Molecular and Cellular Approaches to Extrapolation for Risk Assessment

Thomas R. Sutter
Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205 USA

A workshop, “Molecular and Cellular Approaches to Extrapolation for Risk Assessment,” was held in Baltimore, Maryland, USA, 5–6 May 1993. The workshop was hosted by the Johns Hopkins Center for Alternatives to Animal Testing and was sponsored by the American Forest and Paper Association, Washington, DC. Forty representatives of government, industry, and academia met to discuss the opportunity to use in vitro data to improve evaluations of human risk, citing information gaps between animal and human data sets, and the relatively limited use of in vitro data in risk assessments.

The scientific methods of toxicology are most often used to identify potential hazards or to evaluate the safety of specific substances under certain experimental conditions. Although current scientific risk assessments are quantitative in nature, they are based upon assumptions inherent in methods of safety evaluation and hazard identification.

To date, the predominant method is to test animals and then to extrapolate the results to humans. Interspecies extrapolations include route of exposure, dose, and response. Due to the lack of information about relevant human responses to chemical exposures, such extrapolations lead to uncertainties; these uncertainties result in decreased confidence in risk estimates. Even in tier test systems that are significantly based on alternative tests, confirmation is obtained through selective testing in animal species. In vitro data are often viewed as an additional level of extrapolation. In such schemes, in vitro data may be used to bolster knowledge about specific issues of extrapolation, but the data themselves represent an additional source of uncertainty (Fig. 1A) (1–6).

In this workshop we considered an alternate scheme, based on the parallelogram approach to extrapolation to man, that was proposed in the late 1970s by Sobels (7–9). Although this approach was originally described for its application to chemical mutagenesis, its underlying principal, “to obtain information on damage that is hard to measure directly” (8), is relevant to most, if not all, biological endpoints of toxicity. In Figure 1B, the parallelogram has been modified to emphasize two important issues that were considered in this workshop: interspecies and in vitro–in vivo extrapolation. This parallelogram provides a framework for the discussion of molecular and cellular approaches to extrapolation for risk assessment and provides a process for systematic, comparative biology. In vitro data are used to support investigations of mechanism of action and, more specifically, to evaluate the assumption of conserved mechanism of action among different species. By superimposing the parallelogram onto the components of toxicity identified in Figure 2, a rationale is established for systematic stepwise comparisons of specific mechanism of action. Through such comparisons, it should be possible to establish whether specific mechanistic steps are conserved among species. Furthermore, once conservation of mechanism is established, subsequent studies can be used to determine and to compare quantitative aspects of dose–response relationships between species. Thus, as previously noted by Sobels, the parallelogram approach can be used to provide both qualitative and quantitative information that is directly relevant to estimates of human risk.

Workshop sessions were organized to emphasize each corner of the parallelogram or to highlight related issues. Before the meeting, each speaker provided a statement of his or her beliefs concerning the three most relevant issues and opportunities associated with molecular and cellular approaches to extrapolation for risk assessment. Compilation of these statements identified issues related to four major topics: 1) predictions, 2) humans, 3) mechanisms, and 4) regulatory agencies and risk assessment.

Predictions
In vitro responses are often the result of complex pathological processes, i.e., the long-term result of multiple factor, multicellular interactions. Even when using sensitive molecular and cellular approaches, can in vitro data be used to predict likely outcomes of such processes? For example, can early markers predict chronic toxicity? A related issue of prediction concerns the equivalency of sensitive biological responses. If different concentration–response curves are determined for different markers, which one(s) predicts in vivo toxicity?

These issues related to in vitro–in vivo extrapolations are significant. When viewed within the context of a complex process, e.g., carcinogenesis, it is difficult to conceptualize an approach for addressing these difficulties. However, if complex biological processes are broken down into a biologically based dose–response paradigm, then the specific in vitro–in vivo comparisons become more focused. As shown in Figure 2, complex processes can be subdivided into discrete components that provide a context for investigations of specific mechanistic steps. While each individual component is truly a connected series of mechanistic steps, the broader picture depicted in Figure 2 emphasizes that, for the current status of risk assessments, it may be more useful to obtain increased knowledge of the entire process, albeit at a less comprehensive level, than it is to have complete knowledge about one or more steps in the process, with little knowledge of others. In general, the overall assessment will only be as good as the least understood component in the mechanism of the endpoint of interest.

