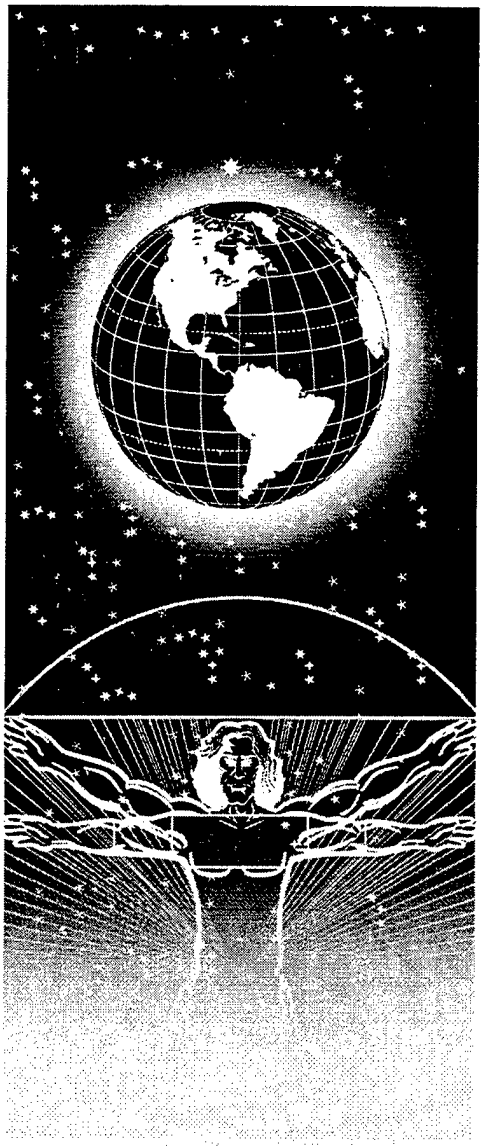
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UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Proceedings Of The 1993 Conference On Toxicology-The Risk Assessment Paradigm After Ten Years: Policy and Practice Then, Now, and In The Future

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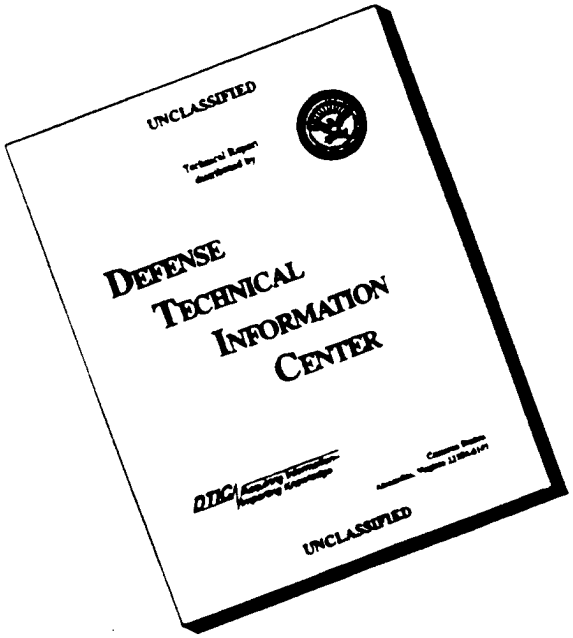
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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Childress

TERRY A. CHILDRESS, Lt Col, USAF, BSC
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The conference on the Risk Assessment Paradigm After Ten Years covered a large number of topics of interest to toxicologists, risk assessors, and environmental managers. Leading scientists presented a range of topics in six sessions. These included introductions to the past, present, and future of the basics of risk assessment and a case study on new approaches to the risk assessment of a Halon replacement (HCFC-123). Possibilities for advancing the science of risk assessment were considered, including the use of physiologically based pharmacokinetic modeling for noncancer risk assessment dermal exposure, and lactational transfer. Other mathematical approaches were considered for cancer and noncancer risk assessment. Mechanisms of biological action responsible for injury were considered for their implications. Finally, critical issues in risk communication were discussed. A poster session presenting research in risk assessment and toxicology supplemented the platform presentations. This conference was supported by the Air Force, Army, Navy, and Environmental Protection Agency and was attended by representatives of government, industry, and academia.

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PREFACE

The Conference on "The Risk Assessment Paradigm After Ten Years: Policy and Practice Then, Now, and in the Future" was held at the Hope Hotel and Conference Center at Wright-Patterson Air Force Base, OH, from 5 through 8 April 1993. The Conference was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, U.S. Air Force; the Naval Medical Research Institute Detachment (Toxicology); the Army Biomedical Research and Development Laboratory; and the Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, with the cooperation of the National Research Council Committee on Toxicology. The conference was coordinated by ManTech Environmental Technology, Inc., Toxic Hazards Research Unit, under Department of the Air Force Contract No. F33615-90-C-0532. Lt Col Terry A. Childress served as Contract Technical Monitor.

Over 290 representatives of government, industry, and academia attended the Conference, which featured invited presentations by noted individuals in the field of toxicology as well as a poster session on topics relevant to the theme of the Conference. The papers and abstracts in this volume span the wide range of topics presented. Conference sessions were held on

- The Basics of Risk Assessment
- Case Comparisons — Issues/Lessons Learned
- Where the Paradigm Needs Change
- Advancing the Science of Risk Assessment
- Risk Communication

We would like to thank the authors for contributing a written document as well as making a presentation at the Conference. We would also like to thank our colleagues who reviewed the manuscripts; Sheila Brooks, JoAnne Barker, and Sheila Elliott for word processing; Tanya Isley for editing; and Patty Fleenor for her invaluable assistance in technical editing and coordinating the review, compiling, and editing processes that resulted in the publication of these proceedings. We also would like to thank Lois Doncaster, Jim Stokes, and the THRU, Air Force, Navy, and EPA personnel who participated in the preparation and coordination of the Conference.

The proceedings of this Conference have been accepted for publication in the journal *Risk Analysis*. A June 1994 (Vol. 14, No. 3) publication is anticipated.

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INTRODUCTION

Hugh A. Barton[1], David R. Mattie[2], and W. Bruce Peirano[3]

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The Conference on "The Risk Assessment Paradigm After Ten Years: Policy and Practice Then, Now, and in the Future" was held at Wright-Patterson Air Force Base, Ohio, 5 through 8 April 1993. It provided an opportunity for research scientists, risk assessment practitioners, and users of risk analysis to evaluate the state-of-the-art of risk assessment. The varied backgrounds of the attendees, in part, reflected the Conference's unique cosponsorship by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, U.S. Air Force; the Naval Medical Research Institute Detachment (Toxicology); the Army Biomedical Research and Development Laboratory; and the Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, with the cooperation of the National Research Council Committee on Toxicology.

The rapid increase in the utilization of risk assessment, since the presentation by the National Academy of Science in 1983 of an analytical paradigm, has raised numerous and difficult scientific and policy issues. The Basics of Risk Assessment (Session I) reviewed the use of paradigm. This was followed by consideration of Case Comparisons – Issues/Lessons Learned (Session II), and suggestions for Where the Paradigm Needs Change (Session III). Selected articles are presented from each of these sessions.

These articles describe insights gained as risk assessment has moved from relatively simple default approaches toward addressing the complexities of exposure scenarios and chemical toxicities. This move toward complexity and flexibility represents a natural progression for the field. Risk assessors often began by screening for those situations that might be of concern by using health-protective assumptions and simplifications. Realities are inevitably different than the simplified case, so additional information and methodologies to utilize it must be developed and implemented. This presents a challenge to researchers, practitioners, and users due to their significantly different time frames, technical backgrounds, and policy perspectives. Risk assessment must improve to assist in decision making despite limitations of available information and human understanding.

The next two sessions described Advancing the Science of Risk Assessment (Sessions IV and V). Several authors discussed scientific advances that have implications for risk assessment, particularly of carcinogens. As our knowledge of cancer processes improves, it reinforces the fact that there are several diseases we refer to collectively as cancer. Any single quantitative risk assessment methodology shows its weaknesses when it tries to address all of these identically. Other authors addressed issues associated with mathematical modeling for exposure routes, carcinogenicity data, or noncarcinogenic effects.

Risk assessment has thrived in this age of computerization. Early risk assessments used simplified assumptions and approaches, in part, due to limitations of access to computational power. The papers in Sessions IV and V show how risk assessment can gain from increasing modeling sophistication. But, they also contain indications that mathematical methods can only move us a short way in the absence of either concrete information (e.g., mechanisms of toxicity) or clear policy decisions (e.g., how to protect various populations).

Much of the Conference focused on how new scientific information and improved methodologies are increasing the sophistication of risk assessment, but the final session addressed the challenges of Risk Communication (Session VI). Mathematical modeling, Monte Carlo simulation, pharmacokinetics, mechanisms of toxicity – these must be pathways to greater clarity and conciseness, not just complexity and technical sophistication.

The public arena in which risk assessment exists is one of human concerns and desires for an improved life. It is this context that makes risk assessment such a challenging multidisciplinary field. The papers from this Conference demonstrate that developing a flow from information gathering to analysis to discussion to decision is still a challenge, even within the risk assessment/management/ communication fields. The languages of mathematics and human speech must come together to facilitate discussion and decision making if risk assessment and risk management are to achieve the success that society desires from them.

SESSION I

BASICS OF RISK ASSESSMENT

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"Times are Tough; Brother, Can You Paradigm?"

Donald G. Barnes
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ABSTRACT

Ten years ago, the National Academy of Sciences released its risk assessment/risk management (RA/RM) "paradigm" that served to crystalize much of the early thinking about these concepts. By defining RA as a four-step process, operationally independent from RM, the paradigm has presented society with a scheme, or a conceptually commmon framework, for addressing many risky situations (e.g., carcinogens, noncarcinogens, and chemical mixtures). The procedure has facilitated decision making in a wide variety of situations and has identified the most important research needs.

However, the past decade has revealed that additional progress is needed. These areas include addressing the appropriate interaction (not isolation) between RA and RM, improving the methods for assessing risks from mixtures, dealing with "adversity of effect," deciding whether "hazard" should imply an exposure to environmental conditions or to laboratory conditions, and evolving the concept to include both health and ecological risk.

Interest in and expectations of risk assessment are increasing rapidly. The emerging concept of "comparative risk" (i.e., distinguishing between large risks and smaller risks that may be qualitatively different) is at a level comparable to that held by the concept of "risk" just 10 years ago. Comparative risk stands in need of a paradigm of its own, especially given the current economic limitations. "Times are tough; Brother, can you paradigm?"

INTRODUCTION

According to many pundits, the central issue of the last presidential election was the economy. A sluggish recovery, characterized by a persistent unemployment – despite the lowest interests rates in decades – raises the possibility of decreased economic performance well into the future, as well as the specter of the Great Depression in the minds of today's great-grandparents.

According to some, former President Bush should be faulted for not having done more during his "watch" to prod the country out of its economic doldrums. According to others, President Clinton should thank former President Bush for taking appropriate action – he simply waited too long for that action to benefit him. This situation illustrates that old aphorism of politics: "Timing is everything," and Washington – along with the rest of the country – often has difficulty with its timing.

On occasion, however, Washington does get the timing right. I submit that the National Academy of Sciences (NAS) got the timing nearly perfect with the release of its report on risk assessment (1). This report presented a conceptual scheme, a "paradigm," that helped to focus and crystalize the discussion about risk assessment just at a time when the practice was being adopted and adapted by more and more individuals and institutions.

It is the tenth anniversary of that paradigm that we celebrate today by reexamining its origins, adaptations, and possible future.

Figure 1 succinctly presents the risk assessment/risk management (RA/RM) paradigm set forth by the NAS in 1983. The four steps in the risk assessment process and the perhaps indelicately posed questions they address are as follows.

