mutagenicity risk assessment guidelines and provide the basis for the Agency's risk assessments for mutagenicity. These Guidelines, which adopt the general approach set forth in the 1980 proposal, reflect additional changes made in response to the comments and to new scientific information generated since the time of the proposal.

The current proposed Guidelines reflect changes made in response to the public comments to the proposed guidelines of 1980. These changes dealt primarily with the section addressing the weight-of-evidence approach. This section has been expanded to define "sufficient," "suggestive," and "limited" evidence for potential human germ-cell mutagenicity and to include two categories of evidence, "sufficient" and "suggestive" for chemical interaction with the gonads. Also, in the quantitative assessment section, the dominant skeletal and dominant cataract tests have been added to the list of systems for possible use in estimating the magnitude of genetic risks. Other minor changes have been made in the text for clarification.

A draft of the current proposed Guidelines was submitted for review to individuals from industry, educational institutions, enivornmental groups, and other government agencies. These reviews were useful in revising the Guidelines.

The Agency has not attempted to provide in the current proposed 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 mutagenicity and mutagenic test systems is available in "Identifying and Estimating the Genetic Impact of Chemical Environmental Mutagens," National Academy of Sciences (NAS) Committee on Chemical Environmental Mutagens (*2*), as well as in other recent publications(*3, 4*).

For the information of the reviewer, Chapter II discusses the comments that were received in response to the proposed guidelines of 1980 and the Agency's responses to those comments. The current proposed Guidelines for Mutagenicity Risk Assessment, for which comments are currently invited, are described in Chapter III. The Agency anticipates that, as methods for mutagenicity risk assessment are

refined, and more information becomes available in the area of mutagenicity, revisions to these Guidelines may be desirable or necessary.

II. Comments Received From the Federal Register Publication of the Proposed 1980 Guidelines and Agency Responses to These Comments

As stated in the Introduction, the current Guidelines are being proposed to encourage further public comment. For the information of the reviewer, a summary of the public comments received in response to the proposed guidelines of 1980 and the Agency responses to those comments are presented here.

A total of 34 comments were received, 17 from manufacturers of regulated products, eight from associations, four from individuals, three from educational institutions, and one each from a private consulting laboratory and a government agency. Many responses noted that the proposed guidelines of 1980 were timely and appropriate and praised the Agency for initiating procedures for scientific evaluation of mutagenicity data. Other commenters felt that the proposed guidelines were "premature." Various reasons were given for this position: (1) The mechanisms by which mutations occur are not understood; (2) the data bases for many mutagenicity tests are limited, and hence the tests have not been validated; (3) the Agency should wait until the EPA Gene-Tox Program is completed; and (4) epidemiologic studies have failed to document chemically-induced mutations in humans.

It is the opinion of the Agency that there is a need for mutagenicity guidelines because various statutes administered by the Agency provide the authority to regulate chemicals on the basis of mutagenicity. The purpose of the current proposed Guidelines is to promote Agency-wide consistency in the evaluation of mutagenicity data. In response to the specific concerns enumerated above relating to the issue of prematurity, the Agency has concluded that the comments do not provide an adequate basis for delaying the development of mutagenicity guidelines. Specifically, with regard to the first comment that the mechanisms by which mutations occur are not understood, the Agency does not believe that a full understanding of all aspects of these mechanisms is necessary to evaluate the mutagenic potential of chemicals in the environment. Additionally, the comment ignores the extensive body of data on specific chemical DNA adducts, repair processes, and mutational expression that enable description of the mutational process in specific physiochemical terms (2).

With regard to the second comment, the Agency agrees that the data bases for many mutagenicity tests are limited; however, the Agency does not agree that the validity of a test is a function of the size of the data base. Validity is the extent to which a test measures the particular biological end point of interest and should not be confused with sensitivity, the proportion of known mutagens that are positive in a system, or specificity, the proportion of nonmutagens that are negative. Hence, a mutagenesis assay is validated when its ability to detect a heritable genetic change is demonstrated.