Several speakers raised an interesting question concerning prediction: are the responses observed in rodents valid predictors of human toxicity? As discussed above, the parallelogram provides a framework in which to test the hypothesis of conserved mechanism of action among different species.

Molecular and cellular approaches, combined with comparative in vitro systems, provide a method to explore early biological responses to chemical or physical agents and the role of these early effects in altered cellular structure and function. Such studies may lead to an improved understanding of mechanism of action and biological determinants of specificity. Also,

Address correspondence to T.R. Sutter, Division of Toxicological Sciences, Johns Hopkins Medical Institutions, Hygiene, Room 7032, 615 N. Wolfe Street, Baltimore, MD 21205 USA

I thank the members of Center for Alternatives to Animal Testing that contributed to this workshop and report: A. Goldberg, J. Zurlo, J. Frazier, D. Rudacille, M. Principe, A. Kerr, and R. Lewis. I thank R. Hill for his discussions of the parallelogram approach and the members of my laboratory that assisted with this report: C. Hayes, J. Gastel, and N. Walker.

To obtain a complete technical report of this workshop, contact Richelle Lewis, Johns Hopkins Center for Alternatives to Animal Testing, 111 Market Place, Suite 840, Baltimore, MD 21202-6709 USA.
studies of the relationship between concentration and biologically effective dose may provide insights into the shape of the dose–response curve in humans, including even lower levels of exposure. The potential for this latter opportunity (high to low dose) comes from the sensitivity of biological endpoints that are based on specific molecular and cellular targets.

In terms of linking exposure to dose–response relationships, several significant advancements have been made in the area of physiologically based pharmacokinetic (PBPK) and pharmacodynamic modeling. The concept of surrogate dose, or dose at the site of molecular action, provides a bridge between in vivo exposure and specific biological responses measured either in vivo or in vitro. As such, these modeling techniques may provide a continuum in investigations of mechanism, as experimental systems move between animals and cells in culture (10).

**Human Cells**

At present, the mechanism of action of many chemicals in humans is not fully understood. This includes knowledge of distribution, metabolism, specific cellular targets, sensitivity of specific cell populations, and repair capacity. This general lack of information concerning toxicity in humans is further complicated by the exposure of people to multiple chemicals over a lifetime that is considerably longer than that of rodents. A second issue relates to the limited availability of human specimens.

Human specimens, including tissues, slices, organ cultures, cocultures, or primary cells in culture, provide tremendous opportunity to investigate human biological response(s) to a variety of chemical and physical agents (11). When combined with modern methods of molecular biology and biochemistry to provide human recombinant DNA probes and expressed and purified human proteins, such studies can be used to identify primary biological endpoints relevant to human exposures (12) and to determine if the same critical cellular target (13) and mechanism (14) responsible for toxicity in animals exist in people. Corollary to this approach is the understanding that the methods developed using human in vitro systems can be easily imported as biomarkers into human epidemiology studies. Thus human in vitro studies support both human corners of the parallelogram and provide an opportunity for improved understanding of human in vivo responses (15,16). Without such improved sensitivity of the methods of human epidemiology or the incorporation of human in vitro data into the risk characterization process, biologically based risk assessments will simply represent improved models for the interpretation of data generated by animal experimentation.

**Mechanisms**

Should all tests be relevant mechanistically? Is correlation sufficient, especially as it relates to screens, or is it necessary to demonstrate a mechanistic link to biological response? Considerable discussion centered on the importance of knowledge of mechanism in decision-making processes. Both lectures on strategies for implementation made it clear that correlation is sufficient as a criteria for the application of screens (rapid tests to determine general or specific toxicity). In these cases knowledge of the mechanism resulting in the endpoint of toxicity is not required. For screens, current and future uses of in vitro data offer great potential to reduce, or eventually eliminate, the use of animals (17,18). The importance of these advancements should not be understated. However, it should be noted that while correlative studies may provide useful in-house information for decision-making, they advance neither the specific understanding of the endpoint of toxicity nor the methods to detect and quantitate such toxicity. For example, the Draize test, an in vivo screen for ocular and dermal irritancy, was widely used as a correlative screen for human-use product safety assessments. If more emphasis had been placed on obtaining a mechanistic understanding of this test, its replacement by cell or organ culture methods would have been greatly facilitated. Correlative studies do not provide a foundation for scientific advancement and, as such, should be used judiciously to immediately reduce the use of animals, while mechanistically based screen replacements with inherent potential for continued improvement are developed.