1. Hazard Identification;
   Is this stuff toxic?

2. Dose–Response Assessment:
   How toxic is it?

3. Exposure Assessment:
   Who is exposed to this stuff, how long, how often?

4. Risk Characterization:
   So what?

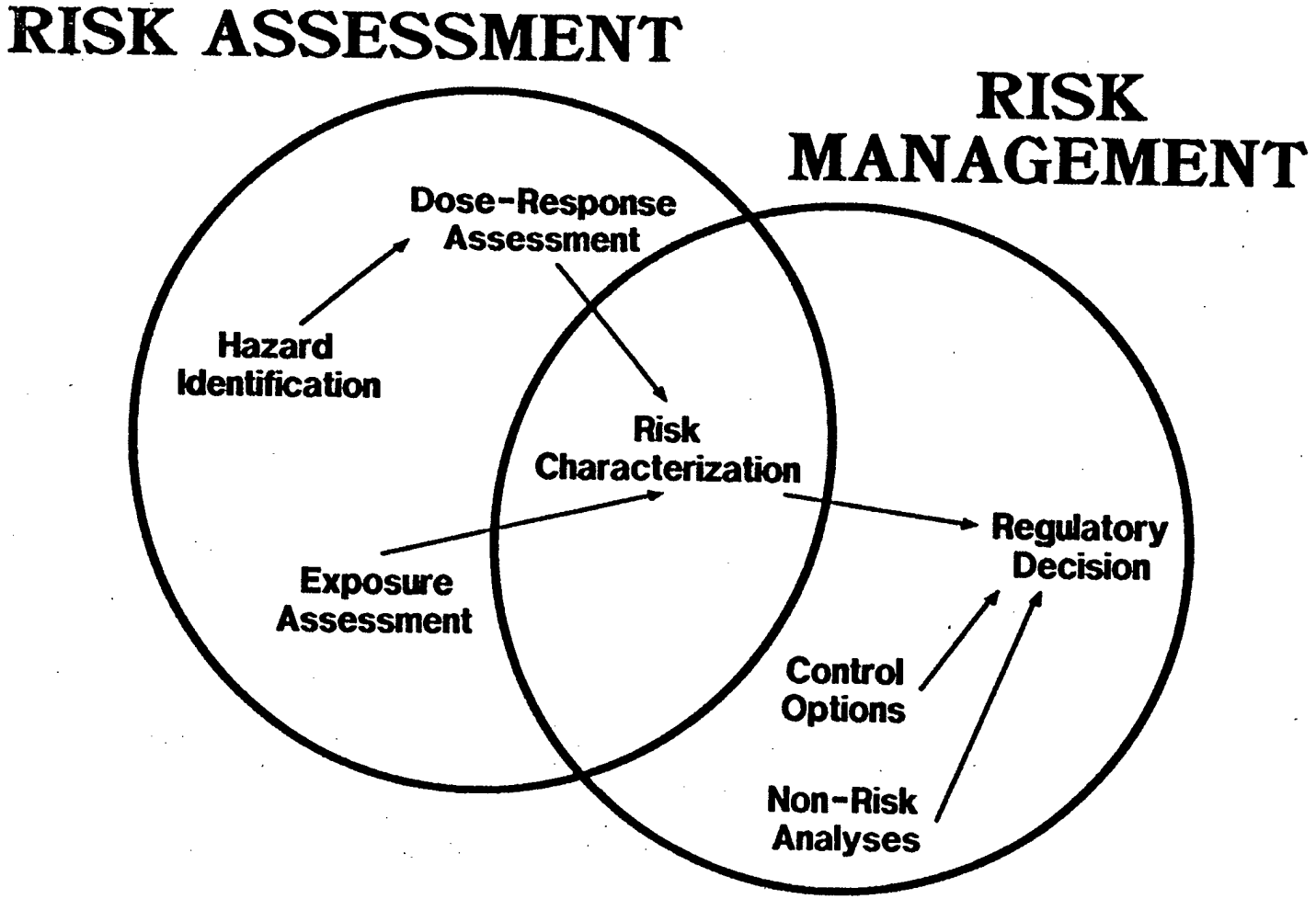


Figure 1. The NAS Paradigm.

This risk information is one of several factors that the decision maker must consider in answering the risk management question posed by an always intense, if not always admiring, public: "So what are you going to do about it?"

In the remainder of the paper, I will describe the impact of the NAS paradigm, some of its strengths and weaknesses, and some suggestions about what the future might hold for the paradigm and for us.

THE STRENGTHS AND IMPACT OF THE NAS PARADIGM

A key development in the early history of the assessment of chemical risks in the decision making process was the announcement of cancer risk assessment guidelines by the U.S. Environmental Protection Agency (EPA) in 1976 (2). That document, forged in the fires of legal action taken against chlorinated pesticides, succinctly laid out the approach that the EPA would take in dealing with chemicals that pose carcinogenic risks. Although quite useful in its own right, that early set of guidelines lacked the intellectual construct (paradigm) that could serve to frame the thinking and discussion about risk. Consequently, the vigorous developments in RA that took place in the late 1970s and early 1980s shimmered and sparkled but, like so much Jello, lacked a unifying, undergirding structure. It was the NAS paradigm that finally succeeded in nailing much – but not all – of this Jello® to the wall.

For example (to borrow from the comparison of the sacred and the secular), the paradigm provides a common, somewhat demystified language which both anointed practitioners (risk assessors) and laypeople (the rest of us) can use when communicating about risk. Both groups can appreciate that all scriptures (risk assessments) should have a common underlying structure (i.e., the four elements in the paradigm).

Further, the paradigm lays out the moral equivalent of the Prime Directive (i.e., the separation of church [RA] and state [RM]). If the decision making process is to be credible to members of the public, they must have an assured faith that the RA process is not tainted by the involvement of those who might have a stake in the RM decision.

In addition, experience has shown that the paradigm has broad applications. Although used most often at EPA to reach conclusions on carcinogenic compounds, the scheme is equally applicable to

noncarcinogens; mixtures (e.g., environmental tobacco smoke [3]); nonchemicals (e.g., electromagnetic fields); and site-specific situations, such as Superfund sites or military base cleanups.

Finally, the paradigm causes us to clearly distinguish between "fact" (scientific data) and "faith" (scientific inferences and "default positions"). In so doing, we are led to identify those data gaps that have the greatest potential effect on the RA. Through disciplined application of the paradigm, we can best target our research resources to have the maximum impact on the decision making process. For example, much of EPA's research on "dioxin" has been explicitly guided by the impact that these results might have on the reassessment of "dioxin" risk, slated for release in late 1993.

The influence and impact of the RA/RM approach to decision making has been growing steadily within EPA. For example, the Agency has issued several RA guidelines beyond those for cancer (4), most of them structured according to the paradigm.

The Agency also has summarized its Hazard Identification and Dose–Response Assessments and made them available to the public through the Integrated Risk Information System (IRIS). Through IRIS, states, localities, and individual citizens have access to two of the fundamental components of the paradigm. Coupled with the publicly available software called RISK*ASSISTANT (5), the ability to generate simple, but effective, risk assessments is available to anyone with a personal computer.

In addition, the EPA is becoming more sophisticated in its exposure assessments through the use of Monte Carlo computer techniques. More attention also is being focused on ways to improve the final risk characterization step in the RA process.

The paradigm has served to sort out the respective roles and responsibilities of risk assessors and risk managers. This has sheltered the risk assessor from some political pressures, while exposing risk managers to the need to make difficult decisions and to communicate them in a comprehensible fashion to the public.

The concept of RA/RM decision making continues to spread beyond the Agency. Conferences and publications on the subject are found almost everywhere. A professional Society for Risk Analysis has now formed, with organizational units throughout the United States, in Asia, and in Europe –

including the emerging democracies of Eastern Europe. Agencies that once denigrated RA as being as accurate as a 5-year weather forecast are adopting the paradigm for their own purposes.

STRAINS AND WEAKNESSES OF THE NAS APPROACH

Total Separation of RA and RM is Not Possible – or Even Desirable in Many Instances.

Some degree of interaction between RA and RM is essential if the RA answers are going to address – let alone satisfy – the RM questions. In some instances, for example, a qualitative answer will suffice (e.g., Could the pollutant run off into the stream and bioaccumulate in aquatic organisms?). In other instances, a more detailed answer is needed (e.g., What are the remedial options that would prevent runoff of the pollutant to such an extent that bioaccumulation would be maintained at levels below a 10^{-5} risk level to the sportfishing population?).

In fact, the NAS considered the option of separating the RA operations totally from the RM operations (e.g., establish a separate agency to conduct such analyses). To their credit, the NAS panel rejected this option as being infeasible, while spotlighting the importance of separating RA and RM functions within a single agency.

In finding the proper balance, there will – and should be – a continual tension between the need for good communication between the customer (RM) and supplier (RA).

As Used, the Paradigm Favors a Reductionist – Single Chemical (Stressor) – Approach (i.e., Prejudices the Risk Assessor against Considering Mixtures in a Holistic Manner).

Risk assessment/risk management decision making is most easily applied to the case of a single chemical. Therefore, there is a tendency to ignore "the mixtures problem" and to attempt instead to focus on the few chemicals that pose the largest individual risks in a given situation. This approach carries the implicit assumption that individual chemical risks will generally exceed those posed by simultaneous exposures to combinations of risky materials. We know of instances in which this simplifying assumption does not hold (e.g., the response of the exposure of tobacco smokers to asbestos).

The Current Approach Does Not Address Adversity of Effect.

A perennial chestnut, the issue of adversity of effect (e.g., Which is worse: Cancer or development effects?) has withstood all attempts at definitive resolution by the Agency, the NAS, and

anyone else for that matter. At bottom, the question of the relative concern to give to leukemia versus missing limbs, or reproductive effects versus stratospheric ozone depletion appears to be a value judgment to be made somewhere other than in the RA arena. Although such a statement may be true, it reflects a limitation in the ability of RA to answer the RM question: What are you going to do about these two risks?

There is increasing recognition that resources to address environmental problems are limited. Consequently, trade-offs have to be made in many cases, including those in which a variety of risks estimated via the NAS paradigm are generally equal. In order to reach decisions in such cases, therefore, something beyond the current NAS paradigm is needed. (See "Where Do We Go From Here?")

As Used, There is Ambiguity about Whether Hazard Identification Should Relate to Effects Observed at "Exposures Under the Condition of the Test" or "Exposure Likely to be Encountered in the Environment."

The traditional 2-year bioassay for carcinogenicity utilizes high doses (e.g., the maximum tolerated dose [MTD]) to compensate for the limited number of animals used in the assay. Increasingly, questions are being raised about the relevance of results from such high-dose assays in anticipating the consequence of exposures at much lower levels likely to be encountered in the environment (6).

The issue is being confronted even more directly in the case of noncancer end points. For example, the Agency's RA guidelines for reproductive and developmental effects explicitly consider anticipated levels of exposure in reaching a conclusion in the Hazard Identification step, which is in contrast with the current practice for cancer RA.

Although the paradigm itself is silent on this issue, any improvement in the scheme should explicitly address this matter.

Ecological Paradigm is Purported to Be Different.

Recently, the Agency issued an Ecological Risk Assessment Framework document containing a paradigm for eco-RA that is somewhat different from that proposed by the NAS a decade ago (7). The reasons for these differences include the following.

- We know more about RA than we did 10 years ago.
- The ecological problems are qualitatively different from the health problems, which were the focus of the original NAS concerns.
- The ecological community wants to leave their distinctive mark along the RA trail.

In any event, there should be a single RA paradigm that is sufficiently broad to encompass both health and ecological concerns. Currently, there is the danger that eco-RAers and health-RAers will evolve in different – and possibly opposing – directions, to the detriment of both groups and the public.

WHERE DO WE GO FROM HERE?

The Clean Air Act of 1990 contains a provision for the NAS to revisit the manner and methods of RA. The economic and social implications of the Act are of such magnitude that the Congress felt it was important to reassess the technical aspects of the decision making process. The NAS report, perhaps with an updated paradigm, should be released during 1993.

In addition to NAS effort, however, events are already under way that may well expand the paradigm. Just as the NAS's 1983 effort helped to legitimize the practice of RA, the 1988 EPA *Unfinished Business* report and the 1990 Science Advisory Board *Reducing Risk* report highlighted the concept of relative risk analysis (8, 9). These reports demonstrated that it is possible – even with today's limited data – to distinguish on a technical basis between high risk problems and lower risk problems.

Although not universally accepted at this point (10), relative risk holds the promise of being a valuable tool in shaping a national environmental agenda. The EPA has expanded the concept even further by successfully enlisting a broader range of parties – those with interests in the technical, social, political, and other spheres – to work collegially in establishing environmental agendas at the regional, state, and local levels through a comparative risk process.

In fact, the city of Columbus, OH, has established its equivalent of a Science Advisory Board to advise the mayor on the relative risks facing the city. In addition, Senator Daniel Moynihan of New York held hearings on the potential use of these comparative risk ideas in 1990 and 1992. In 1993, he

introduced S. 110 (the Environmental Risk Reduction bill), which directs the Agency to generate a comparative ranking of environmental risks on a biennial basis for submission to the EPA and Congress.

In my view, comparative RA is at a stage of development similar to that of RA in the late 1970s. Once again, we stand in need of a defining paradigm that will structure our thought, our discourse, and our progress. May we respond to this challenge in 1993 as well as those who responded to the challenge in 1983.

As I said, "Times are tough; Brother, can you paradigm?"

REFERENCES

1. National Research Council, *Risk Assessment in the Federal Government: Managing the Process* (National Academy Press, Washington, D.C., 1983).

2. U. S. Environmental Protection Agency, "Interim procedures and guidelines for health risk and economic impact assessments of suspected carcinogens," *Federal Register* **41**: 21402-21405 (1975).

3. U. S. Environmental Protection Agency, *Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders* (EPA/600/6-90/006F, December 1992).

4. U. S. Environmental Protection Agency, "Guidelines for Carcinogen Risk Assessment," *Federal Register* **51**, 22992-34003 (September 24, 1986a).

   U. S. Environmental Protection Agency, "Guidelines for Mutagenicity Risk Assessment," *Federal Register* **51**, 34006-34012 (September 24, 1986b).

   U. S. Environmental Protection Agency, "Guidelines for the Health Risk Assessment of Chemical Mixtures," *Federal Register* **51**, 34014-34025 (September 24, 1986c).

   U. S. Environmental Protection Agency, "Guidelines for Health of Suspect Developmental Toxicants," *Federal Register* **51**, 34028-34040 (September 24, 1986d).

   U. S. Environmental Protection Agency, "Guidelines for Estimating Exposure," *Federal Register* **51**, 34042-34054 (September 24, 1986e).

   U. S. Environmental Protection Agency, "Proposed Guidelines for Assessing Female Reproductive Risk: Notice," *Federal Register* **53**, 24834-24847 (June 30, 1988).

   U. S. Environmental Protection Agency, "Proposed Guidelines for Assessing Male Reproductive Risk and Request for Comments," *Federal Register* **53**, 24850-24869 (June 30, 1988).

U. S. Environmental Protection Agency, "Guidelines for Developmental Toxicity Risk Assessment," *Federal Register* **56**, 63798-63826 (December 5, 1991).

