Introduction and Summary of the 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies

William S. Stokes¹ and Erminio Marafante²

¹Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; ²European Centre for the Validation of Alternative Methods, Environment Institute, Ispra, Italy

A workshop on alternative toxicological testing methodologies was convened by the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) 26–31 January 1997 in Ispra, Italy, at the European Centre for the Validation of Alternative Methods. The purpose of the workshop was to assess the current status of alternative testing methodologies available to evaluate adverse human health and environmental effects of chemicals. Another objective of the workshop was to identify and recommend research needed to fill knowledge gaps that would lead to new test methodologies. Four work groups were established to address conceptual issues, acute toxicity, organ toxicity, and ecotoxicology. A joint workshop report was prepared for each topic and included recommendations for the development and use of alternative methods. Participants concluded that alternative methods and approaches are available that can be incorporated into tiered strategies for toxicological assessments. Use of these methods will reduce the numbers of animals required, and in some instances reduce animal pain and distress. It was recommended that future efforts to develop test methods should emphasize mechanism-based methods that can provide improved predictions of toxicity. Continued international cooperation was encouraged to facilitate future progress in the development of alternative toxicological testing methods. These methods will provide for improvements in human health protection, environmental protection, and animal welfare. — Environ Health Perspect 106(Suppl 2):405–412 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/405-412stokes/abstract.html

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Background

The Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) is an international organization whose objective is to contribute to the reduction and prevention of human health and environmental risks caused by the introduction of natural and man-made chemicals into the environment. To assess the current status of alternative testing methodologies available to evaluate such risks, a SGOMSEC workshop was organized and convened 26–31 January 1997, in Ispra, Italy, at the European Centre for the Validation of Alternative Methods.

Knowledgeable scientists from the international community were invited to participate and prepare comprehensive review papers on specific topics for discussion at the workshop.

The workshop focused on new or revised testing methods for which there are planned, ongoing, or completed validation studies, and those which will likely be considered for regulatory use. An additional objective of the workshop was to identify and recommend research needed to fill knowledge gaps that would lead to new test methodologies.

Workshop participants were assigned to four work groups: conceptual issues, acute toxicity, organ toxicity, and ecotoxicology. Each group prepared a joint report on the status of alternative test methods and issues relevant to their topic, as well as recommendations for their future development and use. The workshop report, published in this Environmental Health Perspectives Supplement, consists of this overview, four joint reports, and background papers prepared by workshop participants.

The purposes of this workshop overview is to provide an introduction about alternative testing methods, and to summarize the conclusions and recommendations of the four joint reports (1–4). Additional details about specific issues and methods can be found in the joint work group reports and the individual review papers.

Introduction

It is estimated that over 80,000 chemicals are currently in use and that an average of 2000 new ones are introduced annually (5). The public and the environment may be exposed to these chemicals during their manufacture, distribution, use, and disposal. Chemicals are present in pharmaceuticals, foods, personal care products, pesticides, industrial solutions, and cleaning agents, and exposure may occur in the home and workplace. Exposure may also occur from chemicals in the environment as pollutants in water, air, or soil. To safeguard human health and the environment, governments adopt toxicological testing methods to evaluate the potential hazardous effects of chemicals or to demonstrate the safety of such chemicals. These test methods generate information used for premarketing evaluation of new products, hazard classification, and risk assessment. Depending upon testing outcomes, industry and regulatory agencies may implement prevention and risk management practices to protect public health and the environment.
The potential adverse effects of chemicals are currently assessed largely by tests involving laboratory animals. However, several factors have influenced the scientific community to develop new alternative test methods. One major factor influencing the development of new test methods is the enormous advances in the understanding of molecular and cellular mechanisms of toxicity. These advances have been matched by technological advances in tissue culture, genetic engineering of cells and whole animals, and high throughput automated testing equipment. Much of this new knowledge and technology is being incorporated into alternative testing methodologies.

Another factor affecting the development of new test methods is the desire for tests that will be more predictive of potential chemical toxicity and thereby support improved risk assessment. There is also great interest on the part of both government and industry to develop tests that are more cost and time efficient. For instance, the current rodent bioassay for assessing carcinogenicity costs $1 million to 3 million and requires at least 3 years to complete. More efficient testing methods may reduce the time required to bring new products to the marketplace and increase the amount of useful information that can be obtained.