Mechanism-based approaches to risk assessment tend toward identification of true risk. Risk assessments that are based on such information will be based on the best available science. In turn, this should motivate good research and promote a self-advancing field that provides an improved understanding of human risk. Computer-based chemical databases facilitate the collection, storage, and retrieval of large amounts of information. Inherent in these chemical structures are features that determine biological activity (19). Studies of structure activity relationships provide the opportunity to advance from chemical specific risk assessments to chemical class-based risk assessments. Both the concepts of structure activity and surrogate dose imply the presence of a critical cellular target. Mechanism-based approaches implore the identification of such targets and raise the question of their conservation among species.

**Regulatory Agencies and Risk Assessment**

Several issues were identified that relate to certainty and uncertainty in risk estimates. Currently, both the regulatory and legal systems attempt to classify everything as safe or hazardous. Is it possible to move away from this toward a weight-of-eva-
Molecular and Cellular Approaches to Extrapolation for Risk Assessment

Program Committee: A.M. Goldberg, J. Zurlo, and T.R. Sutter

Program Sessions

Plenary Lecture
J. M. Frazier
New perspectives on in vitro/in vivo extrapolation for risk assessment

In Vivo Responses: From Other Animals to Humans
G.A. Boorman
Animal toxicity/carcinogenicity studies
J.D. Groopman
Human epidemiology/biomarkers: aflatoxin and liver cancer as a model

Research Supporting Extrapolations
M.E. Andersen
How will we know whether in vitro and molecular approaches really tell us about what goes on in the living animal?
B.J. Smith
Ovarian toxicity of 4-vinylcyclohexene and related compounds
L.D. Lehman-McKeeman
Male rat specific o2U-globulin nephropathy: in vivo and in vitro assessment
R.B. Conolly
Pharmacodynamic modeling: quantitative descriptions of the linkage between tissue dose and toxic response
H.S. Rosenkrantz
Application of SAR to extrapolation from in vitro to in vivo assays

Case Study: Dioxin
W.H. Farland
Dioxin: current and future uses of in vitro data
C.A. Bradfield
Molecular modeling of dioxin action
B.D. Abbott
Palatal organ culture in the study of dioxin-induced cleft palate
W.F. Greenlee
Human responses to dioxin: identification of interspecies determinants of specificity

Strategies for Implementation
K.A. Sittzel
Current and future uses of in vitro data
S. Green
Current and future uses of in vitro data

Current and Future Uses of In Vitro Data

Several current uses of in vitro data exist: 1) to select the most appropriate animal model of humans; 2) to provide mechanistic information about in vivo responses; 3) to screen series of toxicants rapidly; 4) to screen for ocular, dermal, neurological, and developmental toxicity; and 5) to establish potential mutagenicity and carcinogenicity; and 6) to further document the hazardous nature of a carcinogen.

Future uses of in vitro data include: 1) expanded use as screens; 2) reduction or elimination of the use of animals for assessments of dermal irritation; 3) determination of specific parameters for PBPK models; and 4) expanded use in investigations of mechanism of action, specifically as such information relates to risk assessment.

Conclusions

This workshop explored many aspects of the complex issues related to interspecies extrapolation. The parallelogram approach provides a rationale for systematic step-wise comparisons, including in vitro–in vivo comparisons of rodent and human biology that provide knowledge of response and sensitivity to chemical action. Applications of modeling provide important methods to link in vivo exposures to other endpoints of in vivo and in vitro biological response. In reviewing the available methods and experimental systems, a major informational gap was identified concerning the events that mechanistically link altered structure and function to toxicity or disease. Future studies need to focus on this important area of limited knowledge, as it appears to be rate limiting in the overall process to determine accurate risk estimates.

Given the understanding that chemical-specific risk assessments are both time consuming and expensive, considerable concern remains about the issue of selective versus universal mechanisms of toxicity. For now, no simple solution is evident. Minimally, advancements in structure activity relationships should permit us to move from chemical-specific risk assessments to those based on chemical class. Moreover, from the history of mutagenesis, it is clear that complete knowledge of specific mechanisms is not required for effective determinations of risk estimates. As in the case of chemical mutagenesis, unifying concepts of general mechanisms may make it possible to develop systems to
detect and quantify specific chemical activity. It remains possible that such unifying concepts are inherent in other complex biological process such as dermal irritancy or even cancer, and that such concepts will supersede the need for complete and specific knowledge of mechanism of action and permit the development of effective, general screens based on common mechanism.