U. S. Environmental Protection Agency, "Guidelines for Exposure Assessment," *Federal Register* **57**, 22888-22938 (May 29, 1992).

5. RISK*ASSISTANT is available from Thistle Publishing, P.O. Box 1327, Alexandria, VA, 22313.

6. National Research Council, *Issues in Risk Assessment* (National Academy Press, Washington, D.C., 1993).

7. U. S. Environmental Protection Agency, *Framework for Ecological Risk Assessment* (EPA/630/R-92/001, Risk Assessment Forum, Washington, D.C., February 1992).

8. U. S. Environmental Protection Agency, *Unfinished Business: A Comparative Assessment of Environmental Problems* (Office of Policy, Planning, and Evaluation, Washington, D.C., February, 1987).

9. U. S. Environmental Protection Agency, *Reducing Risk* (Science Advisory Board, SAB-EC-90021, Washington, D.C., September, 1990. Available from the National Technical Information Service as Publication No. PB91-155242, Springfield, VA).

10. Resources for the Future Conference on "Setting National Environmental Priorities," Historic Inns of Annapolis, Annapolis, MD, November 15-17, 1993.

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Hazard Evaluation and Dose Response

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Although we define toxicology as the study of the adverse effects of chemicals on biologic systems, toxicology is clearly more than just the science by which we identify and characterize adverse effects. Toxicology, like medicine, is both a science and an art and it is the art or predictive aspect of toxicology that I have been asked to discuss this afternoon.

Toxicologic predictions are traditionally based on three kinds of information. First, we need to know something about the agent or chemical and its adverse effects. Second, we need information about the dose and/or exposure conditions. Third, we need information about the target or exposure subjects. These same three end points: the type of adverse effect, the exposure scenario, and the susceptibility of the exposed population are also the basic input for assessing the risk of exposure to chemicals in our environment and for setting limits on exposure to chemicals in the workplace as well as in our food, drugs, water, soil, and elsewhere.

Although it is evident that the quality of our predictions will depend on the quality of each of the three inputs, it should also be evident that the only absolutely reliable basis for predicting adverse effects in humans is data obtained with the desired chemical and the correct exposure scenario in humans. Any prediction based on less than this will be constrained by the need to extrapolate the input data.

Further, even when we have these ideal conditions of correct agent, species, and exposure, because our predictions are for the average or typical population, the recommendation may not be predictive for every individual in the population because of differences in age, sex, occupation, dietary and personal habits, and other factors. The next best option for insuring predictive validity is human data with the right chemical and exposure or dose information that can be reliably interpolated or extrapolated. The third option is human data for a surrogate that has chemical and biologic properties that are similar to those of the target chemical. In principle, it is only when we do not have adequate human data on the target chemical or a reasonable surrogate that we use animal studies for estimating risk but, in practice,

studies in animals are almost always used as a part of the predictive process because of their role in validating the human studies and because of their importance as the basic input for both the hazard evaluation and the quantitative risk assessment approach.

The first step in what is traditionally referred to in toxicology as "hazard evaluation" is to identify all of the adverse effects that can be produced by either acute or chronic exposure to the chemical and the second step is to establish dose−response relationships for each of these adverse effects. Ideally, these studies would also provide information on the effects of administration by different routes, at different rates and durations of exposure, and information on other test species. This information together with data on the chemical and physical-chemical properties of the chemical, kinetic data in various species, gene-tox studies, teratology, reproduction, and other types of end-organ damage, plus the mechanistic information, constitute what is referred to as the tox-database for the chemical. The next step is to determine whether this information is relevant to the target species and to the exposure scenario. In those cases where the information is relevant and where there is a threshold or no-observable-adverse-effect level (NOAEL), then the final step is to divide the NOAEL by an appropriate safety factor to assess risk and establish the exposure limit. A modification of this approach has been developed by the Environmental Protection Agency in which the slope of the dose−response curve and its confidence limits are used to calculate benchmark or reference doses for low incidence responses. Although this approach is more responsive to the number of animals at each test dosage level and avoids the use of a single threshold value, it is basically an extension or enhancement of the traditional threshold approach. The advantage of the threshold approach is that it is simple, easy to understand, and that it has been widely used for over 50 years to assess and regulate the hazards of chemicals in our environment. However, this approach cannot be used with nonthreshold effects and it requires the exercise of judgement or a weight of evidence evaluation in three areas: the selection of the test data to be used, the decision about the relevance of the data, and in the selection of the safety or uncertainty factor.

When Lehman and Fitzhugh introduced this approach at the Food and Drug Administration (FDA), they used a single value of 100 which was intended to include all of the uncertainties and the confidence level of the predictor. However, over the years, it has become customary to divide this value into two factors of 10 and to assign these to the intraspecies and the interspecies variation. More recently, additional modifying factors have been recommended as part of the benchmark or reference dose approach to adjust for lowest-observable-adverse-effect level-to-NOAEL conversion, subchronic-to-

chronic extrapolation, route-to-route extrapolation, differences in susceptibility of the target population, and other factors. Although the intent of these changes is to enhance the precision of the transspecies prediction, the net result is that we now have a cascade of these multiplicative uncertainty factors defined largely by guidelines rather than actual data. Efforts are under way to reduce this problem by using actual data rather than default assumptions whenever possible and to provide documentation of the uncertainties and alternatives for all such assumptions; particularly those which are arbitrarily added to increase the margin of safety rather than because they are essential for the transspecies prediction.

In contrast to this approach, the risk assessment paradigm for carcinogens is currently based on the use of quantitative risk assessment models that focus on the high-dose to low-dose extrapolation rather than on the transspecies extrapolation. Currently, the most popular of these is the linear multistage model in which the upper bound confidence limit of the maximum tolerated dose is extrapolated to zero and the slope or potency for the chemical is estimated from the low-dose region of this line. This approach would be more acceptable to toxicologists if it used the actual dose–response data to calculate potency rather than this hypothetical line. This approach would also be more rational if it used a finite value rather than zero as the origin and thus did not exclude the possibility of a threshold parameter in the extrapolation equation. Because the smallest possible biologic unit is one molecule, the origin cannot be zero, and slopes or potencies based on this assumption will be incorrect. Another way to significantly improve the risk assessment paradigm for carcinogens and noncarcinogens would be to move the responsibility for all of the issues relating directly to conservatism from the risk assessor to the risk manager. The job of the risk assessor is to provide the best estimate or central tendency, or most scientifically defendable estimate of the risk and to indicate the uncertainties and variabilities associated with this estimate. When we arbitrarily include conservatism in a default assumption or guideline for risk assessment, we are usurping the role of the risk manager and mixing policy with science. My final recommendation has more to do with risk communication than with risk assessment, and it is that we focus more on the use of a risk-risk analysis rather than on the risk-benefit equation because we can do a better job of quantitatively assessing and communicating relative risk than we can do with benefits. This presentation has focused on the development and application of the hazard evaluation or threshold approach to risk assessment. The use of this approach to establish exposure limits for workers, military personnel, and other population groups exposed to chemicals in their environment represents the major practical application of the risk assessment paradigm. Regulatory groups such as the FDA and the Occupational Safety and Health Administration and professional groups such as the Committee on Toxicology of the National

Academy of Sciences, the Threshold Limit Value Committee of the American Conference of Governmental Hygienists, and others have used and validated the process. The basis elements of this approach are (1) the priority of human over animal data, (2) the case-by-case evaluation, (3) the use of a threshold concept, and (4) the reliance on good science rather than rigid protocols to achieve an optimal balance between competing risks. In our effort to find ways to improve the risk assessment paradigm, we need to recognize the advantages of our current approach and to ensure that all such recommended changes are beneficial to public health.

Exposure Assessment: Then, Now, and Quantum Leaps in the Future

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ABSTRACT

Health risk assessments have become so widely accepted in the United States that their conclusions are a major factor in many environmental decisions. Although the risk assessment paradigm is 10 years old, the basic risk assessment process has been used by certain regulatory agencies for nearly 40 years. Each of the four components of the paradigm has undergone significant refinements, particularly during the last five years. A recent step in the development of the exposure assessment component can be found in the 1992 EPA *Guidelines for Exposure Assessment*. Rather than assuming worst-case or hypothetical maximum exposures, these guidelines are designed to lead to an accurate characterization, making use of a number of scientific advances. Many exposure parameters have become better defined, and more sensitive techniques now exist for measuring concentrations of contaminants in the environment. Statistical procedures for characterizing variability, using Monte Carlo or similar approaches, eliminate the need to select point estimates for all individual exposure parameters. These probabilistic models can more accurately characterize the full range of exposures that may potentially be encountered by a given population at a particular site, reducing the need to select highly conservative values to account for this form of uncertainty in the exposure estimate. Lastly, our awareness of the uncertainties in the exposure assessment, as well as our knowledge as to how best to characterize them, will almost certainly provide evaluations that will be more credible and, therein, more useful to risk managers. If these refinements are incorporated into future exposure assessments, it is likely that our resources will be devoted to problems that, when resolved, will yield the largest improvement in public health.

INTRODUCTION

During the last 10 years, the risk assessment paradigm has served to evaluate hazards posed by exposure to developmental and reproductive toxicants, mutagens, carcinogens, and systemic toxicants. Many existing environmental criteria and some of our nation's occupational health standards have, at least in part, been based on the results of low-dose extrapolation models and exposure assessments (1-4). Tolerances for pesticides residues, drinking water guidelines, ambient water quality criteria, air standards, and exposure limits for contaminants found in indoor air, consumer products, and other media have been developed that embrace these techniques (5). Most recently, the paradigm has been modified to evaluate potential impacts on fish, wildlife, endangered species, and selected flora due to chemicals or other stressors (6).

The goal of any risk assessment is to estimate the likelihood of an adverse effect on humans, domestic animals, wildlife, or ecological systems from possible exposures to chemical or physical agents. Each of the four components of the risk assessment paradigm has been refined during the last five years, dramatically so in the case of the exposure assessment. A growing acceptance and application of these advances can be found in the 1992 Environmental Protection Agency (EPA) *Guidelines for Exposure Assessment* (7) and in the 1993 Science Advisory Board draft review of the *Risk Assessment Guidance for Superfund: Volume I – Human Health Evaluation Manual* (8). This paper discusses these scientific advances and presents a viewpoint on the way in which exposure assessments should be conducted from this point forth.

EXPOSURE ASSESSMENT

Risk assessments of hazardous waste sites, airborne emissions, effluent discharges, or those conducted as a part of the process for permitting new facilities are often plagued by serious shortcomings in the exposure assessment phase of the analysis. Indeed, this appears to be the most easily mishandled of the four components of the paradigm (Figure 2) (9). It is, however, the one for which we are best prepared to make significant improvements, especially with respect to determining health-based cleanup levels.

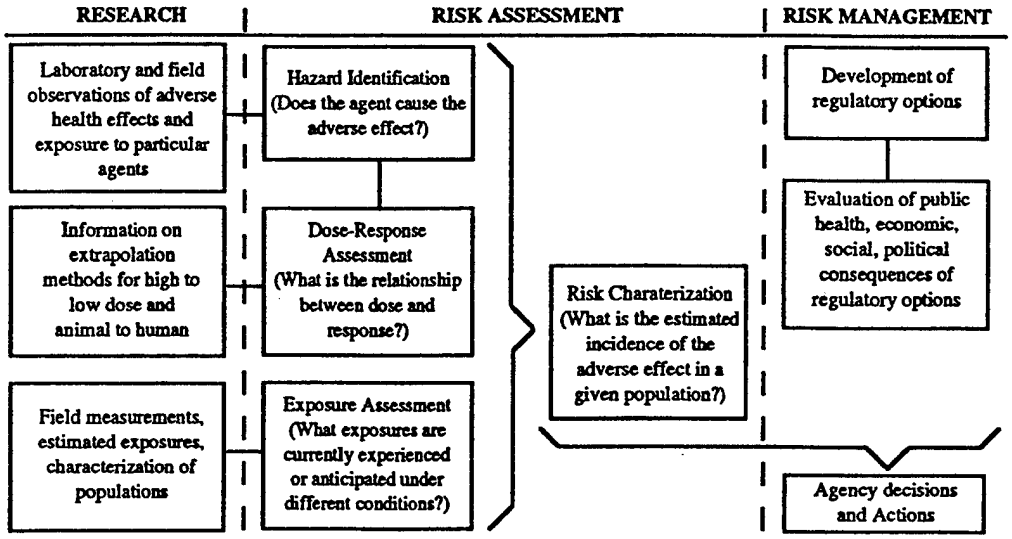


Figure 2. Elements of Risk Assessment and Risk Management (From: National Academy of Science, 1983).

Although there have been numerous claims that exposure assessments are exceedingly difficult and uncertain, this component often contains much less uncertainty than other steps in the process. Admittedly, there are a large number of factors to consider when estimating exposure, and it is a complicated procedure to understand the transport and distribution of a chemical that has been released into the environment (10,11). Nonetheless, the available data indicate that scientists can do an adequate job of quantifying the concentration of chemicals in various media and the resulting uptake by exposed persons provided that they account for all of the important exposure factors (10-14). For some chemicals, the actual uptake need not be estimated using numerous assumptions, but rather can often be measured directly in body fluids, excrement, or hair (15, 39).