New test method development is also being stimulated by public concern about the use of animals for testing. This concern has resulted in legislative mandates in the United States and Europe to develop alternative test methods. In 1986 laws were enacted in the United States that required consideration of alternatives prior to the use of animals in research, testing, or education. A component of the U.S. National Toxicology Program, the National Institute of Environmental Health Sciences, was directed by a 1993 law to develop and validate alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing (6). The institute was also directed to develop criteria and processes for the validation and acceptance of test methods by regulatory agencies. In 1986 the European Union (EU) issued a directive that states that an animal procedure shall not be performed if nonanimal procedures are reasonably and practically available (7). A 1993 EU directive would have prohibited the testing of cosmetics in animals as of January 1998, if scientifically validated nonanimal alternative methods were available (8).

Alternative test methods are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use. These include methods that use nonanimal systems or lower species to partially or fully replace higher animals, methods that reduce the number of animals required, and methods that refine animal use by lessening or eliminating pain or distress and enhancing animal well-being. Virtually every new testing method incorporates some aspect of refinement, reduction, or replacement compared to the corresponding traditional test. Thus in this report, alternative methods and new methods will be used interchangeably.

**Alternative Methodologies and Conceptual Issues**

This work group addressed broad issues associated with the application and use of alternative testing methods for risk assessment (1). New testing models and approaches are available that can provide considerable information about chemical toxicity. Examples presented include genetically engineered animal models, quantitative structure–activity relationship (QSAR) models, and physiologically based biokinetic (PBPK) models. Considerations for integrating new test methods into testing strategies were also discussed.

**Risk Assessment**

The risk assessment process evaluates potential adverse health effects that may arise from exposure to a chemical. This assessment includes a qualitative judgment of the potential for the chemical to produce adverse effects, and a quantitative judgment of the influence of dose on the potential for adverse effects. Alternative test methods may recapitulate the initial toxic event, referred to as the mechanism of toxicity, or they may recapitulate one or more subsequent events leading to injury or disease, referred to as the mode of action. Improved understanding of toxic mechanisms and modes of action will influence the type and amount of data considered most useful for determining potential toxicity. Additional alternative methods likely will be needed to generate this data.

**Mechanistic Approaches**

Mechanistic test methods are based on an understanding of the biologic events responsible for, or associated with, the effect observed. These tests provide information concerning the molecular, cellular, or physiological mechanisms by which substances exert their effects on living cells and organisms. They are contrasted to correlative test methods, which are based on statistical relationships of phenomena that cannot be explained on a mechanistic basis. Correlative tests are unlikely to lead to widespread acceptance, and it was recommended that future efforts should be directed at the development of mechanistic test methods. There was also consensus that development of relevant and reliable mechanistic tests is highly dependent on progress in the fundamental science of toxicology.

**Molecular Biology**

Molecular biology techniques are being used to develop new animal models for toxicity testing. These models include transgenic and "knockout" animals that have genetic material introduced or deleted, respectively, to generate models that have greater sensitivity and specificity for the toxic end point of interest. Currently, product development and public health decisions on carcinogenicity depend on the expensive and time-consuming chronic 2-year rodent bioassay. However, rodent models have been created in which critical genes serve as specific targets for potential chemical carcinogens. Examples include three models being evaluated in an international multilaboratory effort: the p53 tumor-suppressor gene deficient mouse, the Tg.AC mouse that carries an activated ras oncogene, and the ras H12 mouse which carries a human ras oncogene. Ongoing and planned studies on these test models will provide information on their potential usefulness for risk assessment purposes.

**Computer Algorithms**

Computer-based analysis of diverse chemical properties is being used to create algorithms for predicting toxicological effects of structurally related chemicals. An example is the use of QSAR models, which have been extensively developed and used. This model is based on the premise that the biologic properties of a chemical are inherently linked to its molecular structure. QSAR models are currently being incorporated in the initial stages of tiered testing approaches to estimate the likelihood of a toxic effect. Information is used to determine candidates for additional toxicity testing or further product development.