In response to the third comment, the Agency does not believe it is necessary to wait for completion of the Gene-Tox Program before issuing guidelines for evaluating mutagenicity data. The Agency acknowledges that future scientific developments can be expected to affect the methods for the evaluation of mutagenicity data. Such developments may stem from phase II of the Gene-Tox Program (which focuses on test applications) as well as from other collaborative activities in basic and applied research. However, the Agency believes that the current Guidelines, as written, can accommodate new information.

With respect to the fourth comment, the Agency does not agree that the failure to identify a chemical as a known human mutagen is justification for not proposing guidelines to evaluate mutagenicity data. Despite the difficulty in translating changes in mutation rate to alterations in disease frequency, the NAS Committee on Chemical Environmental Mutagens has concluded that the net effect of an increase in mutation rate is harmful because almost all mutants with any detectable effect are deleterious (2).

A. Comments on the Introduction

Many commenters on the proposed guidelines of 1980 were critical of the statement, "Since the prospect of curing most heritable diseases caused by mutagens in the near future is unlikely, minimizing exposure to mutagens is among the best available means to protect against further deterioration of the human gene pool." At the present time there is no direct evidence in humans that heritable diseases are being caused by chemical mutagens, and there is no evidence of deterioration of the gene pool. This sentence has been deleted.

Several commenters objected to the statement, "Mutations are largely recognized as being deleterious," and pointed out that many mutations are silent or have no effect. In the current proposed Guidelines, this sentence has been changed to read, "It is generally recognized that most mutations that are phenotypically expressed are in some ways deleterious to the organism carrying them."

One commenter requested an explanation of how mutagenicity guidelines would be administered and requested a statement indicating requirements for genetic toxicology testing in premarket manufacturing notices. The Agency believes that the language in the current proposed Guidelines clearly states that they will be used to assess risks associated with human exposure to chemical mutagens. Requirements for genetic toxicology testing are the responsibility of the appropriate Agency office.

B. Concepts Relating to Heritable Genetic Risk

One commenter objected to the definition of a mutagen because it was not limited to stable and heritable alternations in the DNA. The Agency agrees that the ultimate end point of concern for the purpose of the current proposed Guidelines is heritable and stable mutation. For gene mutations, heritability is an obvious and necessary component, since all tests used to detect gene mutations actually detect mutant cells or organisms that are descendants of the treated cells. The same is not always true for certain cytogenetic end points, such as chromatid breaks, etc., which may be detected in the same cell generation in which they occur. Since these latter end points provide information relevant to heritable mutation, they will be considered in any mutagenicity assessment. As a result, the Agency feels that the general definition of a mutagen as used in these Guidelines is appropriate.

C. Testing Systems

One commenter felt that most cytogenetic end points that are routinely evaluated (e.g., chromosome breaks, micronuclei) are not transmitted, and therefore, are not germane to the issue of heritable mutation. The Agency disagrees. Although it is clear that cells that carry such aberrations generally do not reproduce, other related aberrations (i.e., balanced translocations, inversions, small duplications, and deficiencies) are compatible with cell survival in germ cells and can be transmitted. Additionally, there is no evidence indicating that the non-transmissible aberrations occur by

mechanisms different from transmissible aberrations.

Several commenters requested that the Agency establish minimal criteria by which assays are to be judged for use in risk assessment determinations. The Agency believes that to list a specific set of criteria that must be met for each assay before the Agency evaluates data would be overly restrictive and inappropriate. Data generated in any system that measures or correlates with a true genetic end point may provide some useful information. The Agency believes that the general protocols and criteria for data evaluation established by the expert committees of the Phase-I Gene-Tox Program as well as other sources provide sufficient guidance for those planning to conduct mutagenicity tests.