There are at least six major pitfalls in the exposure assessment process that should be avoided. First, too much emphasis has been placed on the so-called maximally exposed individual (MEI), and the results of such analyses are often misinterpreted and/or misrepresented. For example, many risk assessments only discuss the MEI using the rationale that this should be the focus of the analysis. However, the EPA *Guidelines for Exposure Assessment* (7) note that a worst-case or MEI analysis should be used only as a screening tool to determine whether exposure is insignificant, not as the basis for characterizing the actual or plausible human health risks (7,16,17). Upon review of these new guidelines, it is clear that the sole use of an MEI analysis is outdated and should be discouraged.

The recently released final *Guidelines for Exposure Assessment* (7) define exposure descriptors that characterize individual, population, and subpopulation groups that may be exposed. The guidelines stress that the high end exposure (HEE) estimate should be a plausible estimate of the individual exposure for a person at the upper end (90th percentile or greater) of an exposure distribution. The HEE should not be an estimate that is beyond the true distribution of exposures. The EPA (7) points out that the high-end exposures should not be defined as those that merely are postulated or hypothesized to occur (i.e., the homeowner living on the fenceline for 70 years). Characterization of the HEE should represent an effort to define the likelihood of individuals falling within the specified range of actual exposure distributions, or attempt to define how many persons (if any) might actually be exposed under the hypothetical scenario.

In earlier exposure assessments, the failure to evaluate different groups of exposed individuals was a serious shortcoming. Under the new Guidelines (7), subpopulations consisting of sensitive or more highly exposed individuals may serve as the basis for developing different exposure scenarios and their resulting risk estimates. For example, recreational anglers may consume more freshwater fish than the general population (18), requiring a separate exposure scenario. In its *Guidance on Risk Characterization for Risk Managers and Risk Assessors*, EPA (6) strongly recommends the use of multiple estimates of risk and exposure in decision-making, stating that all Agency decisions must consider these multiple characterizations.

A second potential pitfall involves the repeated use of conservative assumptions and default exposure scenarios. Several investigators have discussed this issue and have demonstrated its impact on estimates of intake (19-21). The problem can be illustrated by a recent attempt to assess the dioxin hazard associated with the application of pulp and paper mill sludge on agricultural lands (22). An agency evaluated the theoretical cancer risk for a subsistence farm family living on a sludge-amended field. At first review, the analysis of the food consumption pathway appeared reasonable, until one noted that each member of the farmer's family ate more than 8.2 lbs per day of home-raised beef, pork, chicken, dairy products, and contaminated soybean-fed catfish over a lifetime of 70 years. Furthermore, the subsistence food consumption pathway was based on sludge application frequencies 25 times greater than actually practiced; on fish bioaccumulation factors at least 30 times higher than the maximum values reported in the literature; and on sludge dioxin levels as much as 40 times greater than the analytical data would indicate. After estimating the resulting carcinogenic risks in the vicinity of 10^{-2}, it would have

been appropriate for the risk assessors to reconsider whether the exposure scenarios and selected parameters were reasonable. Regrettably, it was this type of exposure assessment that led the agency to go forth and propose a national rule for land application of pulp and paper mill sludge (23,24), one that eventually was withdrawn (25).

The example described above illustrates how exposure analyses that are built on single point estimates often result in conservative, unrealistic characterizations of actual exposure conditions. A solution to these problems is the use of Monte Carlo simulations of exposure. In contrast to the point estimate type of risk assessment that is most often performed, Monte Carlo simulations have become an accepted and rapidly developing technique for characterizing the full range and variation of exposures that may potentially be encountered by a given human population at a particular site. In fact, as voiced by Henry Habicht, Deputy Administrator of the EPA, in his memo accompanying the new *Guidance on Risk Characterization for Risk Managers and Risk Assessors* (26), EPA now is requiring more detailed information on the range of individual and population risks in its decision-making. In the new *Guidelines for Exposure Assessment*, EPA (7) endorsed the use of simulated distributions, such as those produced by the Monte Carlo model, as appropriate means of determining typical and higher end individual and population exposures and risks (26). This approach provides more realistic estimates of exposure and minimizes the unnecessary overstatement of risks.

The third potential pitfall is the improper use and statistical evaluation of environmental data, which may result in inaccurate exposure estimates. As has been shown repeatedly, most environmental and occupational data are log-normally distributed and not Gaussian-distributed (27-28). This observation is relevant in light of earlier Superfund guidance (41) that called for the use in risk assessments of the 95% upper confidence limit of the arithmetic mean concentration. For this recommendation to have relevance, the data must be normally distributed. When data are lognormally distributed, the approach results in greatly overestimated concentrations. In contrast, the EPA Science Advisory Board (8) criticizes such practices and instead recommends that a full distributional approach be embraced. This is possible via Monte Carlo exposure assessment and statistical methods such as kriging or triangulation for quantifying the spatial distribution of environmental concentrations. As noted by EPA, inappropriate statistical analysis of environmental data represents one of the most easily corrected of the common errors in exposure analysis (16).

Statistical handling of samples that have no detectable level of a contaminant (29-31) may also lead to errors. Often, the majority of samples collected at a site will contain no measurable amount of contaminant. Frequently, regulatory agencies will use the minimum detection limit (MDL) of the analysis in the calculations on the premise that the contaminant might be present at that level. Some agencies have suggested that 50% of the MDL should be used to calculate the plausible degree of human exposure. When such an approach is used on a site that is only 2 to 10% contaminated (surface area), the predicted average level of contamination will be much higher than what is likely. A convenient approach to help avoid this problem was presented by Travis and co-workers (42).

The fourth potential pitfall is to conduct an exposure assessment without considering the environmental fate of the chemical, usually resulting in overstated exposure estimates. Many factors such as degradation by sunlight, soil and water microbes, and evaporation can influence the degree of human exposure (5). For instance, the public health hazard posed by the potential release of dioxin vapors from incinerators was evaluated. It was alleged that the vapors posed a serious health hazard to surrounding residents, and a risk assessment was conducted. It was soon recognized that the environmental half-life of dioxin (as a vapor) was a critical factor in this analysis because it had a half-life of only 90 min. In contrast, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in soil and fly-ash may have an environmental half-life of 12 to 50 years. What had been portrayed as a potentially serious health hazard was shown to be insignificant when half-life was considered.

An agency Phase I risk assessment for the upper Hudson River (32) provides a second illustration of this critical principle. By considering the rate of decline of polychlorinated biphenyl levels in Hudson River fish, it was shown that estimated risks would be reduced by a factor of eight. Environmental fate should be factored into any assessment that considers exposure opportunities over any appreciable length of time. Failure to do so can result in a significant overstatement of uptake when the exposure duration exceeds a time period of two-to-three times the half-life of the chemical of concern.

The fifth potential pitfall is the failure to validate exposure assumptions or model estimates by using actual measurements or biological monitoring to validate or confirm the predicted degree of human exposure. Although, in the past, there were inadequate sampling and analytical procedures to measure the low levels of toxicants found in the environment, better techniques have become available. As these

field measurements are refined, less reliance should be placed on mathematical models for predicting the distribution of chemicals in the environment.

Consideration should also be given to the use of biological monitoring to validate or confirm the predicted degree of human exposure (9). Over the past five years, analytical chemists have increased their ability to detect very small quantities of non-natural chemicals in blood, urine, hair, feces, breath, and fat. Measurement of parts per trillion and parts per quadrillion is now possible. For many chemicals, the results represent a direct indicator of either recent or chronic exposure to a chemical. For example, the uptake of dioxin by Vietnam veterans exposed to 2,4,5-trichlorophenoxyacetic acid herbicides was evaluated by analyzing the amount of dioxin in their blood. This study, conducted almost 15 to 20 years after the last day of service in Vietnam, allowed epidemiologists to conclude that the vast majority of veterans had only a modest degree of exposure to this chemical which has been alleged to produce numerous adverse health effects in field soldiers (33).

In any exposure assessment, a validation should be performed to ensure that the assumptions and results are reasonable, such as use of mass balance "reality checks" or other methods of substantiating assumptions. An example of this problem was the evaluation of the cancer hazard posed by dioxin-contaminated soot following an office building fire (20). One well-known assessment assumed that office workers might be exposed to the dioxin in the soot for the entire 46 years that they might work in the building and that the dioxin would be released through volatilization at a particular rate and then inhaled. It was estimated that persons who worked in the office building would be exposed to an increased cancer risk much greater than 1 in 1,000,000 and, as a result, the building was not reoccupied.

After further evaluation, it was shown that at the assumed rate of volatilization, virtually all the dioxin would have been volatilized and removed by the ventilation system only four years after reoccupation. In short, the assessment assumed exposure was to occur for 46 years, yet inconsequential amounts of dioxin were available during 42 of these years.

Another example was the agency risk assessment performed to evaluate the need for regulating the land application of pulp and paper mill sludge (22). In this risk assessment, the purpose of which was to define a maximum acceptable level of TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) in landspread sludge, mass balance considerations were ignored. For the inhalation pathway of exposure,

the vapor emissions model not only neglected mass balance but, also, it assumed an infinite depth of contamination through the soil column. These unreasonable modeling assumptions resulted in a prediction that 100 times more TCDF would leave the site through volatilization than the amount that was originally applied via landspreading. The lesson is that in any exposure assessment, a validation should be performed to ensure that the assumptions and results are reasonable.

The sixth potential pitfall is to neglect indirect pathways of exposure. For example, the uptake of a contaminant in water via ingestion is obvious (and direct), but the uptake by garden vegetables due to watering or uptake via the inhalation of volatile contaminants while showering are indirect pathways not always evaluated in an assessment (34). Perhaps the most important indirect route of exposure to be considered when regulating airborne nonvolatile chemicals is through the ingestion of particulate emissions that have deposited onto soil and plants and are subsequently eaten by grazing animals (10,35). The ingestion of the meat and milk from these animals can produce risks 200- to 500-fold greater than those resulting from inhalation (35). Methods for estimating uptake through many of these indirect routes have been developed (9,10,36).

CONCLUSION

Exposure assessment procedures have matured a great deal over the past five years. Reliance on worst-case exposure scenarios is no longer necessary in light of better information on specific exposure parameters and more sensitive techniques for measuring concentrations of contaminants in the environment. New and highly sensitive analytical procedures will permit us to assay hair, blood, urine, adipose, and other biologic media to validate the reasonableness of the exposure estimates. Statistical procedures that account for the distribution of the various factors within the exposed population will almost certainly be an integral portion of risk assessments conducted under the new EPA guidance and guidelines (7,26). Lastly, our awareness of the numerous uncertainties in the process, as well as our knowledge of how to best characterize them, will almost certainly provide assessments that will be more credible and, therein, more useful to risk managers (37,38). If these refinements are incorporated into future exposure assessments, it is likely that our resources will be devoted to problems that, when resolved, will yield the largest improvement in public health and the environment.

REFERENCES

1. B. N. Ames, "Six common errors relating to environmental pollution," *Reg. Toxicol. Pharmacol.* **7**, 379 (1987).

2. W. D. Ruckelshaus, "Science, risk, and public policy," *Science* **221**, 1026 (1984).

3. Office of Science and Technology Policy (OSTP), "Chemical carcinogens: A review of the science and its associated principles," *Federal Register* **50**, 10372 (1985).

4. P. W. Preuss and A. M. Ehrlich, The Environmental Protection Agency's risk assessment guidelines, *J. Air Poll. Cont. Assoc.* **37**, 784 (1987).

5. D. J. Paustenbach, "A Survey of Health Risk Assessment, in D.J. Paustenbach (ed.), *The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies*, (John Wiley and Sons, New York, 1989a), pp. 27-125.

6. U. S. Environmental Protection Agency (EPA). *Framework for Ecological Risk Assessment* (Report No. EPA/630/R-92/001. Risk Assessment Forum, U. S. Environmental Protection Agency, Washington, D.C., 1992a).

7. U. S. Environmental Protection Agency (EPA). *Final Guidelines for Exposure Assessment. Federal Register* 57, 104. (U. S. Environmental Protection Agency, Washington, D.C., 1992b).

8. Science Advisory Board (SAB). *Draft Review of the Risk Assessment Guidance for Superfund: Volume I - Human Health Evaluation Manual (RAGS).* (U. S. Environmental Protection Agency, Washington, D.C., 1993).

9. P. Lioy, "Assessing total human exposure to contaminants," *Environ. Sci. Technol.* **24**, 938 (1990).

10. G. F. Fries and D. J. Paustenbach. "Evaluation of potential transmission of 2,3,7,8-TCDD contaminated incinerator emissions to humans via food," *J. Toxicol. Environ. Health* **29**, 1 (1989).

11. A. di Domenico, "Guidelines for the definition of environmental action alert thresholds for polychlorinated dibenzodioxins and polychlorinated dibenzofurans," *Reg. Toxicol. Pharmacol.* **11**, 8 (1990).

12. A. Eschenroeder, R. J. Jaeger, J. J. Ospital, and C. Doyle. "Health risk analysis of human exposures to soil amended with sewage sludge contaminated with polychlorinated dibenzodioxins and dibenzofurans," *Vet. Hum. Toxicol.* **28**, 435 (1986).

13. T. J. Smith, "Exposure assessment for occupational epidemiology," *Am. J. Ind. Med.* **12**, 249 (1987).

14. D. J. Paustenbach, "A comprehensive methodology for assessing the risk to humans and wildlife posed by contaminated soil," in D. J. Paustenbach (ed.), *The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies* (John Wiley and Sons, New York, NY, 1989b), pp. 296-330.

15. A. Lynch, *Methods in Biological Monitoring* (John Wiley and Sons, New York, NY, 1987).

16. U. S. Environmental Protection Agency (EPA). *Proposed Guidelines on Exposure Related Measurements. Federal Register* **53**, 232 (U. S. Environmental Protection Agency, Washington, D.C., 1988).