**Exposure–Effect Relationships**

Determination of the absorption, distribution, metabolism, and excretion of chemicals is necessary to fully characterize their potential toxicity. This information can be
used to create PBBK models that predict target organ concentrations and toxicity for specified external doses. In PBBK modeling, physiological and chemical-specific parameters are combined to predict tissue concentrations. The model integrates data from in vivo and in vitro testing methods with data from the target species, such as human blood levels from exposed populations. PBBK modeling is also useful for selecting the most appropriate test models and for estimating chemical doses for in vitro and in vivo testing.

Integration into Testing Strategies
The development of an ever-increasing number of new test methods is likely to continue. The usefulness of these methods must be carefully assessed and the methods integrated into logical, stepwise testing strategies that provide reliable and relevant information. Care must be taken to avoid the adoption of methods that do not provide meaningful information useful for decision-making. An approach for integrating new test methods for carcinogenicity testing is discussed in the joint report by Blaauboer et al. (1).

Validation and Acceptance
Requirements for the use of alternative testing methods are validation and acceptance. Validation is the process of establishing the reliability and the relevance of a test method. Reliability is the reproducibility of results within and between laboratories, and relevance is the extent to which a test is meaningful and useful for a particular purpose. Acceptance occurs when the test method is considered suitable for risk assessment purposes aimed at the protection of human health or the environment. New test methods evolve through a series of steps from development of the method to validation and finally acceptance. Validation of a new test method in accordance with established criteria is a prerequisite for regulatory acceptance (9). Furthermore, separate criteria must be met to achieve regulatory acceptance (9). Specific validation and regulatory acceptance criteria were developed at an Organisation for Economic Cooperation and Development (OECD) international workshop in 1996 (9) and have been described in another recent report on this subject (10).

Validation Criteria
The following criteria (9) should be met before new tests are accepted by regulatory agencies:

- A rationale for the test method should be available, including a clear statement of scientific need and regulatory purpose.
- The relationship of the end point(s) determined by the test method to the in vivo biological effect and to the toxicity of interest must be addressed and limitations of the method must be described.
- A formal detailed protocol must be provided and should be readily available in the public domain. It should be sufficiently detailed to enable replication by other users and should include data analysis and decision criteria. Test methods and results should be available, preferably in a peer-reviewed publication; and test results should have been subjected to independent scientific review.
- Intratess variability, repeatability, and reproducibility of the test method within and among laboratories should have been demonstrated. Data should be provided describing the level of intra- and interlaboratory variability and how these vary with time.
- The performance of the test method must have been demonstrated using a series of reference chemicals, preferably coded to exclude bias.
- The performance of the test method should have been evaluated in relation to existing relevant toxicity data and information from the relevant target species.
- All data supporting the assessment of the validity of the test method, including the full dataset collected in the validation study, must be available for review.
- Normally, these data should have been obtained in accordance with OECD principles of Good Laboratory Practice.

Acceptance Criteria
The following criteria (9) should be met before new tests are accepted by regulatory agencies:

- Application of the method provides data that adequately predict the end point of interest in that the data demonstrate a linkage between either the new test and an existing test method, or the new test and effects in the target species.
- Use of the method generates data for risk assessment purposes that are at least as useful as, and preferably better than, those obtained using existing methods. This will give a comparable or better level of protection for human health or the environment.
- Adequate testing data for chemicals and products representative of the type of chemicals administered by the regulatory program or agency (e.g., pesticides, cosmetics) are provided.
- The test must be robust and transferable and allow for standardization. If highly specialized equipment, materials, or expertise are required, efforts should be sought to facilitate transferability. This is an important criterion to be considered at an early stage of a validation study.
- The test is cost effective and likely to be used.
- Justification (scientific, ethical, economic) should be provided for the new method with respect to any existing methods available. In this respect due consideration should be given to animal welfare, including the 3Rs (refinement, reduction, and replacement of animal use).

Recommendations
The following are recommendations from the SGOMSEC workshop (1) for the future development and use of alternative methods.

- The rate of development of alternative tests for use in toxicological assessments should be increased.
- Increased emphasis should be placed on the development of mechanism-based methods for specific aspects of toxicity.
- Investment in the development of fundamental research that underpins toxicology and toxicity testing should be increased.
- Training institutions, granting bodies, and regulatory agencies should be encouraged to support research and training that will enhance the development and use of alternative systems.
- Accepted alternative methods should be integrated into toxicity assessment of chemicals.
- International cooperation in development, validation, and acceptance of alternative methods should be encouraged.
- In the interest of the most effective development and use of alternative methods, international harmonization of chemical toxicity classification schemes should be encouraged.