D. Weight-of-Evidence Approach

Several commentes suggested that the weight-of-evidence section required clarification of the phrase, "positive response in any two different point mutation test systems," because this phrase may be subject to various interpretations. The Agency agrees that the section as proposed may have been subject to misinterpretation. Therefore, the current proposed Guidelines define sufficient evidence of potential human mutagenicity to include positive responses in any two different gene mutation test systems (one of which utilized mammalian cells) or positive responses in two different somatic cytogenetic tests (one of which utilizes mammalian cells), coupled with sufficient evidence of germ-cell interaction in both caes. Alternatively, the combination of a positive finding in one mammalian gene mutation assay and one mammalian cytogenetics test and sufficient evidence of germ-cell interaction also provides sufficient evidence of potential human mutagenicity. The demostration of heritable effects induced in mammalian germ cells is by itself sufficient evidence for mutagenicity.

Many commenters objected to the criterion that considers a chemical mutagen a potential human germ-cell mutagen if there is "evidence for the presence of the test substance and/or its matabolites in mammalian gonadal organs." First, they pointed out that the presence of a chemical in the testis or ovary does not necessarily mean it has reacted with germ-cell DNA. Such studies are generally performed with radiolabeled chemicals, and it is possible that metabolism of the compound could result in incorporation of the radiolabel into normal cellular macromolecules. The Agency recognizes the shortcomings in the various criteria used to determine whether a mutagen interacts with germ-cell DNA. As a result, in the current Guidelines, two categories of such evidence have been adopted. Sufficient evidence that a mutagen interacts in the mammalian gonad will be the demonstration that an agent interacts with germ-cell DNA or other chromatin constituents, or that it induces such end points as unscheduled DNA synthesis, sister chromatid exchange (SCE), or chromosomal aberrations in germinal cells. Suggestive evidence will include advese gonadal effects following acute, subchronic, or chronic toxicity testing or adverse reproductive effects, such as decreased fertilization index, reduced sperm count, or abnormal sperm morphology.

One commenter suggested that the Agency develop a scale of weighting tests which would place more emphasis on test systems more relevant to human beings. The Agency has explored the possibility of developing such a scale and has concluded that the assignment of fixed values for each test system could be overly simplistic and might not allow for the consideration of such variables as dose range, route of exposure, and magnitude of respone. The Agency believes that the scheme in the current proposed Guidelines, which generally gives greater weight to mammalian rather than submammalian assays and to germ cell rather than somatic cell data, is currently the most appropriate way to evaluate the information from a variety of systems.

E. Quantitative Assessment of Results

Several commenters expressed the opinion that it is not possible to quantitatively express the risk of genetic disease from exposure to a chemical, and therefore no attempt should be made to do so. The Agency does not suggest that it is necessarily possible to generate a numerical estimate of the genetic risk that will result from exposure to any particular chemical. It is well-recognized and documented that the mutational component of certain categories of human genetic disease is not known. However, mutagenicity data have been used to generate semi-quantitative estimates of the impact of ionizing radiation on genetic disease(*5, 6*). The current proposed Guidelines state the Agency's commitment to utilize existing relevant mutagenicity data to give some estimate of potential human mutagenicity. All such estimates will include a careful delineation of the assumptions and uncertainties associated with the assessment.

Many commenters objected to the use of "linear or nonthreshold models" for low-dose extrapolation on point mutation rates. The Agency acknowledges that linearity and the presence or absence of a threshold are separate issues. The Agency will strive to use the most appropriate extrapolation model for risk analysis and will be guided by the available data in this selection. However, it is anticipated that for whole-animal germ-cell assays, few dose points will be available to define a dose-response function. In these situations there is a theoretical basis for a linear, nonthreshold extrapolation provided that no major germ-cell killing (and thus possible cell selection) has occurred(*2, 7*).

One commenter suggested that for quantitative risk it is more appropriate to rely on tests for structural chromosomal aberrations than on gene mutations, particularly since many diseases can be more readily associated with an identifiable chromosome abnormality. The Agency agrees that associations between diseases and specific chromosomal changes can be estimated. This concept is well documented and has been discussed at length in the NAS report(*2*). However, similar estimates can be made for gene mutations, and such techniques have been used for some time for effects of ionizing radiation(*5, 6*). Because the spectrum of mutational effects induced by different chemicals is known to be variable, the Agency believes that it is necessary to perform estimates on all end points.