17. B. Goldstein, "The problem with the margin of safety: Toward the concept of protection," *Risk Anal.* **10**, 7 (1990).

18. E. S. Ebert, J. W. Knight, N. W. Harrington, R. E. Keenan, and K. J. Boyle. Accepted for publication. Estimating Consumption of Freshwater Fish. *North Am. J. Fisheries Management.*

19. E. K. Silbergeld, "Five types of ambiguity: Scientific uncertainty in risk assessment," *Haz. Waste Haz. Materials* **4**, 139 (1987).

20. D. Maxim, "Problems associated with the use of conservative assumptions in exposure and risk analysis," in D. J. Paustenbach (ed.) *The Risk Assessment of Environmental and Occupational Health Hazards: A Textbook of Case Studies* (John Wiley and Sons, New York, NY, 1989), pp. 525-560.

21. T. E. McKone and K. T. Bogen. "Predicting the uncertainties in risk assessment," *Environ. Sci. Technol.* **25**(10) 1674-1681 (1991).

22. U. S. Environmental Protection Agency (EPA), *Assessment of Risks from Exposure of Humans, Terrestrial and Avain Wildlife, and Aquatic Life to Dioxins and Furans from Disposal and Use of Sludge from Bleached Kraft and Sulfite Pulp and Paper Mils,* Report No. 560/5-90-013 (Office of Toxic Substances, Office of Solid Waste, U. S. Environmental Protection Agency, Washington, D.C., 1990).

23. U. S. Environmental Protection Agency (EPA), *Human Health Risk Assessment for Dioxin in Pulp and Paper Sludge: Technical Support Document for the Proposed Land Application Rule,* Contract No. 68-DO-0020 (Office of Solid Waste, U. S. Environmental Protection Agency, Washington, D.C., 1991).

24. U. S. Environmental Protection Agency (EPA), *Land Application of Sludge from Pulp and Paper Mills Using Chlorine and Chlorine Derivative Bleaching Processes: Proposed Rules. Federal Register* **56**, 21802 (U. S. Environmental Protection Agency, Washington, D.C., 1991b).

25. U. S. Environmental Protection Agency (EPA), *Regulatory Investigation of Dioxin in Pulp and Paper Mill Sludge. Federal Register* **58**, 78 (U. S. Environmental Protection Agency, Washington, D.C., 1993).

26. U. S. Environmental Protection Agency (EPA), Memo to Assistant Administrators and Regional Administrators from F.H. Habicht, Re: *Guidance on Risk Characterization for Risk Managers and Risk Assessors* (U. S. Environmental Protection Agency, Washington, D.C., 1992c).

27. W. R. Ott, "A physical explanation of the lognormality of pollutant concentrations," *J. Air Waste Manage. Assoc.* **40**, 1378-1383 (1990).

28. R. L. Sielken, "Statistical evaluations reflecting the skewness in the distribution of TCDD levels in human adipose tissue," *Chemosphere* **16**, 2135 (1987).

29. T. B. Parkin, J. J. Melsinger, S. T. Chester, J. L. Starr, and J. A. Robinson, "Evaluation of statistical estimation methods for lognormally distributed variables," *Soil Sci. J.* **52**, 323 (1988).

30. C. C. Travis, M. L. Land, and H. Hattemer-Frey, "Estimating the mean of data sets with nondetectable values," *Environ. Sci. Technol.* **24**, 981 (1990).

31. C. N. Haas and P. A. Scheff, "Estimation of averages in truncated samples," *Environ. Sci. Technol.* **24**, 912 (1990).

32. U. S. Environmental Protection Agency (EPA), *Phase I Report - Review Copy Interim Characterization and Evaluation Hudson River PCB Reassessment RI/FS: Alternative Remedial Contracting Strategy (ARCS) for Hazardous Waste Remedial Services.* EPA Contract No. 68-S9-2001 (U. S. Environmental Protection Agency, Washington, D.C., 1991c).

33. L. Needham, "Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in U. S. Army Vietnam-era veterans: CDC Veterans health studies," *JAMA* **260**, 1249 (1988).

34. J. Byard, "Hazard assessment of 1,1,1-Trichloroethane in groundwater," in D.J. Paustenbach (ed.), *The Risk Assessment of Environmental Hazards: A Textbook of Case Studies,* (John Wiley and Sons, New York, NY, 1989), pp. 331-344.

35. J. B. Stevens and E. N. Gerbec, "Dioxin in the agricultural food chain," *Risk Anal.* **8**, 329 (1986).

36. R. E. Keenan, M. M. Sauer, F. H. Lawrence, E. R. Rand. and D. W. Crawford, "Examination of Potential Risks from Exposure to Dioxin in Sludge Used to Reclaim Abandoned Strip Mines," in D. J. Paustenbach (ed.), *The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies,* (John Wiley and Sons, New York, NY, 1989), pp. 935-998.

37. C. Whipple, "Dealing with uncertainty about risk in risk management," *Hazards: Technology and Fairness* (National Academy Press, Washington, D.C., 1986), pp. 44-60.

38. C. Whipple, "Nonpessimistic risk assessment and de minimis risk as risk management tools," in D. Paustenbach (ed.), *The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies*, (John Wiley and Sons, New York, NY, 1989), pp. 1105-1120.

39. C. Rappe and R. Andersson, "Levels of PCDDs and PCDFs in human feces," *Proceedings of the 12th International Symposium on Dioxins and Related Compounds* (1992), pp. 195-198.

40. National Academy of Sciencies (NAS), Risk Assessment in the Federal Government: Managing the Process (National Academy Press, Washington, D.C., 1983).

41. U. S. Environmental Protection Agency (EPA), *Risk Assessment Guidance for Superfund: Human Health Evaluation Manual, Part 1,* Report No. 9285.701A (Office of Emergency and Remedial Response, U. S. Environmental Protection Agency, Washington, D.C., 1990).

42. C. C. Travis, M. L. Land, and H. Hattemer-Frey, "Estimating the mean of data sets with nondetectable values," *Environ. Sci. Technol.* **24**, 981 (1990).

SESSION II

CASE COMPARISONS – ISSUES/LESSONS LEARNED

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Development of an Oral Reference Dose (RfD) for Essential Nutrients: A Comparison of Selenium and Zinc

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ABSTRACT

The quality and breadth of toxicity data available for essential trace elements (ETEs) affects the ability of risk assessors to establish a safe level of chronic, daily exposure to these potentially toxic substances. This process is further complicated by the fact that ETEs have been found to meet a level of intake essential to the nutrient requirements of practically all healthy people, as defined by the Recommended Dietary Allowance (RDA). The examples presented in this paper illustrate the need for well-defined data for both the essential and toxicologic components of the ETEs. The current RDA for selenium (Se) in the United States reference adult is 0.87 μg/kg. At Se intake levels above the RDA, but below conditions characterized as clinical selenosis, there are no reliable indicators of Se toxicity. However, a no-observable-adverse-effect level (NOAEL) of 15 μg Se/kg-day and a lowest-observable-adverse-effect level (LOAEL) of 23 μg Se/kg-day was established based on a regression analysis in Chinese subjects living in geographical areas with low, medium and high selenium levels in the soil and food supply (Yang et al. 1989). The NOAEL and LOAEL were determined from the lowest correlative Se intake that produced no clinical signs of selenosis and early clinical signs of selenosis, respectively. To account for sensitive individuals, an uncertainty factor of 3 was applied to the NOAEL yielding an oral RfD for Se of 5 μg/kg-day. This RfD accounts for the essentiality of Se, is protective for all ages and physiologic groups, and does not encroach the RDA.

A less robust toxicity database is available for zinc (Zn). Based on data from Yardrick et al. (1989), a clinical reduction in erythrocyte superoxide dismutase (ESOD) occurred following administration of zinc carbonate supplements to healthy women. It is well-documented that excessive zinc alters copper status which is manifested by ESOD enzyme activity. From this study, a LOAEL of 1 mg Zn/kg-day for effects on human health is established. To account for both the minimal LOAEL from a

moderate duration study and sensitive human populations, an uncertainty factor of 3 was applied, yielding an RfD for Zn of 0.3 mg/kg-day. This RfD is protective from chronic toxicity and meets the essential dietary needs for adults (0.2 mg Zn/kg-day), but is lower that the dietary needs of infants, young children, and pregnant or lactating women. Unlike the RfD for Se which has a high confidence, the RfD for Zn is rated medium. However, the RfD for the soluble salts of Zn is sufficient to meet the requirements in adolescents and adults, but does not meet the RDA for those who have greater requirements for a short, less-than-lifetime duration. The RDA is recommended to be used for short-term requirements. The ramifications of these two risk assessments will be discussed.

Respiratory Health Effects of Passive Smoking: Risk Assessment Methodologies

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ABSTRACT

The U.S. Environmental Protection Agency (EPA) has published a major assessment of the respiratory health risks of passive smoking (1). The report concludes that exposure to environmental tobacco smoke (ETS) – commonly known as secondhand smoke – is responsible for approximately 3,000 lung cancer deaths each year in nonsmoking adults in the United States and seriously affects the respiratory health of hundreds of thousands of children. This paper summarizes the methodologies used in the EPA report and the major findings.

BACKGROUND

In recent years, comparative risk studies performed by the Environmental Protection Agency (EPA) and its Science Advisory Board have consistently ranked indoor air pollution among the top five environmental risks to human health (2,3). Environmental tobacco smoke (ETS) is one of the major indoor air pollutants and, given the known health impact of tobacco smoking, there has been concern that nonsmokers may also be at risk of serious health effects (4,5,6,7,8). As part of its efforts to address all types of indoor air pollution, the EPA conducted a risk assessment of the respiratory health effects of passive smoking.

MAJOR CONCLUSIONS

Based on the weight of the available scientific evidence, EPA has concluded that the widespread exposure to ETS in the United States presents a serious and substantial public health risk.

In adults:

- ETS is a human lung carcinogen, responsible for approximately 3,000 lung cancer deaths annually in United States nonsmokers. Environmental tobacco smoke has been classified as a known human or Group A carcinogen under EPA's carcinogen assessment guidelines (9). This classification is reserved for those compounds or mixtures that have the strongest data to determine a cause-and-effect relationship, including data from human populations. Only 10 other agents, including asbestos and radon, have been classified by EPA as Group A carcinogens, and ETS is the only one for which cancer has been observed at typical non-occupational environmental levels (10).

- ETS has subtle but significant effects on the respiratory health of nonsmokers, including coughing, phlegm production, chest discomfort, and reduced lung function.

In children:

- ETS exposure increases the risk of lower respiratory tract infections such as bronchitis and pneumonia. The EPA estimates that between 150,000 and 300,000 of these cases annually in infants and young children up to 18 months of age are attributable to exposure to ETS. Of these, between 7,500 and 15,000 will result in hospitalization.

- ETS exposure increases the prevalence of fluid in the middle ear, a sign of chronic middle ear disease. Fluid in the middle ear is the major cause of hospitalization of young children for an operation in the United States.

- ETS exposure in children irritates the upper respiratory tract and is associated with a small but significant reduction in lung function.

- ETS exposure increases the frequency of episodes and severity of symptoms in asthmatic children. The report estimates that 200,000 to 1,000,000 asthmatic children have their condition worsened by exposure to ETS.

- ETS exposure is a risk factor for new cases of asthma in children who have not previously displayed symptoms. The EPA estimates that ETS exposure may be responsible for 8,000 to 26,000 such new cases of asthma annually.

SCIENTIFIC APPROACH

The EPA's methodology for hazard identification of health effects is based on a total weight-of-evidence approach, which encompasses evidence on exposure, physical and chemical properties, and toxicology, including animal and human studies. Because ETS contains over 4,000 individual components, including over 40 known human and animal carcinogens, examining components individually would be prohibitive. Instead, ETS was evaluated as a complex mixture. Also, emphasis was focused on the respiratory system because that provided the largest database.

The methodologies used for the assessment of lung cancer and respiratory effects in the EPA report differ somewhat. First, lung cancer is only seen in adults and is thought to represent the effect of long-term exposure. The noncancer respiratory effects examined are most apparent in children, and some of these are irritation effects associated with acute exposures. Second, for lung cancer less is known about mechanisms than is the case for some of the childhood respiratory effects, and this leads to differences in the development of the evidence. Third, because there were 30 studies on lung cancer and ETS, this database was analyzed several different ways before arriving at an overall conclusion. For the various childhood respiratory effects that were examined, there were fewer studies of any one effect, and analysis was more limited.

For all effects, studies examine home smoking patterns as a surrogate for ETS exposure. The exposure surrogate in the studies of lung cancer among nonsmokers is spousal smoking patterns. For childhood respiratory effects, parental smoking is the most common surrogate, although recent studies have also shown high correlations between body metabolites of ETS and pneumonia, bronchitis, asthma, and fluid in the middle ear.

There is nearly universal exposure to ETS, which often clouds the distinction between "exposed" and "unexposed" subjects and makes any potential effects difficult to observe. To try to eliminate the effect of some of these misclassified exposures, two methods are used. For hazard identification purposes, trend analysis and analyses comparing high exposure groups with controls are conducted. For population risk estimates, a model which adjusts for background (i.e., non-home) exposures is used.