Alternative Testing Methodologies for Acute Toxicity
Acute toxicity testing determines the potential for injury or death from intentional or
accidental single or short-term chemical exposure. This information is needed to properly classify and label potential hazards. Acute toxicity testing data are also used to determine the appropriate protective equipment or procedures needed to prevent human exposure and subsequent injury or death. Three important end points for acute toxicity were addressed at the workshop (2): ocular toxicity, dermal toxicity, and acute systemic toxicity. In addition, the validation process and algorithms for interpretation of data from in vitro methods were addressed.

General Testing Strategy
The incorporation of alternative methods into tiered testing approaches has resulted in the reduction, and to some extent, refinement of animal use. This tiered approach involves a decision after each level of testing as to whether there is sufficient information for hazard classification, or whether additional testing is necessary. The process begins with information about a chemical's properties, a review of the scientific literature, and QSAR assessment. Subsequent stages of testing may involve in vitro tests, laboratory animals, or humans. Human volunteers are used only when ethically feasible and after approval by an ethical review committee. The extent of testing at each stage and the decisions reached will depend on the test outcome and the usefulness of the test for predicting toxicity or lack of toxicity. There are currently no scientifically adequate methods that can totally replace animals for acute toxicity testing. However, alternative methods are available that can and should be incorporated into tiered testing strategies to reduce the number of animals needed and to reduce the severity of potential pain and distress.

Ocular Toxicity
Determining whether a chemical will cause reversible or irreversible damage to the human eye has traditionally been accomplished by using laboratory animals. The test method assesses damage to three tissues that differ in structure and function: the cornea, the conjunctiva, and the iris. The standard test method has been revised to reduce the number of animals required to as few as one. The test protocol has also been refined to allow for termination of the test whenever severe pain or distress occurs. In vitro tests have been developed that model one or two, but not all three tissues. Test methods that use reconstructed tissue equivalents or isolated eye tissues are available that can and should be used in tiered testing approaches. Further progress in ocular toxicity test methods will require additional research to better understand the mechanisms involved in ocular injury and recovery, and to identify end points predictive of permanent injury.

Dermal Toxicity
Dermal toxicity can occur as dermal corrosion, dermal irritation, phototoxicity, or allergic contact sensitization. Traditional test methods for these end points use laboratory animals. However, tiered testing approaches can reduce, and in some instances eliminate, the need for the animal test. For some products, decisions can be made to proceed to human testing without prior animal testing, but only after approval by an ethical review committee. In vitro methods currently used or under evaluation in tiered testing approaches include cell cultures, reconstructed tissue equivalent cultures, and skin explants. In some countries, in vitro methods have been approved to assess the dermal corrosion potential for some chemicals for transportation purposes. In the area of allergic contact dermatitis, advances in the understanding of cellular and molecular mechanisms are expected to lead to the development of useful in vitro test methods.

Acute Systemic Toxicity
Acute systemic toxicity testing is conducted to assess the potential for a chemical to cause systemic toxicity or death following an acute oral, dermal, or inhalation exposure. This testing currently requires the use of laboratory animals. However, the original LD_{50} test, which used a large number of animals in each dose group, is no longer required or necessary. New methods that provide for the reduction and refinement of laboratory animal use are now approved as international test guidelines and should be used to meet acute toxicity testing requirements. Testing for acute systemic toxicity should be accomplished in a stepwise, tiered testing approach that begins with the collection and integration of information on physical and chemical properties, information from literature reviews, and an analysis of structure-activity relationships. In vitro cytotoxicity determinations can aid in predicting acute toxicity and should be considered in a tiered testing approach.

