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COMPLEX MIXTURES
Methods for In Vivo Toxicity Testing

Committee on Methods for the
In Vivo Toxicity Testing
of Complex Mixtures
Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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Preface

The National Institute of Environmental Health Sciences asked the National Research Council to evaluate the toxicity testing of mixtures and make recommendations for improving that testing. The National Research Council responded to the request by appointing 16 scientists to serve on the Committee on Methods for the In Vivo Toxicity Testing of Complex Mixtures, in the Board on Environmental Studies and Toxicology of the National Research Council's Commission on Life Sciences. The committee membership represented the disciplines of toxicology, pathology, biochemistry, analytic chemistry, environmental sciences, biostatistics, and epidemiology.

Toxicity testing is a subject of major current concern to both the scientific .d regulatory communities and has been the subject of several recent workshops, reports, and discussions. Consequently, many of the committee members were concerned about our ability to "break new ground" or to provide significant new insights for either the toxicologic methods for testing mixtures or the use of resulting data to protect human and environmental health.

After reviewing both the toxicologic and epidemiologic data on the adverse health effects of mixtures, the committee concluded that a new approach, rather than new methods, is the primary need. This report describes our rationale for this conclusion and presents a series of recommended strategies for testing mixtures.

As the chairman and vice-chairman of this committee, we want to thank Drs. Morton Lippmann, Robert A. Scala, Edo D. Pellizzari, and Ronald Wyzga for chairing task groups that focused on concepts for analyzing human exposure to complex mixtures, testing strategies and methods, sampling and chemical characterization, and interpretation and modeling of toxicity-test results, respectively. Working with this distinguished committee was an honor and a

pleasure, and we thank the members for their contributions and hard work in completing this report. Dr. Devra Davis deserves special thanks for the cor stant encouragement and insight that she communicated to a diverse and larg. committee. On behalf of the committee, we would also like to thank Drs. Bruce K. Bernard and Richard D. Thomas for their sustained and patient staff effort in support of the committee.

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Committee on Methods for the In Vivo Toxicity Testing of Complex Mixtures

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Executive Summary

People are seldom exposed to single chemicals. Most substances to which people are exposed, whether naturally or artificially produced, are mixtures of chemicals. Mixtures that are of particular concern include chemicals generated in fire, hazardous wastes, pesticides, drinking water, fuels, and fuel combustion products.

This report was prepared in response to a request from the National Institute of Environmental Health Sciences to the Board on Toxicology and Environmental Health Hazards (now the Board on Environmental Studies and Toxicology) of the National Research Council's Commission of Life Sciences to evaluate problems associated with the determination of the toxicity of mixtures. The mandate of the Committee on Methods for the In Vivo Toxicity Testing of Complex Mixtures was to develop strategies and experimental approaches for evaluating the toxicity of mixtures, on the basis of a selective review of the available literature.

The committee reviewed the epidemiologic evidence of effects of exposure to mixtures and found that much of this evidence was derived from exposures to relatively high doses of substances in the workplace. To detect the effects of environmental exposures to low doses, epidemiologic studies will need better methods for documenting relevant exposures and better ways to avoid misclassification of both exposures and outcomes. Epidemiologic investigations generally are able only to confirm past risks. Nonetheless, they can be useful for estimating risks associated with new exposures that are analogous to conditions previously studied. Owing to the various limitations inherent in the available data on human experience, toxicologic studies of mixtures are essential for estimating human risks and can provide strong clues for anticipating, and hence preventing, illness in the human population.

Current toxicologic test methods are applicable primarily to studies of target organ effects, mechanistic hypotheses, and general systemic toxicity. This report focuses on the effects of exposures of mammals, particularly humans, to mixtures. The committee has not considered the larger issues involved in environmental effects or other aspects of general ecology. It recommends that such broad issues be reviewed by another scientific group, and it suggests that more research is warranted on the effects and mechanisms of toxicity in nonmammals, including aquatic species.

Testing complex mixtures present a formidable scientific problem. The key to attacking this problem lies in the analysis and planning of the strategy or experimental approach. The extent and nature of testing should be guided closely by recognition of what is known and what needs to be learned. Selection of any future testing strategy will be linked strongly to questions related to expected exposure, to the toxic end points of interest, and to the likely use and predictive value of the results.