Lung Cancer

The conclusion that ETS is a human lung carcinogen is based on the total weight of the available scientific evidence. This evidence includes:

- the exposure-related lung carcinogenicity of mainstream smoke in active smokers, with no evidence of an exposure threshold;
- the chemical similarity of mainstream smoke and ETS, both of which contain over 40 carcinogens;
- supporting evidence of ETS carcinogenicity from animal bioassays and genotoxicity studies;
- evidence of ETS exposure and uptake by nonsmokers; and
- the statistically significant exposure-related increase in lung cancer risk observed in an analysis of 30 epidemiology studies of ETS and lung cancer from eight different countries.

The epidemiology studies attempt to estimate the relative risk of lung cancer from actual environmental levels of ETS. Such investigations are inherently difficult for a variety of reasons, not the least of which is the fact that virtually everyone is exposed to some level of ETS from a variety of different sources. Therefore, the studies try to compare risks in people with greater versus lesser exposures. All 30 epidemiology studies provide data on female never-smokers classified as "exposed" or "unexposed" to ETS on the basis of whether or not their husbands smoke. Spousal smoking is a major source of exposure that is relatively stable over time, and these data on female never-smokers and spousal smoking status comprise the largest database for analyzing the lung cancer risks from ETS exposure. Nevertheless, spousal smoking status is a crude exposure measure, and the studies are prone to exposure misclassification which decreases their ability to detect an increased risk if one exists. Furthermore, many of the studies are of small size and have a low statistical power to detect an increased risk.

In the EPA report, the epidemiologic data are analyzed a variety of different ways, and each analysis demonstrates an association between ETS and lung cancer. First, the studies were analyzed individually. Twenty-four of the 30 studies found an increased risk of lung cancer in the exposed group; nine of these were statistically significant. This proportion (9/30) of significant studies is highly unlikely to have occurred by chance (probability $<$ 1-in-10,000). In addition, ALL 17 studies with data categorized by exposure level (i.e., amount of spousal smoking) found an increased risk of lung cancer in the highest

exposure group, and 9 of the 17 were statistically significant (probability < 1-in-10,000,000), despite most having a small sample size. Examining only the highest exposure group helps to minimize exposure misclassification in the "exposed" group, because women whose spouses smoke a lot are more likely to be exposed to substantial amounts of ETS. Finally, 10 of the 14 studies with sufficient data for a trend test showed a statistically significant exposure-response relationship (probability < 1in-10,000,000,000) such as increasing risk of lung cancer with increasing ETS exposure.

The study data were also combined by country, using a statistical procedure called "meta-analysis" to pool the data. Combining datasets increases the ability to detect an effect, if one is present, and provides an objective means of including all studies, both with positive and non-positive results, in the analysis. This combined analysis also showed increased risks, consistent with the analyses of the individual studies.

A number of potential modifying factors, such as diet and occupation, were also examined, and it was determined that they could not account for the observed increased risks. Furthermore, the consistency of the results across numerous independent studies from different countries argues against the existence of any one factor other than exposure to ETS as an explanation for the observed results.

In summary, the total weight of the evidence is overwhelmingly supportive of a conclusion that ETS causes lung cancer in humans.

The population risk estimate of approximately 3,000 lung cancer deaths per year in United States nonsmokers is based on the pooled relative risk estimate for the 11 United States epidemiology studies on ETS and lung cancer, with an adjustment for other sources of ETS exposure in addition to spousal smoking. The adjustment uses biological markers of ETS exposure to assess relative ETS exposure between nonsmokers with and without spousal exposure. The estimate of 3,000 is consistent with estimates generated in an alternative analysis based on the Fontham et al. study (11), the only study that provided data on both relative risk and relative exposure.

The overall estimate of 3,000 lung cancer deaths is a composite of estimates of 1,500 for female never-smokers, 500 for male never-smokers, and 1,000 for long-term former smokers of both sexes. To extend the analyses of female never-smokers to male never-smokers and to long-term former smokers,

the estimated relative risks were converted to excess risks, and these excess risks were assumed to apply to the male never-smokers and the former smokers. This assumption may underestimate the risk in male never-smokers and long-term former smokers, since, for example, males are exposed to greater levels of background ETS. An alternate breakdown of the estimated 3,000 lung cancer deaths attributes 800 deaths to "spousal" (or home) exposure and 2,200 deaths to other sources of exposure, such as work and public places. The EPA has relatively high confidence in these estimates, especially those for female never-smokers, because they are based on increased risks observed in humans exposed to ETS at actual environmental levels.

Noncancer Respiratory Disorders

The weight of evidence for the noncancer respiratory disorders includes mechanistic information on tobacco smoke's effects on the lung, as well as data from over 100 epidemiological studies. Both maternal smoking during pregnancy and postnatal exposure to ETS can predispose a child to a variety of respiratory effects that can themselves have long-term consequences. Maternal smoking during pregnancy can affect the developing lung, causing permanent changes in lung structure and function, (e.g. decreased lung elasticity). Postnatal exposures to ETS may similarly affect lung development, as well as increase bronchial responsiveness and enhance the process of allergic sensitization of the lung. These changes may predispose children to acute lower respiratory tract infections early in life, and to asthma, lower levels of lung function, and chronic airflow limitation later in life.

Epidemiology studies have consistently demonstrated increased risks of lower respiratory tract infections in young children whose parents smoke. In addition, epidemiology studies of children show that ETS exposure is causally associated with increased prevalence of fluid in the middle ear, symptoms of upper respiratory tract irritation (e.g., coughing and wheezing), and reductions in lung function. Environmental tobacco smoke exposure is also causally associated with additional episodes and increased severity of symptoms in children with asthma. Furthermore, the data are suggestive that ETS exposure can cause new cases of asthma in children who have not previously displayed symptoms; however, there were too few studies to make a conclusive determination. No conclusions could be drawn about upper respiratory tract infections (i.e., colds and sore throats) or middle ear infections in children. The epidemiology studies of noncancer respiratory disorders in nonsmoking adults generally relied on spousal smoking as a surrogate for ETS exposure, and also demonstrated significant effects, including coughing, phlegm production, chest discomfort, and reduced lung function.

Because of the widespread exposure to ETS and the high incidence rates for respiratory illnesses and disorders, even small increases in risk can result in substantial numbers of cases being attributable to ETS. For example, acute lower respiratory tract infections are one of the leading causes of morbidity and mortality during infancy and childhood, and the EPA report estimates that ETS exposure is responsible for 150,000 to 300,000 cases in children up to 18 months, resulting in 7,500 to 15,000 hospitalizations, each year. Fluid in the middle ear is another common affliction in young children and is the most common reason for hospitalization of young children for an operation. As a final example of the public health impacts of ETS exposure, the EPA estimates that as many as one million asthmatic children have their condition worsened by exposure to ETS.

REFERENCES

1. U. S. Environmental Protection Agency, "Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders," EPA/600/6-90/006F, (Washington, D.C., 1992).

2. U. S. Environmental Protection Agency, "Unfinished Business: A Comparative Assessment of Environmental Problems," (Washington, D.C., 1987).

3. Science Advisory Board, U. S. Environmental Protection Agency, "Reducing Risk: Setting Priorities and Strategies for Environmental Protection," SAB-EC-90-021, (Washington, D.C., 1990).

4. U. S. Department of Health and Human Services (U. S. DHHS), "The Health Consequences of Smoking: Cancer. A Report of the Surgeon General," (U. S. DHHS, Public Health Service, Washington, D.C., 1982).

5. U. S. Department of Health and Human Services, "The Health Consequences of Smoking: Chronic Obstructive Lung Disease. A Report of the Surgeon General," DHHS Pub. No. (PHS) 84-50205, (U. S. DHHS, Public Health Service, Office of the Assistant Secretary for Health, Office of Smoking and Health, Washington, D.C., 1984).

6. International Agency for Research on Cancer, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 38, Tobacco Smoking," (Lyon, France: World Health Organization, 1986).

7. U. S. Department of Health and Human Services, "The Health Consequences of Involuntary Smoking. A Report of the Surgeon General," DHHS Pub. No. (PHS) 87-8398, (U. S. DHHS, Public Health Service, Office of the Assistant Secretary for Health, Office of Smoking and Health, Washington, D.C., 1986).

8. National Research Council, "Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects," (National Academy Press, Washington, D.C., 1986).

9. U. S. Environmental Protection Agency, "Guidelines for Carcinogen Risk Assessment," *Federal Register* **51**, 33992-34003 (1986).

10. Integrated Risk Information System (IRIS), U. S. Environmental Protection Agency. (Washington, D.C., 1993).

11. E.T.H. Fontham, P. Correa, A. Wu-Williams, P. Reynolds, R.S. Greenberg, P.A. Buffler, V.W. Chen, P. Boyd, T. Alterman, D.F. Austin, J. Liff, and S.D. Greenberg, "Lung Cancer in Nonsmoking Women: A Multicenter Case-Control Study. *Cancer Epidemiol. Biomarkers Prev.* **1**(1):35-43 (1991).

Beryllium: Health Risk Assessment and Risk Characterization Issues

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ABSTRACT

In 1983, the National Academy of Sciences developed a generic approach to evaluating risks associated with exposure to environmental pollutants. The process by which this evaluation occurs would appear to be relatively straightforward especially in the case of a well-studied compound where both human and animal data and multiple route exposure information exists. The U.S. Environmental Protection Agency has recently conducted an extensive review of the health effects data and exposure information on Beryllium (Be). Based on the amount of human and animal data available, Be would be an "ideal" compound for an holistic risk analysis and risk characterization. This paper discusses how health and exposure assessment data on Be examines the inherent difficulties in the identification and quantification of risks and the uncertainties underlying the agency risk characterization of Be. Included in this review will be a discussion of the lack of quantifiable exposure levels, multiple and concurrent human exposures, as well as the use of supportive multiple route, multiple exposure animal data.

Mechanistic Insights Aid the Search for CFC Substitutes: Risk Assessment of HCFC-123 as an Example

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ABSTRACT

An international consensus on the need to reduce the use of chlorofluorocarbons (CFCs) and other ozone-depleting gases, such as the halons, led to the adoptions of the 1987 Montreal Protocol and Title VI of the 1990 Clean Air Act Amendments, "Protecting Stratospheric Ozone". These agreements included major provisions for reducing and eventually phasing out production and use of CFCs and halons as well as advancing the development of replacement chemicals. Because of the ubiquitous use and benefits of CFCs and halons, an expeditious search for safe replacements to meet the legislative deadlines is of critical importance. Toxicity testing and health risk assessment programs were established to evaluate the health and environmental impact of these replacement chemicals. Development and implementation of these programs as well as the structural−activity relationships significant for the development of the replacement chemicals are described below. A dose−response evaluation for the health risk assessment of the replacement chemical HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) is also presented to show an innovative use of physiologically based pharmacokinetic (PBPK) modeling. This is based on a parallelogram approach using data on the anesthetic gas halothane, a structural analog to HCFC-123. Halothane and HCFC-123 both form the same metabolite, trifluoroacetic acid, indicative of the same metabolic oxidative pathway attributed to hepatotoxicity. The parallelogram approach demonstrates the application of template model structures and shows how PBPK modeling, together with judicious

experimental design, can be used to improve the accuracy of health risk assessment and to decrease the need for extensive laboratory animal testing.

INTRODUCTION

Concern about ozone depletion in the stratosphere was first raised in 1974 with publication of research by Molina and Rowland(1). They theorized that chlorofluorocarbons (CFCs), due to their unique stability, do not decompose in the lower atmosphere but instead slowly migrate to the stratosphere. Ultraviolet (UV) radiation breaks the molecules apart and releases chlorine which then reacts with ozone. In the 13 years following that original proposal, a consensus emerged that chlorine derived from the CFCs, as well as bromine from halons, decrease ozone in the stratosphere. The Protocol on Substances that Deplete the Ozone Layer was signed by 24 nations and the European Economic Community in Montreal, Canada. Known as the Montreal Protocol, this landmark international agreement sets a schedule to control the production and consumption of CFCs and halons. Consequently, a search for substitute chemicals for CFCs and halons ensued. This search for substitutes required toxicity testing of the chemicals under consideration and a consortium of participating manufacturing companies known as the Programme for Alternative Fluorocarbon Toxicity Testing (PAFT) was developed to provide test results in an expedited fashion. These data may be the basis for deriving dose–response estimates needed to characterize the potential health risk of the substitutes under the Significant New Alternatives Policy Program (SNAP) of the U.S. Environmental Protection Agency (EPA).

In this paper, the dose–response analysis for both short- and long-term exposures to a single compound replacement, HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane), is presented in the context of the Montreal Protocol, domestic regulations, structural–activity relationships of potential replacement chemicals, toxicity testing programs, and health risk characterization scenarios defined by the SNAP. Due to its structure and efficacy, HCFC-123 is a key candidate for the replacement of Halon-1211 as a liquid stream fire-fighting agent and of CFCs in chillers and rigid foam insulation. HCFC-123 is a structural analog to the anesthetic gas halothane and both are metabolized to potentially toxic intermediates via the same pathways. An innovative use of physiologically based pharmacokinetic (PBPK) modeling is presented that is based on a parallelogram approach using data on halothane. The parallelogram approach demonstrates the potential application of template model structures and how PBPK modeling, together with judicious experimental design, can be used to improve the accuracy of health risk assessment and decrease the need for extensive laboratory animal testing.