Validation and Prediction Models
Extensive efforts have been made to validate replacement tests for skin and eye irritation during recent years. Experience from these efforts has led to the recommendation that algorithms, or prediction models, be established as an integral part of the test method to facilitate interpretation of the data. Validation studies may demonstrate that a new test method is useful for determining the toxicity of certain groups or classes of chemicals, but not the entire universe of chemicals. Validation studies may also demonstrate that a new method is useful for detecting toxicity, but not useful for detecting lack of toxicity (high degree of sensitivity, but poor specificity). The converse is also a possible outcome of validation studies (poor sensitivity, but high specificity). As a test method is used and its performance database enlarged, there is the possibility that its usefulness for predicting toxicity or nontoxicity may change.

Recommendations
The following are recommendations from the SGOMSEC workshop (2) relating to the development and use of alternative methods for acute toxicity:

- Continued support of the development and validation of alternative methods is essential and is expected to lead to continued improvement in the safety evaluation of chemicals.
- Research should be directed toward a better understanding of the relevant biology (especially human) of toxic events and mechanisms.
- Better methods are needed to synthesize information from batteries of alternative tests and to improve interpretation of the integrated results.
- Users of alternative methods should adopt the general scheme for toxicity testing (2).
- Validated and accepted alternative methods should be used immediately. Implementation of methods providing equivalent information should be prioritized according to the degree that they use fewer animals or cause less stress.
- Given the expected rapid progress in the development of new alternative methods, it is recommended that scientists, regulators, potential users, and policy makers establish and use continuing education and information distribution programs to stay current with progress.

Alternative Testing Methodologies for Organ Toxicity
The detection of organ toxicity is a critical component of toxicological testing. This
toxicity is usually assessed in subchronic repeat-dose testing using laboratory animals. However, in recent years there has been substantial progress in the development of in vitro test methods that are helpful in assessing organ toxicity. Model systems cover the full range of organizational structure, and include the use of isolated perfused whole organs, tissue slices, isolated suspended cells, primary cells and early subcultures, established cell lines, subcellular fractions, and genetically engineered cell systems. A significant advantage of some of these systems is that they use human tissues, which may provide data of greater relevance for human toxicity. The parallel use of both animal tissues and human tissues can provide comparative data helpful in interpreting the relevance of animal data for human toxicity.

Liver Toxicity
The liver is both a major detoxification organ and a major target organ for toxicity for systemically absorbed chemicals. The liver can metabolically activate chemicals to forms that are hepatotoxic or toxic to other organs and tissues. The liver can also metabolically inactivate chemicals that might otherwise appear toxic to other tissues in in vitro test systems. In vitro liver preparations can be used to obtain information on chemical metabolism, enzyme induction and inhibition, and drug-drug interactions. The most useful in vitro liver preparations are tissue slices and isolated hepatocytes. Species differences are well documented in liver-metabolizing enzymes and this often creates uncertainties in extrapolating animal test data to humans. Human and rodent liver slices can be used to assess the comparative metabolism of chemicals. This information can then be helpful in interpreting species differences in toxicity and in selecting the appropriate animal model. Liver microsome preparations can be used for in vitro systems to simulate hepatic metabolic activation and inactivation of chemicals. For example, microsome preparations have been adapted for use in the Salmonella mutagenicity assay and the Frog Embryo Teratogenesis Assay in Xenopus developmental toxicity assay. Specific human cytochrome P450 enzymes produced in genetically engineered cells are also now available for use in in vitro systems.

Renal Toxicity
The kidney is especially susceptible to injury from bloodborne toxics and their metabolites due to the high rate of renal blood flow and the potential for concentration in the kidney. Enzymes located in renal epithelial cells may bioactivate drugs and other xenobiotics to toxic forms. Several in vitro preparations are available for the study of the mechanistic and comparative aspects of renal toxicity, including isolated perfused kidneys, renal tissue slices, tubular fragments, primary cell cultures, and established renal epithelial cell lines. Toxic mechanisms can be studied by assessing various kidney-specific parameters such as uptake, interaction with cellular target sites, and cellular responses. In vitro nephrotoxicity methods are being improved to generate useful information regarding toxic mechanisms, interspecies differences, and in vitro–in vivo extrapolations.

