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Project Summary

Workshop Proceedings: Approaches for Improving the Assessment of Human Genetic Risk—Human Biomonitoring

A workshop entitled *Approaches for Improving the Assessment of Human Genetic Risk: Human Biomonitoring* was held in December 1982 to identify the types of experimental approaches required to eliminate some of the assumptions and uncertainties of mutagenicity risk assessment. The approaches identified for using biomonitoring data as a basis for building bridges between experimental mammals and humans are discussed in the full workshop proceedings in order to provide direction for the future research required to improve the scientific basis for mutagenicity risk assessment. Emphasis was placed on practical ways to obtain useful data for estimating mutation induction. The workshop participants analyzed available techniques, their applicability, their limitations, and possible methods for their improvement. Discussions were limited to approaches to identify mutations. The impact of increases in mutation frequency on the incidence of human genetic disease was not considered to be within the scope of the workshop.

This Project Summary was developed by EPA's Office of Health and Environmental Assessment, Washington, DC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

A considerable genetic disease burden has been recognized in the human population, and it is estimated that perhaps 10% of all human disease has a significant genetic component. Humans are exposed to a large and increasing number of chemical substances, some of which have mutagenic effects in other organisms and may pose a genetic risk to people. Because of the adverse consequences of mutation induction including genetic diseases and perhaps cancer, much effort has gone into designing methods for detecting mutagenic agents. Recently, combinations of tests that are quite effective at identifying mutagenic chemicals have been developed. However, these tests are not useful in monitoring humans for heritable mutations, and, thus, the magnitude of the contribution that chemical mutagens may make to human genetic disease is highly debated.

Concerns about the ability of man-made chemical substances to alter the environment led to the passage of federal laws to protect against such effects. All of these laws require a consideration of adverse health effects in arriving at regulatory decisions. Some, such as the Toxic Substances Control Act, require that specific effects of chemical substances, including mutagenicity, be considered in light of the benefits provided by those chemicals in order to ensure that human exposure does not result in an unreasonable risk. This means that the extent of the potential risk must be quantified before decisions are made.

The task of quantifying potential mutagenic risks associated with exposure to chemical mutagens is complex, and many assumptions must be made. Extrapolations must be made between species if

animal data are used to estimate human genetic risk or between tissues in order to use data from somatic cell biomonitoring to estimate heritable genetic risk. In addition, uncertainties about the exposures that humans receive and extrapolations from effects at high experimental levels to the types of effects expected from much lower environmental levels further compound the problems. Thus, quantitative mutagenicity risk assessments are not scientifically rigorous, because the data base needed to support the extrapolations is not adequate.

The workshop to which the full report is addressed was convened to discuss both direct and indirect methods for biomonitoring exposed populations, the biomonitoring assays available for mammalian experimentation, the identification of human populations exposed to chemical mutagens, and approaches toward improving mutagenicity risk assessment.

Methods for Biomonitoring Exposed Populations

Methods for monitoring human populations to assess genetic risk may be classified as direct or indirect, depending on whether extrapolations are necessary for estimating an effect in humans.

The direct method involves the search for mutational effects in human populations exposed to a potential mutagen. It can be used when there is a large population of children of exposed persons available for study. The observations that might be made on children are of three types: morphological; cytological; and biochemical. Morphological observations include the frequency of dominant mutations, congenital defects and stillbirths, altered physical growth and development, and reduced survival. The cytological data include scoring for an array of chromosomal abnormalities. The biochemical approach involves a search for mutant proteins not present in either parent. Because most genetic diseases involve protein alterations, the biochemical approach yields less ambiguous results than the morphological and cytological approaches. However, this method requires a higher level of technology.

Until recently, the biochemical approach in humans and experimental animals has employed one-dimensional electrophoresis and quantitative enzyme level determinations. The development of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), however, has permitted the separation of proteins on the basis of both charge and size on a slab gel; as many as 1000 different polypeptides contained in a single cell-type can now be detected. Although not all of these can be scored unequivocally for genetic variation, at least 200 polypeptides potentially suitable for monitoring can be identified from the components of a venous blood sample, and computer algorithms for both the enhancement of these images and their scoring are under development.

These developments may dramatically improve the monitoring of human populations for genetic damage. There are limitations, however. The population size required for an adequate test of an altered mutation rate is massive in cases of low-level exposures. Because of this requirement and the fact that most exposures to mutagens will involve low dosages, this method is expensive and will be of limited value scientifically in routine biomonitoring of small groups. However, with appropriate exposures, it may be useful in a coordinated effort pooling offspring from several high-risk groups.