Theory of Stratospheric Ozone Depletion

Unlike many other environmental issues, stratospheric ozone protection is a truly global issue requiring an international resolution. Given that the consumption of CFCs and halons is ubiquitous, numerous developed nations and even some developing nations are involved in their production. Due to their chemical stability, CFCs and halons become widely dispersed in the atmosphere over time. Thus, the release of these chemicals in one country could adversely affect the health and welfare of other countries. Figure 3 shows the theory of stratospheric ozone depletion. Because the stratospheric ozone layer shields the earth against UV radiation, ozone depletion would allow increased damaging UV radiation to penetrate to the earth's surface. A 1% depletion in ozone has been estimated to increase exposure to UV radiation by 1.5 to 2.0%.(2,3,4)

A risk assessment performed by the EPA to evaluate the impact of such an increase in UV radiation focused on the following areas: (1) increases in skin cancer, (2) increases in cataracts, (3) suppression of the human immune system, (4) damage to crops, and (5) damage to aquatic organisms.(2,3,4) For each area, significant increases in the effects were estimated to result if controls on CFCs and halons were not implemented. Other noted impacts of increased UV radiation include an estimated increase in ground level (tropospheric) ozone and degradation (increased "weathering") of polymers used in outdoor applications. Because these chemicals are also greenhouse gases, contributions to climate change by global warming was also estimated.

Overview of Montreal Protocol

Recognizing the global nature of the stratospheric ozone depletion problem, the Montreal Protocol on Substances that Deplete the Ozone Layer was negotiated and signed by 24 nations and the European Economic Community on September 16, 1987. The signatories included the major CFC and halon producing and consuming nations. The Montreal Protocol entered into force on January 1, 1989 with over 68 nations party to it. The agreement prescribed a timetable for the reduction of specific ozone-depleting substances (ODS) for both the production (i.e., quantity of manufactured regulated chemicals) and consumption (i.e., production plus imports minus exports).

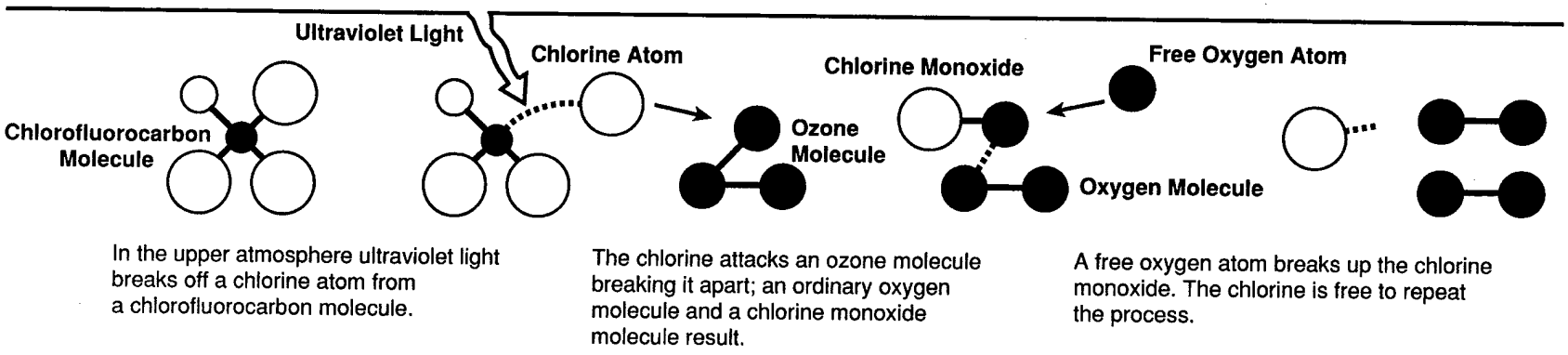


Figure 3. The Theory of Stratospheric Ozone Depletion by CFC Chemicals.

Consumption levels for several CFCs (CFC-11, -12, -113, -114, and -115) were either frozen or scheduled for reduction from the 1986 base year levels. Thus, beginning July 1989 consumption levels were frozen at 1986 levels, by 1993 they were decreased by 20%, and in 1998 consumption levels are scheduled to be decreased by 50%. In 1992, halon consumption levels were frozen also at the 1986 levels. Because of their relative reactivities with ozone, the CFCs (Group I) and halons (Group II) were treated separately. Halons are more potent at ozone depletion although they are produced in much smaller quantities than CFCs (see Table 1).

TABLE 1. OZONE AND GLOBAL WARMING POTENTIALS

Chemical	ODP	GWP (100 year)
Carbon tetrachloride	1.10	1300
Group I: Fully Halogenated Chlorofluorocarbons		
CFC-11 (trichlorofluoromethane)†	1.00	3500
CFC-12 (dichlorodifluoromethane)†	0.80	7300
CFC-113 (1,1,2-trichloro-1,2,2-trifluoroethane)†	1.00	4200
CFC-114 (dichlorotetrafluoroethane)†	0.05	6900
CFC-115 (monochloropentafluoroethane)†	0.6	6900
Group II: Halons		
Halon 1211 (bromochlorodifluroromethane)†	3.00	*
Halon 1301 (bromotrifluoromethane)†	10.00	5800
Halon 2402 (dibromotetrafluoroethane)†	6.00	*
Candidate Replacement Chemicals		
HCFC-22 (chlorodifluoromethane)	0.016	1500
HCFC-123 (2,2 dichloro-1,1,1-trifluoroethane)	0.02	85
HCFC-124 (2-chloro-1,1,1,2-tetrafluoroethane)	0.12	430
HFC-134a (1,1,1,2-tetrafluoroethane)	0.00	1200
HCFC-141b (1,1-dichloro-1-fluoroethane)	0.06	440
HCFC-142b (1-chloro-1,1-difluoroethane)	0.033	1600
HFC-152a (1,1-difluoroethane)	0.00	140
HCFC-225cb (1,3-dichloro-1,1,2,2,3-pentafluoropropane)	0.00	*

*Not available at this time.
†Chemical covered by the 1987 Montreal Protocol.

For calculations in production and consumption quotas, each chemical is assigned an ozone depletion potential (ODP). This value is a measure of a chemical's ability to destroy ozone molecules in the atmosphere relative to CFC-11 (rated at 1.0). For production quotas, the chemicals are interchangeable within Group I or Group II only, provided the ODP weightings (ODP value x amount) are equivalent (see Table I). For example, 1000 kg of Halon 1211 (ODP=3) could be traded for 30 kg of Halon-131 (ODP=10).

Because CFCs and halons are also greenhouse gases, an index called the global warming potential (GWP) was developed by atmospheric scientists in order to assist policymakers with risk management decisions. The GWP quantifies the relative, globally averaged warming capability of emissions (versus atmospheric concentration) of a greenhouse gas compared to that of the reference gas, carbon dioxide (CO_2)[1]. Global warming potential values are also provided in Table 1.

Reassessment of the protocol provisions is regularly scheduled in order to incorporate new data on atmospheric sciences, biological effects, technical control options and to reevaluate the coverage and stringency of the protocol. At the second meeting of the parties to the Montreal Protocol in London on June 27-29, 1990, agreement was reached to phase out the production and consumption of additional CFCs, carbon tetrachloride, and methyl chloroform (1,1,1-trichloroethane) (see Table 2). Compounds with ODP values lower than the ODP values of the ODS, such as the HCFCs, were to be utilized as transitional compounds when other more environmentally suitable compounds were not available. Based on data confirming the contribution of CFCs and other chemicals that release chlorine or bromine to ozone depletion and global warming, revisions were proposed again at the fourth meeting of the parties in Copenhagen on November 23-25, 1992. The Copenhagen revisions included adjustments to the original timetable for reductions and amendments to control the hydrochlorofluorocarbons (HCFCs), hydrobromofluorocarbons (HBFCs), and methyl bromide.

Domestic Regulatory Approach

The United States has been a leading advocate of the Montreal Protocol and its amendments. The EPA proposed domestic regulations on December 14, 1987 with a Notice of Proposed Rulemaking (NPRM) that would ensure U.S. compliance with the Montreal Protocol. The NPRM proposed to adopt the Montreal Protocol's definition of controlled substances and set forth a number of control strategies effective July 1, 1989.

TABLE 2. SUMMARY OF REDUCTION SCHEDULES FOR PRODUCTION AND CONSUMPTION OF OZONE DEPLETING SUBSTANCES UNDER MONTREAL PROTOCOL AND AMENDMENTS

	Montreal Protocol	London Amendments	Copenhagen Amendments
	September 1987	June 1990	November 1992
CFCs 11, 12, 113, 114, 115	Base year 1986 Freeze by 1989 — 20% by 1993 — 50% by 1998	Base year 1986 Freeze by 1989 — 50% by 1995 — 85% by 1997 — 100% by 2000	Base year 1986 Freeze by 1989 — 75% by 1994 — 100% by 1996
Halons 1211, 1301, 2402	Base year 1986 Freeze by 1992	Base year 1986 Freeze by 1992 — 50% by 1995 — 100% by 2000	Base year 1986 Freeze by 1992 — 100% by 1994
Other fully halogenated CFCs 13, 111, 112, 211, 215, 216, 217	No regulation	Base year 1989 — 20% by 1993 — 85% by 1997 — 100% by 2000	Base year 1989 — 85% by 1995 — 100% by 1996
Carbon tetrachloride CCl_4	No regulation	Base year 1989 — 85% by 1995 — 100% by 2000	Base year 1989 — 85% by 1995 — 100% by 1996
Methyl chloroform (CH_3CCl_3)	No regulation	Base year 1989 Freeze by 1993 — 30% by 1995 — 70% by 2000 — 100% by 2005	Base year 1989 Freeze by 1993 — 50% by 1994 — 100% by 1996
HCFCs 21, 22, 31, 121, 122, 123, 124, 131, 132, 133, 141, 142, 151, 221, 222, 223, 224, 225, 226, 231, 232, 233, 234, 235, 241, 242, 243, 244, 251, 252, 253, 261, 262, 271	No regulation	Transitional substances non-binding ban by 2040	Base year 1989[1] Freeze by 1996 — 35% by 2004 — 65% by 2010 — 90% by 2015 — 99% by 2020 — 100% by 2030
HBFCs	No regulation	No regulation	Base year 1989 — 100% by 1996
Methyl bromide (CH_3Br)	No regulation	No regulation	Base year 1991 Freeze by 1995

[1]Base = 3% of calculated level of CFC consumption (ODP weighted) plus calculated level of HCFCs in 1989.

On November 15, 1990, the Clean Air Act Amendments (CAAA) of 1990[2], which included Title VI: "Stratospheric Ozone Protection"(7), were signed into law. Title VI contains 18 sections which provide for: a phase-out schedule for substances with an ODP > 2.0 (Class I); flexibility to tighten or accelerate regulations when evidence necessitates it; the reduction of emissions, establishment of a recycling program and standards for servicing mobile air-conditioning units, establishment of a program to evaluate alternative substances (see "Risk Characterization of the Substitutes – The Snap Program"), and the publication of a list of prohibited and acceptable substances based on overall health and environmental risk.

Partly in response to the National Aeronautics and Space Administration's November 1991 findings regarding the increased severity of ozone depletion, an accelerated phase-out schedule for Class 1 substances was announced on February 11, 1992. An accelerated review of substitutes that do less damage to the ozone layer than do the ODS was also added. On July 30, 1992, the EPA issued regulations implementing the phase-out schedules for Class I substances. A subsequent NPRM, under the legal authority of the CAAA, proposed an amended schedule in order to respond to the President's announcement and to conform with the Copenhagen revisions of the Montreal Protocol(8). The proposed domestic reduction schedules for production and consumption of controlled ODS are provided in Table 3. The effective date of this regulation was January 1, 1994.

SEARCHING FOR SUBSTITUTES

Adoption of the Montreal provisions and the U.S. domestic policy has the potential for a major impact on electric power demand and energy consumption(9). The CFCs are the major chemicals currently used as refrigerants in a wide range of commercial and residential applications, such as blowing agents for rigid and flexible foam insulation as well as cleaning solvents. The use categories for the various CFCs and the replacement candidates currently under consideration are provided in Table 4(3).