One commenter objected to the omission of the dominant skeletal and cataract mutation systems for quantitative risk assessment. The Agency recognizes that these dominant mutation systems do have relevance in the preparation of quantitative risk assessment along with specific-locus test systems. The current proposed Guidelines have been modified to include both types of tests.

III. Proposed Guidelines

A. 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 existing industrial chemicals and to pesticides. 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 with respect to transmitted genetic changes, and, if so, how great an impact it 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 chemnicals. 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(*8*). 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 quantiative 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 goses 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(*9*).

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 esimated increase of generic 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 mutagenci effects. The necessity for a consistent approach to the evaulation 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 Agency-wide approach for performing risk assessments is desirable.

The mutagencity risk assessments prepared pursuant to these Guidelines will be utilized within 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 is concerned with the risk associated with both germ-cell mutations and somatic cell mutations. Mutations carried in germ cells are 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 only concerned 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 proposed Guidelines for Carcinogen Risk Assessment(*10*).

As a result of the progress in the control of infectious diseases, increases in average human life span, 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 states, such as abnormal composition, arrangement, or dosage of genes and chromosomes(*2, 6, 11*). Such genetic diseases 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; they 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 conditions are Down's and Klinefelter's 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(*12*).

Estimation of the fraction of human genetic disease that results from new mutation is difficult, although in certain specific cases insights are available(*13*). It is clear that recurring mutation is important in determining the incidence of certain genetic conditions, such as some chromosomal aberration syndromes (e.g., Down's) and rare dominant and X-linked recessive diseases (e.g., achondroplasia and hemophilia A). For other single-factor conditions (e.g., sickle-cell anemia and color blindness) and certain multifactorial conditions (e.g., pyloric stenosis), the contribution of new mutations to disease frequency is probably very small. However, it is generally recognized that most mutations that are phenotypically expressed are in some ways deleterious to the organism receiving them. 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 above the spontaneous level could lead to an accumulation of deleterious mutations in the human population and, to a varying extent, an increased frequency of expressed genetic disease.

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. As our knowledge of genetics and disease etiology increases, and techniques for detecting mutations in human beings improve, we may become aware of chemically-induced human genetic effects. The extent to which exposure to natural and synthetic environmental agents may have increased the amount of genetic damage 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. Concepts Relating to Heritable Mutagenic Risk

For the purposes of these Guidelines, a mutagen is considered a chemical substance or mixture of substances that can induce alterations in the DNA of either somatic or germinal cells. The mutagenicity of physical agents (e.g., radiations) is not addressed here. There are several mutagenic end points 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, inversions, and translocations, whereas numerical aberrations are gains or losses of whole chromosomes (e.g., trisomy, monosomy) or sets of chromosomes (haploidy, polyploidy).

It is conceivable that only one or a few molecules of an active compound may be sufficient to cause certain types of heritable changes in DNA. Mutagenic effects may also come about through mechanisms other than chemical alterations of DNA. Among these are interference with normal DNA synthesis, or induction of DNA misrepair, DNA methylation, abnormal nuclear division processes, or lesions in non-DNA targets (e.g., protamine, tubulin).

The best evidence that an agent induces heritable mutations in human beings would be epidemiologic data indicating a strong association between chemical exposure and a heritable response. Such data do not exist at this time 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 complicates such studies. In addition, only 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 mutations (which appear to occur more frequently than dominants) or by interaction of multiple factors may go unrecognized for many generations. Therefore, in the absence of human germ-cell data, it is appropriate to rely on data from experimental animal systems.

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. There also exists evidence 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(*14*), sister chromatid exchange(*14*), and, quite probably, point mutations in human lymphocytes exposed *in vivo*(*15*). 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 the 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 data base; and a basis for characterizing genetic events(*10*).