Despite the technical difficulty, studies of human germinal mutation rates are essential in order to understand the increases in transmitted genetic damage following exposure to mutagens and as part of the basis for extrapolating from animal studies to humans. Thus, it is essential that at least one comprehensive study of a sufficiently large or appropriately pooled "worst case" population be conducted in conjunction with observation on a variety of "presumptive" indicators of mutation.

In an indirect approach, tissue samples from exposed persons are analyzed for genotoxic damage, or body fluids are tested for the presence of mutagens. Extrapolations from tissue to tissue are then made in order to predict the risk of genetic disease in future generations. Available methods involve the study of genetic endpoints, including mutations and chromosomal aberrations, or determinations of chemical interactions with DNA.

At least six indirect tests can be performed on germinal tissue from human populations. Four of these measure effects on sperm cells, and the remaining approaches utilize other cytological techniques. Most of the tests for endpoints that may have a genetic basis are not yet well characterized genetically. In addition, not all of these tests are applicable to females because of the inability to study ova. This may be a major limitation, since studies restricted to male germ cells may overestimate the true risk for the entire population. Several genotoxic endpoints can be measured in somatic tissue and the information generated from such measurements can be used to indirectly monitor human populations for heritable genetic damage. Some of the tests measure gene mutations or chromosomal aberrations, while the remainder measure other endpoints indicative of genotoxic damage. Most of these tests can be conducted in both humans and experimental animals, providing a means for correlating epidemiological and clinical data with respect to adverse health outcomes. Because these tests monitor *in vivo* events, they offer several advantages: (i) they detect genotoxicity from agents whose *in vivo* effects are dependent upon metabolic or pharmacokinetic factors; (ii) they potentially are able to determine the effects of complex mixtures; and (iii) for humans and animals, they may detect heterogeneity for individual susceptibilities to genotoxicants. However, for purposes of quantifying heritable genetic risks, the various tests using somatic tissues are limited. The most obvious restriction is that they are performed with somatic tissue. Thus tissue to tissue extrapolations must be made in order to make predictions o transmissible genetic risk. Additionally for risk assessment purposes, mutationa rates rather than mutant cells are o interest, but in somatic cell tests, it i mutant frequencies rather than muta tional rates that are quantified. It woul be difficult to quantify the latter, becaus little is known about *in vivo* cell genera tions or cell kinetics. It is also difficu with somatic cell tests to define th genetic basis of the phenotypic change at the somatic cell level. Although th difficulty has been overcome for TG' 1 lymphocyte and mutant HbG tests, sever potentially useful somatic tests have bee abandoned because of the presence "phenocopies."

Several approaches are available f studying the frequency of chromosom aberrations in peripheral blood lymph cytes, bone marrow cells, and germ cell Such cytogenetic studies allow compa sons between effects in somatic cells a effects in germ cells, as well as compa isons between species. Chromosom aberrations provide unequivocal eviden of genetic damage and thus constitute relevant endpoint for reproductive ha ards. Furthermore, many carcinoge have been shown to be clastoge Generally accepted principles for t conduct of tests and the scoring of resu have been developed. Considerable

search has been conducted to assess spontaneous frequencies, the clastogenic effect of physical and chemical agents, and to define the technical variables in the techniques. The limitations of cytogenic analysis are that it is labor intensive and requires a high level of experience for accurate scoring. In addition, the data base on interindividual variation, persistence of lesions, and the sensitivity of peripheral blood lymphocytes to various classes of chemicals is relatively small. Micronucleus tests are also available; the most common procedure involves the scoring of micronuclei in the polychromatic erythrocytes (PCEs) in mammalian bone marrow. However, because of the short lifespan of PCEs (approximately 24 hr) and the requirement for bone marrow samples, this approach is largely restricted to tests that use acute exposure regimes in experimental mammals.

Although it is possible to perform somatic cell biomonitoring tests on several different cell-types, the difficulty of obtaining most tissues necessitates that peripheral blood will be the main source of tissue for human biomonitoring studies. Blood tissue is readily available, and thus somatic cell systems offer the key advantage of permitting the collection of data from small populations of individuals. However, measurements of mutational events in somatic cells also have deficiencies. Among these are the limited data base for chemicals, the insensitivity of some of the endpoints as indicators of genotoxic damage, and the lack of evidence for a correlation between elevated levels of mutations in somatic cells and an increased risk for adverse health effects (including the lack of appropriate bridging models to predict heritable effects).