Neurotoxicity
Neurotoxicity is commonly assessed using a tiered testing approach that requires laboratory animals at all levels. The first tier consists of data collected during subchronic rodent toxicity testing. The second tier consists of detailed behavioral observations, specific motor and sensory function measurements, and neuropathologic examinations. Third-tier studies are aimed at further characterization of neurotoxic effects and identification of possible mechanisms of action. Several in vitro test methods can be used in third-tier testing to investigate neurotoxic mechanisms at the cellular and molecular level. These include organotypic explants, brain slices, reaggregate cultures, primary cell preparations, and established cell lines. Coculture systems also exist that can be used to mimic the blood–brain barrier. These in vitro systems are amenable to biochemical, electrophysiologic, and morphologic examinations. Furthermore, in vitro methods allow for study of alterations in gene expression caused by toxicants. These in vitro approaches can also be useful as screening tools for particular situations or classes of compounds.

Hematotoxicity
Hematotoxicants can act directly on circulating blood cells or their precursor stem cells in the bone marrow, or they can act indirectly by inducing an immune response against these cell types. One example of an in vitro test being used to study potential toxicity to stem cells is the colony-forming unit–granulocyte/macrophage assay. This assay is being evaluated for its usefulness in predicting the level of chemical exposure that induces severe neutropenia.

Immunotoxicity
Immunotoxicity from chemicals may result from heightened immune activity or decreased immune function. Chemical-induced immunosuppression is manifested by the reduced ability to combat infectious agents or tumors and has traditionally been evaluated using a two-tiered in vitro approach. Each tier consists of at least five assays that depend on evaluation of immune cells from chemically exposed mice. A recent study (11) demonstrated that using only three tests from each tier is sufficient to predict immunotoxicity, thus reducing the number of animals required. Immunotoxicity due to heightened immune activity is manifested by either hypersensitivity or autoimmunity. Evaluation of chemicals for hypersensitivity currently requires animal testing, with the guinea pig as the traditional model. Improved understanding of the mechanisms of hypersensitivity has resulted in the development and validation of the local lymph node assay in mice. This alternative assay requires less time, expense, and animal distress than the traditional test.

Reproductive and Developmental Toxicity
The potential reproductive toxicity of chemicals is evaluated in multigenerational studies using laboratory animals. The lack of validated nonanimal alternatives is attributable to the complexity of reproduction. Reproductive toxicity must evaluate effects on male and female fertility that might occur from chemical exposure to the fetus and during the entire postnatal period through adulthood. In vitro fertilization techniques can be used to assess viability of sperm and oocytes from exposed animals. The potential of a chemical to cause developmental toxicity is evaluated in pregnant laboratory animals. Numerous in vitro culture systems have been evaluated as screening tests for developmental toxicity. These include established cell lines, primary cell cultures, nonmammalian embryos, and mammalian embryos or primordia.

Endocrine Disrupters
Environmental endocrine disrupters have been defined as exogenous agents that interfere with the normal physiological effects of endogenous hormones. Endocrine disrupters exert their effects by mimicking the endogenous hormone or by interfering with its production, release, transport, metabolism, binding, action, or elimination. Multiple organs and cells at numerous sites may be affected. Therefore a battery of in
vivo and in vitro assays may be needed to adequately assess the endocrine-disrupting potential of chemicals. A number of in vivo and in vitro assays have been proposed, including those that evaluate receptor binding and alterations in gene expression. The effects on gene expression can be accurately measured using transcriptional activation assays in mammalian and yeast cells. Competitive binding assays have been developed for several nuclear receptors and binding globulins.

Recommendations

The following are recommendations from the SGOMSEC workshop (3) for the development and use of alternative methods in organ toxicity:

- The infrastructure for alternative toxicity testing should be improved through the following actions: a) establish mechanisms to increase the availability, distribution, and use of human tissues and cells for alternative toxicity testing; b) develop and make available relevant reference compounds for toxicity assessment in each organ system; c) organize and make available via the internet complete in vivo toxicity data, including human data, that contain dose, end points, and toxicokinetics whenever available; and d) establish mechanisms to assure that biological material, reference chemicals, and data are subject to international standards for quality control and assurance.

- Biological end points for target organ toxicity that can be reliably used in developing alternative strategies should be identified and validated.

- Alternative methodologies to predict target organ toxicity should be developed.

- Procedures to assure early standardization of alternative tests should be developed.

- The number of in vitro tests used for target organ toxicity should be minimized by using the most predictive ones.