2. Test Systems

Many test systems are currently available that can contribute information about the mutagenic potential of a test compound with respect to various genetic end points. 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(*16, 17*).

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 (e.g., the mouse specific-locus test). Positive results in a mouse germinal gene-mutation test argue strongly that a chemical is a potential human mutagen because such tests demonstrate that the mutations occur in mammalian germinal cells and are transmitted to the next generation. However, because large numbers of offspring must usually be generated, it is not expected that many chemicals will 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). Nondisjunction and chromosome lagging are recognized sources of numerical aberrations. Aneuploidy can also arise from chromosome breakage and reunion followed by segregation(*15*). The mechanmisms by which nondisjunction occurs are not well understood. However, proteins (e.g., spindle apparatus), rather than DNA, may be

the target molecules for at least some mechanisms of induced nondisjunction.

Other end points 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 end points often correlates positively with the potential of a chemical to induce mutations.

In general, for all three end points (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(*19*); 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 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 other studies for the purpose of 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.

B. 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 its ability to produce these events in the mammalian gonad. All relevant information is then integrated into a weight-of-evidence scheme which 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 reach and 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.

1. Mutagenic Activity

In evaluating chemicals for mutagenic activity, a number of factors will be considered: (1) Genetic end points (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 end point, (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.

The array of mutagenicity tests available will be reviewed within the following qualitative perspective: greater weight will be attributed to tests conducted in germ cells than in somatic cells, to studies in mammalian cells than in submammalian cells, and to studies in eukaryotic cells than in prokaryotic cells.

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 since 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.

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 end points as unscheduled DNA synthesis, sister-chromatid exchange, or chromosomal aberrations in germinal cells. Positive results in a mammalian germ-cell mutation study also demonstrate the action of the chemical in the gonadal target cells.

b. *Suggestive evidence* will include the finding of adverse gonadal effects following acute, subchronic, or chronic toxicity testing, or findings of adverse reproductive effects, which are consistent with interaction with germ cells.

3. Weight-of-Evidence Determination

The evidence for a chemical's ability to produce mutations and to interact with the germinal target are integrated into a weight-of-evidence judgment that the agent may pose a hazard as a potential human germ-cell mutagen. 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.

Information available will vary greatly from chemical to chemical because there are many mutagenicity test systems, and there has been no systematic attempt to develop information on all chemicals of concern. The responses noted for different tests may also vary from chemical to chemical since often one does not find consistent positive or negative results across all tests. Chemicals may show positive effects for some end points 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 (Table 1). It is not possible to illustrate all potential combinations of evidence, and considerable judgment must be exercised in reaching conclusions. The factors illustrated in Table 1 and discussed previously in sections 1, 2, and 3 must all be considered in making an assessment of mutagenicity. In addition, certain responses in tests that do not measure well-defined mutagenic end points (e.g., SCE induction in mammalian germ cells) or germ-cell tests in higher eukaryotes (e.g., Drosophila tests) may provide a basis for raising the weight of evidence from one category to another.

Sufficient evidence for potential human germ-cell mutagenicity would include cases in which positive responses are demonstrated in a mammalian germ-cell test. Also, in general, sufficient evidence exists when there is confirmed mutagenic activity in other test systems (positive responses in at least two different test systems, at least one of which is in mammalian cells), and there is sufficient evidence for germ-cell interaction as defined above.

Suggestive evidence encompasses a weight-of-evidence category between sufficient and limited that includes cases in which there is some evidence for mutagenic activity and for interaction with germ cells.

Limited evidence for potential human germ-cell mutagenicity exists when evidence is available only for mutagenicity tests (other than mammalian germ cells) or only for chemical interactions in the gonad.