Various endpoints that potentially indicate mutagenesis can be detected in both somatic and germinal tissue. These include sister chromatid exchanges (SCEs), chemical interactions with DNA, and DNA repair. Of these three approaches, the detection of SCEs is at the most advanced stage of development. Difficulties are encountered in all of these methods, however, because of varying replication rates and repair capabilities in different cell-types and because of the restriction of germ cell measurements to males. Furthermore, positive findings with these tests cannot be equated with an increase in the frequency of mutations. Nonetheless, the measurement of SCEs in peripheral lymphocytes is a relatively easy and sensitive test, and several sensitive techniques are being developed for measuring DNA damage. These approaches may be used to provide information on internal dosages resulting from human exposures to chemical substances, and as such may be employed to provide a common denominator for tying human biomonitoring and animal testing methods together.

Much remains to be learned about specific DNA damage and its implications for mutagenesis and carcinogenesis, and simple models can prove valuable in making the necessary first steps to improve genetic risk assessment. For initial studies with experimental animals, total DNA alkylation may serve as a useful indicator of dose. Eventually, the specific type of alkylation product, rate of specific adduct repair, amount of cell replication, and the probability of mispairing of specific adducts needs to be considered. Although we are presently incapable of accomplishing this goal, the methodology for conducting such studies is developing rapidly.

Biomonitoring Assays Available for Mammalian Experimentation

A number of direct and indirect methods applicable only to animal experimentation are described in this section of the full report. These methods are useful for defining intertissue relationships and for making comparisons with human data in order to strengthen the basis for extrapolating between species.

Whole mouse tests for putative heritable gene mutations are generally considered the most valid experimental approaches for making quantitative mutagenicity risk assessments. Among these are the morphological and biochemical specific locus tests and tests for dominant mutations causing skeletal defects or cataracts. Other available tests score for chromosomal aberrations; these tests include the heritable translocation test, dominant lethal test, and X chromosome loss test. All of these tests, except perhaps the dominant lethal test, which cannot be shown to respond only to mutagenic events, may be used for quantifying genetic risk. An indirect estimation of heritable genetic effects in mice can be performed using the mouse spot test.

Although the close biological and evolutionary relationship that exists between humans and other mammals is the basis for estimating heritable human genetic risk from mouse and rat data, there are several limitations associated with using studies in animals for predicting human responses. One is the difficulty of accounting for differences in metabolism, repair, and cell cycle kinetics. Another is the need to extrapolate from high acute dosages, after involving long sterile periods, to dosage levels that would be more typical of human exposures. Another limitation in essentially all assays for mutagenesis in germ cells is the shortage of information on females; the great majority of the available information comes from males. Consequently, many assumptions must be made in attempting to project human genetic risk. This leads to the inescapable conclusion that there is no substitute for genetic data from humans to calibrate the experimental systems for risk assessment purposes.

Identification of Human Populations Exposed to Chemical Mutagens

Considerable effort will be required to collect human data for assessing genetic risk for just one chemical substance, and all sources of information ought to be drawn upon to select the appropriate human population for study. A number of populations at greatest risk should be identified for potential studies. One is the children of cancer chemotherapy patients. There are other analogous populations as well, and careful thought should be given in the design of a program to identify and select the most appropriate ones. Information from these studies would be useful for defining the extent of genetic hazard and in validating the animal models as predictors of human risk.

Approaches for the Improvement of Mutagenicity Risk Assessment

Many tests are available for identifying chemical mutagens. Data from combinations of tests provide a basis for making qualitative assessments of the ability of chemical substances to cause gene mutations, chromosomal aberrations, and other effects that are indicative of interaction with DNA. However, only a few tests (i.e., heritable gene mutation and heritable translocation tests in mice) can be used by themselves for quantitatively assessing genetic risk, but they are not routine tests, and they cannot be used to estimate human genetic risk directly. By default, genetic risk must be assessed qualitatively for most chemical substances.

For decision-making by the federal government, it is no longer adequate merely to assess genetic risk quantita-

tively; quantitative assessments are needed to balance the risk associated with exposure to a chemical against the benefit of its use. Because our understanding of interorgan relationships is inadequate, human monitoring data cannot be used for such purposes. Many assumptions have to be made before genetic risk can be estimated quantitatively, and the assessments are not scientifically rigorous. Because of practical and legal considerations, it is important to make optimal use of all sources of information in future genetic risk assessment efforts and to develop the science to a point where rigorous assessments can be made.