- Alternative methodologies to detect toxicity due to multiple organ interactions should be developed.

- Structure–activity relationships and computational models should be given a high priority for development and validation as alternative approaches to reduce animal testing.

- Coculture models involving bioactivating cells, tissues, and subcellular fractions with other target cells in static and perfused systems should be developed, standardized, and validated.

- Development of the battery of transgenic cells that express the range of human cytochrome P450s should be completed and used as an alternative to animal-derived S9 microsomes in assessments to predict human risk.

- The origin and suitability of cultured cell lines to assess particular toxicants should be carefully evaluated. For example, tumor-derived cell lines should only be used in alternative methods if they are derived from tumors that are not resistant to cytotoxic anticancer drugs.

Observation of Community Structure

Methods are available for directly assessing disturbances at the community level. One example is the River Invertebrate Prediction and Classification System, which is used to assess the biological quality of rivers. This system generates site-specific predictions of the invertebrate fauna of a healthy community. This prediction is then compared with the observed fauna of the exposed community. A second example is the Invertebrate Community Index, which is used to assess invertebrate community structure and function. Another example is the Index of Biotic Integrity, which measures the effects of contaminants on aquatic communities. This index derives a quantitative index of aquatic community health from water characteristics and fish populations.

Alternative Systems

Model systems and approaches used for ecotoxicology include cultured cells, transfected cell lines, microorganisms, fish embryos, insects, QSAR, and nondestructive sampling from vertebrate species. Fish hepatocyte cultures have been employed to measure toxicity as evidenced by increases in stress proteins, cytochrome P4501A1, DNA adducts, and vitellogenin levels. A luminescent bacterium, Vibrio fischeri, that responds to toxicants with a change in light emission is being used as an environmental toxicity screen. A 48-hr zebrafish embryo assay has been used to detect developmental toxicity to aquatic species. Insects have numerous advantages for toxicity studies and have been used to assess reproductive toxicity. Several standard mutagenicity tests have been developed in the fruitfly Drosophila melanogaster.

Useful information can also be obtained from nondestructive sampling. Samples such as blood, skin, eggs, and feces can be used to measure biomarker responses to toxicants. Behavioral effects can also be observed in the field and the laboratory.

Recommendations

The following are recommendations from the SGOMSEC workshop (4) for the development and use of alternative methods for ecotoxicology:

- Strategies and goals should be more clearly defined when testing procedures are undertaken.

- In the context of environmental risk assessment, the objectives of the 3Rs will be served by a) development and improvement of assays to incorporate...
new techniques from biochemical/ molecular biology that relate to mechanisms; b) further development of non-destructive assays for vertebrates, and assays for invertebrates; c) selection of the most appropriate species, strains and developmental stages in light of new knowledge (but no additional vertebrate species for basic testing); and d) better integrated approaches incorporating biomarker assays, ecophysiological concepts, and ecological end points. Maximum success depends on a flexible approach and exercising expert judgment in interpretation.

• Testing protocols need to be realistic, taking into account particular problems with mixtures and volatile or insoluble chemicals.

**Summary**

Alternative methods that provide useful information for human health and environmental risk assessments are now available in all areas of toxicity testing. Some methods can replace traditional methods, whereas others are useful in tiered testing approaches. Still other methods provide mechanistic information that is helpful in interpreting data from laboratory animal models. These mechanistically based methods provide improved predictions of toxicity, and some provide savings in time and cost. Alternative methods also reduce the numbers of animals required for toxicological assessments, and many incorporate refined end points that are more humane. Continued international cooperation will facilitate future progress in the development of alternative toxicological test methods. These methods will provide for worldwide improvements in human health protection, environmental protection, and animal welfare.