Table 1.—Classification of Weight of Evidence for Potential Human Germ-Cell Mutagenicity [a]

1. *Sufficient* evidence exists when positive responses are demonstrated in:
   a. at least one *in vivo* mammalian germ-cell mutation test, or
   b. at least two point mutation tests (at least one in mammalian cells) plus sufficient evidence that the chemical interacts with mammalian germ cells, or
   c. least two structural chromosome aberration tests (at least one in mammalian cells) plus sufficient evidence that the chemical interacts with mammalian germ cells, or
   d. one gene mutation assay in mammalian cells and one structural chromosome aberration test in mammalian cells and sufficient evidence for chemical interaction with mammalian germ cells.

2. *Suggestive* evidence exists in those cases in which there are positive data for both mutagenic activity and evidence for chemical interactions in the gonad, but the evidence is less than sufficient. This category is potentially large and heterogeneous in nature and ranges from almost sufficient to essentially limited.

3. *Limited* evidence denotes a situation in which the evidence is limited to information on mutagenic activity or to evidence of chemical reactivity in the target.

[a] Takes into consideration the extent, quality, and consistency of responses bearing on an agent's ability to product mutagenic events and to interact with the mammalian gonadal target. Nonmutagenic test responses (e.g., SCE in germ cells) may help to elevate evidence of mutagenicity from one category to another.

Designation of evidence as limited does not preclude the use of such information to set priorities for further testing or to support a case for potential carcinogenicity.

Although definitive proof of nonmutagenicity is not possible, it seems appropriate that a chemical could be classified operationally as not a human germ-cell mutagen, if it gives negative responses in those test systems that together fulfill the criteria (i.e., all relevant end points) for sufficient evidence of a potential human germ-cell mutagen, providing that all assays have been properly performed. Test systems used to define a negative should be capable of detecting weak responses (adequate statistical power) and should be appropriate for the chemical or class of chemicals under investigation.

Negative evidence of chemical interaction in the gonad in the presence of evidence of mutagenic activity may still signal some concern in regard to somatic effects(*10*). Other combinations of relevant information will most likely require case-by-case evaluation. It may also be possible to operationally define a chemical as not being a human germ-cell mutagen based on negative results from other assays which provide information about mutagenicity and/or interaction with germ-cell chromatin.

C. 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 on mainly qualitative criteria. For quantitative risk assessment, further information is required, namely, determination of the heritable effect per unit of exposure (dose-response) and the relationship between mutation rate and disease incidence. Dose-response information is combined with anticipated levels and patterns of human exposure in order to derive a quantitative assessment (risk characterization).

1. Dose-Response

Two approaches to obtaining dose-response data are available. One approach requires experimental data on germinal mutations induced in intact mammals. Several test systems may provide such information, e.g., the mouse heritable translocation, dominant skeletal, dominant cataract, and specific-locus tests. Although the dominant skeletal and cataract assays have the advantage of measuring dominant mutations, the heritability of observed effects has not been clearly demonstrated. 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.

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. In these situations certain theoretical considerations will apply(*20*). For point mutations, linear extrapolations with no threshold may be used as a conservative approximation, provided the results allow one to rule out major germ-cell selection. For structural chromosome rearrangements such as heritable translocations, linear extrapolation of the experimental data is thought to overestimate the risks at low levels of exposure and use of a multiple-hit model is more appropriate.

The second experimental approach for quantitative assessment of genetic risk uses molecular dosimetry data from intact mammals in conjunction with mutagenicity and dosimetry data from other validated test systems(*21*). 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.

Using 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. This approach is still experimental and its application involves many unknowns, such as possible differences between mammalian germ cells and cells of the reference system with regard to types of genetic damage induced and magnitude of repair.

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.

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[9].

3. Risk Characterization

In performing mutagenicity risk assessments, it is important to consider each genetic end point 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 for individual chemicals and between doses for a given chemical. Furthermore, transmissible chromosomal aberrations appear to be inducible with higher frequencies in meiotic and postmeiotic germ-cell stages, which have a brief life span, 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 end points.

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 per lifetime, or the fractional increase in the assumed background spontaneous mutation rate of humans[5]. Examples of quantitative risk estimates have been published [6, 22]; these examples may be of use in performing quantitative risk assessments for mutagens.