If the question being posed is related to the effects of a mixture, the strategies invoked involve toxicity testing of the mixture itself. The first step should be a careful consideration of the origin of the mixture, because this will provide some basic information on the complexity and the physical and chemical characteristics of the mixture, which might imply the types of anticipated effects. Care needs to be taken in the sampling process: samples must reflect as closely as possible what humans are potentially exposed to; they must be suitable to the types of assay chosen; and their integrity must be maintained at all points, as one means of ensuring relevance to the human situation. Questions regarding stability of mixtures must be considered, because different components age or decompose at different rates, and that creates uncertainty about what is actually being tested, compared to what humans are exposed to. Established and emerging toxicologic methods are as adequate for evaluating complex mixtures as for evaluating single agents.

Toxic-effects strategies are limited by the lack of animal or cellular models for some effects (e.g., cardiovascular and respiratory disease). Therefore, the committee encourages the continued development of animal models that permit critical studies of alien disease. The search for exposure-related effects in human populations must continue, and the resulting information must be integrated into the development of animal models in toxicology.

One of the central questions associated with complex mixtures is related to finding the primary causative agents of specific health effects. The strategies for identifying causative agents depend heavily on the integration of toxicology with chemical characterization. In light of the complexity and diversity of many mixtures, the complete chemical characterization of every sample under study is neither prudent nor possible.

Bioassay-directed fractionation, which is not used for single agents, is the most useful strategy for studying mixtures. Bioassays of fractions derived from

mixtures are used to identify which fractions need chemical characterization; the most toxic fractions are quickly identified.

In dealing with the ability to predict adverse effects of mixtures, the committee distinguished between factors related to effects and factors useful in modeling. The predictive value of an effects strategy centers on the ability to use data from tests of one mixture to predict the likely effects of exposure to a new but similar mixture. Among the effects strategies, comparative-potency studies and matrix testing were designed for such prediction. (Matrix testing involves the identification and manipulation of several variables that are arrayed in a two-dimensional matrix, such as boiling range and aromaticity.) The predictive value of a model strategy is related to the toxicity of components of simple mixtures whose components are all known. In such a case, if the toxicity of each component is known or can readily be determined, it might not always be necessary to test the whole mixture. (This model-driven prediction is described in Chapter 3.)

Simple mathematical models have been used to assess the toxicity of simple mixtures, particularly binary mixtures. Those models suggest that interactions that might be observed at a high dose, such as an experimental dose, do not necessarily occur at lower doses; hence, models might play an important role in permitting the extrapolation of toxicity to lower doses. Although a concentrated effort has been made to define the role of models in assessing the toxicity of more complex mixtures, it is clear that further work is needed to test the validity of models against experimental and epidemiologic data.

Consistency of analytic results between mixtures implies an increase in the predictability of their effects. The more similar two mixtures are chemically, the more similar their toxicologic properties are expected to be. However, even mixtures that are relatively well characterized sometimes have unexpected toxic effects. One must be prepared to look for unexpected results of exposure to complex mixtures, because of the potential disjunction between chemical analysis and biologic effect. Complex chemical mixtures are more likely to produce unexpected results than are individual chemical substances, for several reasons. Mixtures are composed of various substances, exposure to which can be expected to be associated with different toxicities. The constituents of a mixture sometimes combine chemically to produce new compounds with different toxicities. The presence of some materials might mask, dilute, or increase the toxicity of other materials. Such phenomena, referred to as interactions, can amplify or reduce anticipated effects. Moreover, different doses of separate materials might increase the bioavailability of materials that are otherwise nontoxic at the doses present in the mixture.

On the basis of theoretical considerations and its examination of some epidemiologic studies, the committee noted that effects of exposures to agents with low response rates usually appear to be additive. The only examples of interaction that were considered greater than additive occurred in humans exposed to

agents, such as cigarette smoke, that alone produced a high incidence of effects. Current quantitative models used to assess cancer risks support these results. However, the committee did not thoroughly review the toxicologic data on additivity assumptions.

The committee recognizes that several important related issues are not discussed in its report. Its discussion of the testing of complex mixtures deals largely with strategies, rather than with detailed testing methods.