**Appendix. Participants in the 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals**

- **Bernard D. Goldstein**
  - Chair
  - SGOMSEC

- **David Peakall**
  - Vice Chair
  - SGOMSEC

- **Michael Balls**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Martin Barratt**
  - Unilever Environmental Safety Lab
  - Bedford, England

- **George Becking**
  - WHO/IPCS/IRRU
  - Research Triangle Park, NC

- **Bas Blaauwboer**
  - Utrecht University
  - Utrecht, The Netherlands

- **N.P. Bochkov**
  - Russian Academy of Medical Sciences
  - Moscow, Russia

- **Leon Bruner**
  - The Procter & Gamble Company
  - Middlesex, England

- **Silvia Casati**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Sandra Coecke**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Lucio Costa**
  - University of Washington
  - Seattle, WA

- **Rodger Curren**
  - Microbiological Associates
  - Rockville, MD

- **Betty Davis**
  - EOHSI - UMDNJ
  - Robert Wood Johnson Medical School
  - Piscataway, NJ

- **Manjit Dosanjh**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Julia Fernem**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Michela Ferraris**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Alan Goldberg**
  - Johns Hopkins University
  - Baltimore, MD

- **Bernard Goldstein**
  - EOHSI - UMDNJ
  - Robert Wood Johnson Medical School
  - Piscataway, NJ

- **Gareth Green**
  - Harvard School of Public Health
  - Boston, MA

- **Laura Gribaldo**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Andre Guillouzo**
  - INSERM
  - Rennes, France

- **Jerrold Heindel**
  - NIEHS
  - Research Triangle Park, NC

- **Klaus Kaiser**
  - National Water Research Institute
  - Burlington, Canada

- **Meryl Karol**
  - University of Pittsburgh
  - Pittsburgh, PA

- **Werner Klein**
  - Fraunhofer-Institut für Umweltschutz
  - und Okotoxikologie
  - Schmallenberg, Germany

- **Laurent Lagadic**
  - INRA
  - Rennes, France

- **Emirino Marafante**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Aly Massoud**
  - Ain Shams University
  - Cairo, Egypt

- **Mostafa Mohammed**
  - Ain Shams University
  - Cairo, Egypt

- **Jack Moore**
  - Institute for Evaluating Health Risks
  - Washington, DC

- **Canice Nolan**
  - Commission of the European Community
  - Brussels, Belgium

- **Pilar Peraira**
  - ECVAM, Environmental Institute
  - Joint Research Centre
  - Ispra, Italy

- **Ralph Parchment**
  - Wayne State University
  - Detroit, MI

- **David Peakall**
  - Wimbledon
  - London, England

- **Walter Pfäffer**
  - University of Innsbruck
  - Innsbruck, Austria
REFERENCES AND NOTES

1. Blaauwboer BJ, Balls M, Barratt M, Casati S, Cooeke S, Mohamed MK, Moore J, Rall D, Smith KR, Tennant R, et al. 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies and Conceptual Issues. Environ Health Perspect 106(Suppl 2):413–418 (1998).

2. Curren R, Bruner L, Goldberg A, Walum E. 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Validation and Acute Toxicity Testing. Environ Health Perspect 106(Suppl 2):419–425 (1998).

3. Spielmann H, Bochkov NP, Costa L, Gribaldo L, Guillouzo A, Heindel JJ, Karol M, Parchment R, Pfaller W, Prieto Peraia P, Zacharewski T. 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies for Organ Toxicity. Environ Health Perspect 106(Suppl 2):427–439 (1998).

4. Walker C, Kaiser K, Klein W, Lagadic D, Peakall D, Sheffield S, Soldan T, Yasuno M. 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies for Ecotoxicity. Environ Health Perspect 106(Suppl 2):441–451 (1998).

5. OTA. U.S. Congress Screening and Testing Chemicals in Commerce. OTA-BP-ENV-166. Washington: Office of Technology Assessment, 1995.

6. United States Code. NIH/National Institutes of Health Revitalization Act. Public Law 103–43. 42 USC. Washington: U.S. Government Printing Office, 1993.

7. EEC. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other purposes. Off J Eur Comm L352:1–29. (1986).

8. EEC. Council Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of laws of the Member States relating to cosmetic products ("Cosmetic Directive"). Off J Eur Comm L151:32–36. (1993).

9. OECD. Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods. Paris:Organization for Economic Co-operation and Development, 1996.

10. NIEHS. Validation and Regulatory Acceptance of Toxicological Test methods: a Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publ No 97–3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1997.

11. Luster MI, Portier C, Pait DG, White KL, Gennings C, Munson AE, Rosenthal GJ. Risk assessment in immunotoxicity: 1. Sensitivity and predictability of immune tests. Fundam Appl Toxicol 18:200–210 (1992).