IV. References

[1] U.S. Environmental Protection Agency. 1980. Mutagenicity risk assessment: proposed guidelines. Federal Register 45 (221): 74984–74988.

[2] Committee on Chemical Environmental Mutagens. 1982. Identifying and estimating the genetic impact of chemical environmental mutagens. Washington, DC: National Academy Press.

[3] Committee 1 Final Report. 1983. Screening strategy for chemicals that are potential germ-cell mutagens in mammals Mutat. Res. 114: 117–177.

[4] 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.

[5] Committee on the Biological Effects of Ionizing Radiation. 1980. The effects on populations of exposure to low levels of ionizing radiation. National Academy of Sciences. Washington, DC: National Academy Press.

[6] United Nations Scientific Committee on the Effects of Atomic Radiation. 1977. Sources and effects of ionizing radiation Report of the General Assembly, 32nd Session. Supplement No. 40(A/32/40). United Nations, New York.

[7] Ehling, U.H., D. Averback, P.A. Cerutti, J. Friedman, H. Greim, A.C. Kolbye, and M.L. Mendelsohn. 1983. Review of the evidence for the presence or absence of thresholds in the induction of genetic effects by genotoxic chemicals. Mutat. Res. 123:281–341.

[8] Committee on the Institutional Means for the Assessment of Risks to Public Health 1983. Risk assessment in the Federal government: managing the process. Commission on Life Sciences, National Research Council. Washington, DC. National Academy Press.

[9] U.S. Environmental Protection Agency. 1984. Proposed guidelines for exposure assessment. Office of Health and Environmental Assessment.

[10] U.S. Environmental Protection Agency. 1984. Proposed guidelines for carcinogen risk assessment. Office of Health and Environmental Assessment.

[11] Flamm, W.G. 1977. DHEW Report on approaches to determining the mutagenic properties of chemicals: risk to future generations. J. Environ. Pathol. Toxicol. 1:301–352.

[12] McKusick, V.A. 1978. Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive and x-linked phenotypes. Baltimore, MD: Johns Hopkins University Press.

[13] Crow, J.F., and C. Denniston. 1981. The mutation component of genetic damage. Science 212:888–893.

[14] Musilova, J., K. Michalova, and J. Urban. 1979. Sister chromatid exchanges and chromosomal breakage in patient treated with cytostatics. Mutat. Res. 67:289–294.

[15] Strauss, G.H., and R.J. Albertini. 1979. Enumeration of 6-thioguanine-resistant peripheral blood lymphocytes in man as a potential test for somatic cell mutations arising *in vivo*. Mutat. Res. 61:353–379.

[16] U.S. Environmental Protection Agency. 1983. Health effects test guidelines. Office of Toxic Substances. EPA 560/6/82–001. Available from: NTIS, Springfield, VA.

[17] U.S. Environmental Protection Agency. November 24, 1982. Pesticides registration; proposed data requirements. Federal Register 47: 53192–53203.

[18] Parker, D.R., and J.H. Williamson. 1974. Some radiation effects on segregation in Drosophila. Genetics 78:163–171.

[19] Russell, L.B., C.S. Aaron, F. de Serres, W.M. Generoso, K.L. Kannan, M. Shelby, J. Springer, and P. Voytek. Evaluation of existing mutagenicity bioassays for purposes of genetic risk assessment. Mutat. Res. in press.

[20] Ehrenberg L., E. Moustacchi, and S. Osterman-Golkar. 1983. Dosimetry of genotoxic agents and dose-response relationships of their effects. Mutat. Res 123:121–179.

[21] Lee, W.R. 1979. Dosimetry of chemical mutagens in eukaryote germcells. In: A. Hollaender and F.J. de Serres, eds. Chemical mutagens: principles and methods for their detection, Vol. 5. New York: Plenum Press, pp. 177–202.

[22] Ehling, U.H., and A. Neuhauser. 1979. Procarbazine-induced specific-locus mutations in male mice. Mutat. Res. 59:245–258.

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