REFERENCES

1. Huff J. Issues and controversies surrounding qualitative strategies for identifying and forecasting cancer causing agents in the human environment. Pharmacol Toxicol 72(suppl 1):12–27 (1993).
2. Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiwicz S, Anderson B, Minor R. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science 236:933–941 (1987).
3. Davidson JW, Parker JD, Belles RP. Biological basis for extrapolation across mammalian species. Regul Toxicol Pharmacol 6:211–237 (1986).
4. Cohen SM, Ellwein LB. Risk assessment on high-dose animal exposure experiments. Chem Res Toxicol 5:742–748 (1992).
5. Boorman GA, Eustis SL, Elwell MR, Grinemeyer RA. Rodent carcinogenesis studies: their value and limitations. In: Assessment of inhalation hazards (Mohr U, Bates DV, Dungworth DL, Lee PN, McCellan RO, eds). Heidelberg:Springer-Verlag, 1989; 61–68.
6. Frazier JM. Scientific perspectives on the role of in vitro toxicity testing in chemical safety evaluation. In: In vitro methods in toxicology (Jolles G, Cardier A, eds). New York: Academic Press, 1992:521–529.
7. Sobels FH. Some problems associated with the testing for environmental mutagens and a perspective for studies in comparative mutagenesis. Mutat Res 46:245–260 (1977).
8. Sobels FH. Evaluating the mutagenic potential of chemicals: the minimal battery and extrapolation problems. Arch Toxicol 46:21–30 (1980).
9. Sobels FH. Environmental mutagenesis in retrospect. Mutat Res 181:299–310 (1987).
10. Conolly RB, Andersen ME. Biologically-based pharmacodynamic models: tools for toxicological research and risk assessment. Ann Rev Pharmacol Toxicol 31:503–523 (1991).
11. Haris CC. Human tissues and cells in carcinogenesis research. Cancer Res. 47:1–10 (1987).
12. Greenlee WF, Sutter TR, Marcus C. Molecular basis of dioxin actions on rodent and human target tissues. In: Receptor-mediated biological processes: implications for evaluating carcinogenesis, vol 387 (Spitzer HL, Slaga TJ, Greenlee WF, McClain M, eds). NewYork: Wiley-Liss, 1994;47–57.
13. Lehman-McKeeman LD. Male rat-specific light hydrocarbon nephropathy in toxicology of the kidney. In: Toxicology of the kidney (Goldstein RS, Hook, JB, eds). New York: Raven Press, 1934; 477–494.
14. Smith BJ, Sipes IG, Stevens JC, Halpert JR. The biochemical basis for the species difference in hepatic microsomal 4-vinylcyclohexene epoxidation between female mice and rats. Carcinogenesis 11:1951–1957 (1990).
15. Wogan GN. Molecular epidemiology in cancer risk assessment and prevention: recent progress and avenues for future research. Environ Health Perspect 98:167–178 (1992).
16. Groopman JD, Kenzler TW. Molecular biomarkers for human chemical carcinogen exposure. Chem Res Toxicol 6:764–770 (1993).
17. Bruner LH. Alternatives to the use of animals in household products and cosmetic testing. J Am Vet Med Assoc 200:660–673 (1992).
18. Green S, Bradlaw J. Regulatory law and the use of in vitro methods for the assessment of various toxicities. In: In vitro toxicity testing. (Frazier JM, ed), New York: Marcel Dekker, 1992:281–293.
19. Klopman G, Rosenkranz HS. Approaches to SAR in carcinogenesis and mutagenesis-prediction of carcinogenicity/mutagenicity using MULTI-CASE. Mutat Res 305:33–46 (1994).
20. National Research Council Committee on Biological Markers. Biological markers in environmental health research. Environ Health Perspect 74:3–9 (1987).

Biostatistics in the Study of Human Cancer

The conference on Biostatistics in the Study of Human Cancer was held November 9–11, 1993, in Tokyo, Japan. Sponsors were the US-Japan Cooperative Cancer Research Program, the Japan Statistical Society, the Biometric Society of Japan and the Japan Epidemiology Association. The conference was organized by David G. Hoel, Robert Miller, Haruo Sugano, and Takashi Yanagawa.