A. Bridging Human Biomonitoring Endpoints with Animal Experimentation (Defining the Relationships)

Each test system has advantages and limitations for assessing genetic risk to humans. It is only by determining how the endpoints measured in these tests relate to events that occur in humans that full advantage can be taken of each. Comparative experimentation involving different endpoints, test systems, and chemicals is required to build intellectual bridges between the systems.

Studies in mice can be used by themselves to predict mutagenic effects in humans, because the same range of steps between external exposure and production of mutant offspring occurs in all mammals. Measurements of somatic cell and germ cell events should be performed in mice in such a way that the relationships between biomonitoring markers and health outcomes of concern (i.e., genetic disease, cancer, and birth defects) can be determined. Such an approach will provide a basis for using biomonitoring endpoints to predict adverse health effects. It will also enable comparisons to be made between different health outcomes in order to determine their sensitivity of expression and predictability. It is necessary to conduct at least one study in such a manner so that the data generated in an experimental animal study can be compared to the results obtained from similarly exposed humans. Until this is done, the applicability of animal data for human risk assessment will be unknown.

B. Other Types of Testing Needed

Because it is impossible to study genetic damage in more than a very few human populations, it will be necessary to rely heavily on animal experimentation and short-term biomonitoring tests to predict human risk. This requires bridge building between human heritable mutagenicity data, other human biomonitoring data, and animal heritable mutagenicity and biomonitoring data. Although the specific models that can be used to make bridges remain controversial, there is some agreement that ratios (or parallelograms as they are sometimes called) involving dosimetry should be further explored. As such studies are conducted, knowledge of biological processes rather than statistical models can start to drive the risk assessment procedure. Flexibility should be maintained so that the risk assessment process works differently when different amounts of data are available and so that the level of sophistication can be increased as better data become available. In addition, the effects caused by a chemical in one cell-type might not predict its effects in a different cell-type. Thus, it is important to consider cellular specificities of certain chemicals. Attention also should be paid to the homeostatic mechanisms of humans and experimental animals that may be important with respect to disease outcomes, since the stage of the cell cycle, level of differentiation, and location in the body all affect a tissue's response to toxic insults.

Need for Coordination of Efforts

To ensure maximum efficiency in collecting relevant data, it is desirable to search systematically for information on existing efforts related to genetic risk assessment rather than to attempt to set up overlapping independent studies. Although effective collaboration among agencies is still sporadic, there are some promising developments. For example, the coordination between the U.S. Environmental Protection Agency and the National Toxicology Program (NTP) to obtain dosimetric information on chemicals being tested in the mouse specific locus tests should enable existing NTP studies to better be used for genetic risk assessment. It now seems appropriate that an oversight committee be formed for guidance on needs in human biomonitoring and to facilitate the coordination of efforts in genetic risk assessment.

The research effort required to answer major questions in genetic risk assessment must be cumulative rather than episodic, and funding for these efforts should endure over many years. With proper support, an oversight committee could help to ensure that this is accomplished. Investigators studying exposure or genotoxic damage in different tissues and different organisms must somehow integrate and focus their efforts. More effective sharing of valuable materials would certainly be useful in this respect. Within the purview of an oversight committee, a repository for biological materials obtained from animals and humans that have been exposed to putative mutagens should be established.

A series of regularly scheduled workshops should be inaugurated to facilitate collaboration. These workshops should include investigators using the biological materials in the repository and should provide for a cumulative review, comparison of results, and the identification of research needs.

It may be worthwhile to consider the selection of a few key compounds for concentrated, long-term efforts in genetic risk assessment. Since a long-term effort to collect data on chemical mutagenesis in human germ cells is a major undertaking, with important implications for genetic risk assessment, it should not be undertaken lightly. There are other issues that could be considered by an oversight committee.

Efforts should also be coordinated among government agencies, industrial concerns, and the academic community. Some preliminary efforts are underway and it is hoped that these efforts will be expanded.

The final section of the full report contains an extensive reference list documenting scientific opinions expressed by participants in the workshop.

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The complete report, entitled "Workshop Proceedings: Approaches for Improving the Assessment of Human Genetic Risk—Human Biomonitoring," (Order No. PB 85-103 018; Cost: $10.00, subject to change) will be available only from:
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