TABLE 3. PROPOSED DOMESTIC REDUCTION SCHEDULES FOR PRODUCTION AND CONSUMPTION OF OZONE DEPLETING SUBSTANCES

Class I Substances						
Date	**CFCS**	**Halons**	**Carbon Tetrachloride**	**Methyl Chloroform**	**Methyl Bromide**	**HBFCs**
(Jan. 1)						
1994	25%	0%	50%	50%	100%	100%
1995	25%	0%	15%	30%	100%	100%
1996	0%	0%	0%	0%	100%	0%
1997	0%	0%	0%	0%	100%	0%
1998	0%	0%	0%	0%	100%	0%
1999	0%	0%	0%	0%	100%	0%
2000	0%	0%	0%	0%	0%	0%

Class II Substances		
Date	**Affected Compounds**	**Restriction**
January 1, 2003	HCFC-141b	ban on production and consumption
January 1, 2010	HCFC-142b, HCFC-22	production and consumption frozen at baseline levels
	HCFC-142b, HCFC-22	ban on the use of virgin chemical unless used as feedstock or refrigerant in appliances manufactured prior to January 1, 2010.
January 1, 2015	all other HCFCs	production and consumption frozen at baseline levels
	all other HCFCs	ban on the use of virgin chemical unless used as feedstock or refrigerant in appliances manufactured prior to January 1, 2020.
January 1, 2020	HCFC-142b, HCFC-22	ban on production and consumption
January 1, 2030	all other HCFCs	ban on production and consumption

TABLE 4. CFC USE CATEGORIES AND REPLACEMENT CANDIDATES

Use Category	Currently Used	Replacements
Mobile Air Conditioning	CFC-12	HFC-134a
Chillers	CFC-11	HCFC-123
	CFC-12	HFC-134a
	CFC-114	HFC-134a
	R-500 (CFC-12/HFC-152a)	HFC-152a/HCFC-124/HCFC-22 blend
Residential Air Conditioning	HCFC-22	HCFC-22
		HFC-32
		HFC-32/HFC-125 blend
Commercial Low-Temp. Refrigeration (Cold Storage Warehouses, Retail Food Storage, Process Refrigeration)	HCFC-22	
	CFC-12	
	R-502 (CFC-115/HCFC-22)	
Cleaning	CFC-113	HCFC-123/HCFC-141b blends
	Methyl Chloroform	HCFC-225ca
		HCFC-225cb
Aerosols	CFC-11	HCFC-22
	CFC-12	HFC-134a
		HCFC-142b
		HFC-152a
Rigid Foam—Insulation	CFC-11	HCFC-22
	CFC-12	HCFC-123
		HFC-134a
		HCFC-141b
		HCFC-142b
		HCFC-123/141b
		HCFC-22/142b
Flexible Foam	CFC-11	HCFCs/HFCs not needed
Rigid Foam—Packaging	CFC-11	HCFC-22
	CFC-12	HCFC-142b
	CFC-114	HFC-152a
		HFC-134a

Although the halons pose a greater risk to ozone depletion than the CFCs, they are significantly beneficial. Halons are widely used as fire-fighting agents. They represent particularly effective agents for the special applications required by the U.S. Air Force such as protecting electronic equipment in the presence of large amounts of explosive hydrocarbon fuels(11). Halons do not function by smothering fires but instead interfere with the fundamental reactions that cause burning by removing the highly reactive free—radical molecules formed during combustion which propagate this reaction. More importantly, halons do not conduct electricity and are clean agents (i.e., leave no residue on aircraft engines or electronic components). The halons are gaseous substances that can penetrate in and around physical objects, thus, they can be used to extinguish liquid fuel fires as streaming agents (e.g., Halon-1211) or to suppress fires in three-dimensional spaces as flooding agents (e.g., Halon-1301). The halons are highly effective against solid, liquid/gaseous, and electrical fires (Class A, B, and C fires, respectively), requiring 5% or less concentrations in air for fire suppression as compared to 30% required for CO_2. Furthermore, halons offer the advantage of relatively low toxicity (e.g., compared to CO_2), properties making them ideal for commercial aircraft, ships, computer rooms, and electric power facilities in the private sector.

The two halons most widely used in the United States are Halons 1211 (chlorodifluoro-bromomethane) and 1301 (trifluorobromomethane)[4]. HCFC-123 and -22 are candidate replacements for Halon-1211 and -1301, respectively. Although the Air Force is a major user of Halon-1211, it should be noted that the remainder of users when taken together have a much greater demand for Halon-1211 than does the Air Force(11).

The CFC chemical manufacturing companies are now competing to find and develop acceptable substitutes or replacements that can be mass produced at an affordable cost. Longer term objectives include the redesigning of equipment and retooling plants to manufacture compressors, auto air conditioners, commercial chillers, and all kinds of refrigeration systems (including domestic refrigerators and freezers) that will be forced to use less efficient and less-than-ideal substitutes(9). Because the halon market is small compared to the CFC market, private industry is not as vigorously seeking replacements for these chemicals[5].

Structural Considerations

The most common refrigerants used before CFCs included ammonia, CO_2, ethyl chloride, isobutane, methyl chloride, methylene chloride, and sulfur dioxide(9). All had disadvantages related to their physicochemical properties. Isobutane, for example, although relatively nontoxic is highly flammable due to its hydrogen. The chlorinated solvents are toxic. Ammonia and sulfur dioxide are noxious, toxic, and flammable. Although safest to use, CO_2 must operate at high pressure and thus requires heavy construction equipment. By eliminating chemicals that were unstable, insufficiently volatile, or had too low a boiling point, developers of refrigerant gases began focusing on eight elements: carbon, nitrogen, oxygen, sulfur, hydrogen, and the halogens fluorine, chlorine, and bromine. The considerations that the refrigerants be chemically inert and stable, of low flammability, and minimally toxic focused attention on the strong carbon-fluorine bond of the fluorocarbons (also known as halocarbons) which included the CFCs and halons. Fluorocarbons are chemically similar to hydrocarbon molecules except that one or more hydrogen atoms are replaced by chlorine, fluorine, or bromine atoms. CFCs are fully chlorinated fluorocarbons containing no hydrogen, only chlorine, fluorine, and carbon. Halons contain bromine, chlorine, fluorine and carbon.

It has become general practice within the refrigeration industry to designate various halocarbons with a number. This "Halocarbon Numbering System" has now become widely used in both national and international regulations[6]. Figure 4 illustrates the nomenclature system for the halocarbons(12).

Given that they had the desired combination of low toxicity and the required thermodynamic properties, as well as being nonflammable, extremely stable, and unreactive due to the complete lack of hydrogen, the halocarbon compounds were hailed as wonder chemicals in the 1930s. Ironically, it was these same properties of chemical inertness and stability that would prove to contribute to their long atmospheric lifetimes and the resultant stratospheric ozone depletion problem as described above. The search for substitutes to the CFCs and halons also involves an optimization of physicochemical properties (i.e., trade-off of the desirable properties provided by the different elements in proper proportion with the undesirable properties of flammability, reactivity, and toxicity). The pure hydrocarbon, methane, with one carbon and four hydrogens is too flammable. The pure chlorocarbon, carbon tetrachloride, is too toxic. Fluorine makes liver toxicity less likely than does chlorine. The structural considerations of this optimization are summarized in Figure 5.

First Digit: Number of carbon atoms minus 1.
An initial zero (indicating a one-carbon compound) is omitted.

Second Digit: Number of hydrogen atoms plus 1.

Third Digit: Number of fluorine atoms.

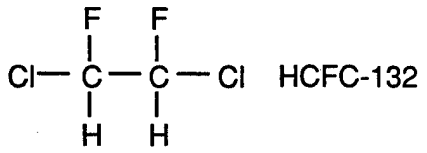
All remaining atoms are assumed to be chlorine atoms.

Examples

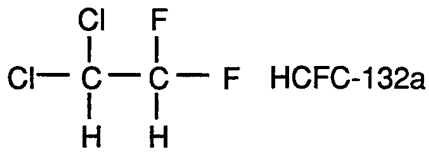
CFC-12: One carbon (initial zero dropped), no hydrogen atoms (0 + 1 =1), two fluorine atoms, and by default, two chlorine atoms. Formula = CF_2Cl_2. Dichlorofluoromethane.

CFC-113: Two carbons, no hydrogen atoms (0 + 1 = 1), three fluorine atoms, and by default, 3 chlorine atoms. Formula = CF_3CCl_3. 1,1,2-Trichloro-1,2,2-trifluoroethane.

For two-carbon compounds, absence of a letter indicates the most symmetrical isomer, while an "a" indicates the next most symmetrical, "b" the next, and so on. The symmetry is determined by adding the atomic masses of the substituents on each carbon atom. The isomer with the smallest difference in the masses on the two-carbon atom receives no letter, the next smallest receives an "a", the next "b", and so on.



HCFC-132



HCFC-132a

Figure 4. Halon Nomenclature System With Examples.

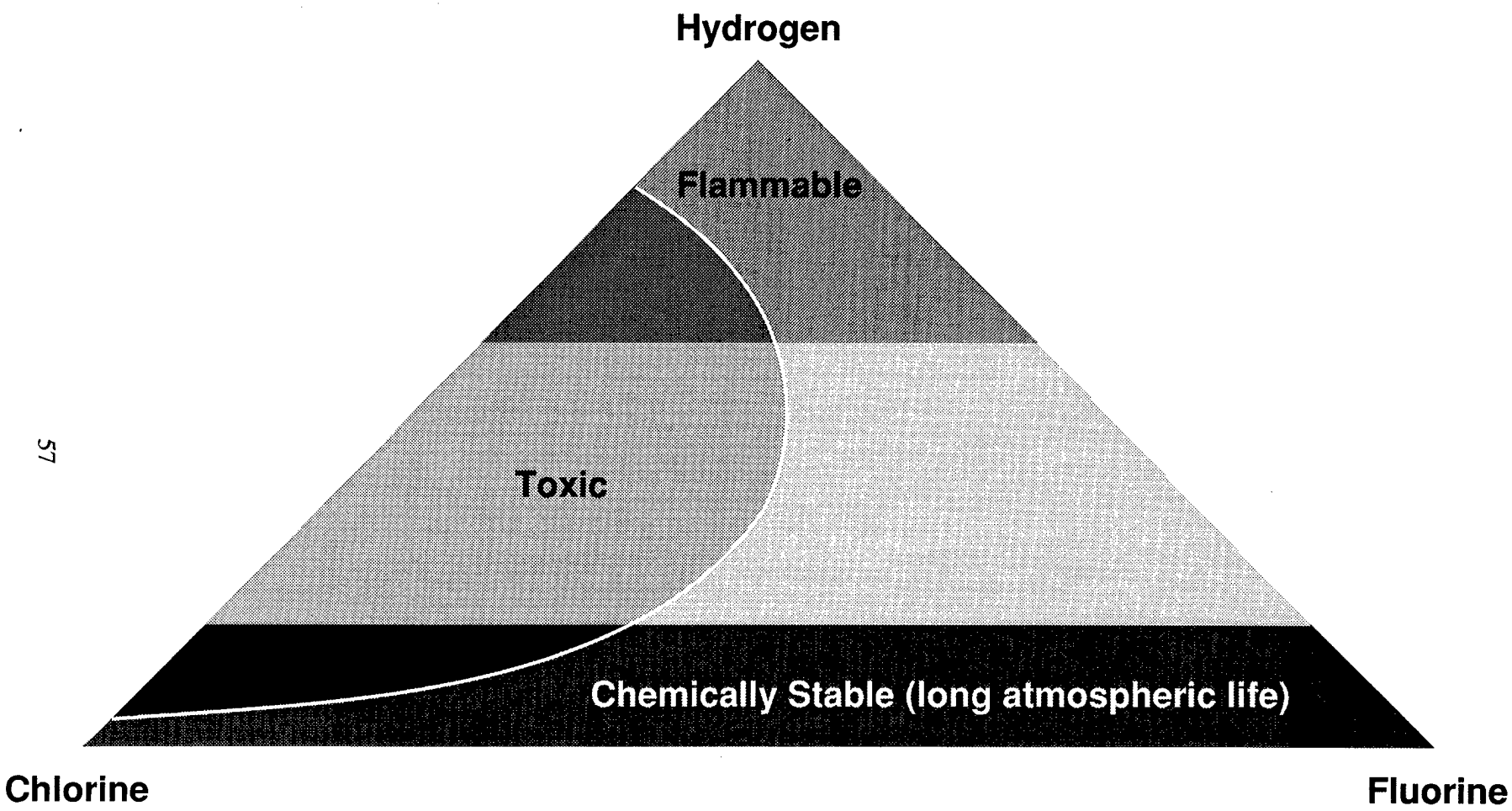


Figure 5. Triangle of Trade-Offs. Proportions of hydrogen, chlorine, and fluorine in various compounds play a critical role in the inherent trade-offs between desirable and undesirable properties. CFC producers are searching for substitutes that they hope will fall in the middle right of the triangle (little or no toxicity, low flammability, and moderate chemical stability) by adding hydrogen and reducing or eliminating chlorine (9).

In the near term, introduction of hydrogen has been a key to the development of replacement chemicals. Introduction of at least one hydrogen atom in place of chlorine or fluorine in a CFC molecule results in retention of some of the desirable thermodynamic properties, but the compound is less stable and more likely to break down in the lower atmosphere. Five such HCFCs have been developed, including HCFC-22, -123, -124, -141b, and -142b. Hydrofluorocarbons (HFCs) containing only carbon, hydrogen, and fluorine (but no chlorine to deplete stratospheric ozone) are also being developed as desirable alternative compounds. HFC-134a and -152a have developed as potential substitutes.

Figure 6 illustrates how these potential substitutes compare to the CFCs with respect to ODP and GWP values as presented in Table 1. Note that the although the HCFCs have ODP values considerably lower than the CFCs or halons, they still have the potential for ozone depletion due to their constituent chlorine. For this reason, they are considered transition chemicals only and are scheduled for eventual phaseout. Hydrofluorocarbons have ODP values of zero but nonetheless can contribute to global warming. Both HCFCs and HFCs act physically (cool, dilute, or smother the fire by separating air and fuel) to extinguish fires, making them less effective and requiring larger storage volumes and higher extinguishing concentrations. Hydrofluorocarbons potentially decompose into greater amounts of hydrogen fluoride than do HCFCs.

Besides evaluation of their efficacy, introduction of these substitute HCFC and HFC compounds to commercial production also requires that they be evaluated for their potential toxicity. Chemical properties that result in decreased stability in the environment are likely to result in increased reactivity (and potential toxicity) in biological systems as well. The remainder of this paper discusses development of the toxicity database for these substitutes and aspects of the dose–response component of risk characterization for the HCFCs and HFCs, in particular that for HCFC-123.

Programme for Alternative Fluorocarbon Toxicity Testing (PAFT)

The recognition of the need to establish the toxicity database and characterize the potential hazard of the candidate compounds in an expedited fashion led to the creation of the PAFT in December 1987. The PAFT represents a unique international effort by most of the CFC producers from Asia, Europe, and the United States aimed at evaluating the toxicology of limited production or research chemicals in order to support the introduction and use of these chemicals as substitutes for widely used compounds(13,14,15).

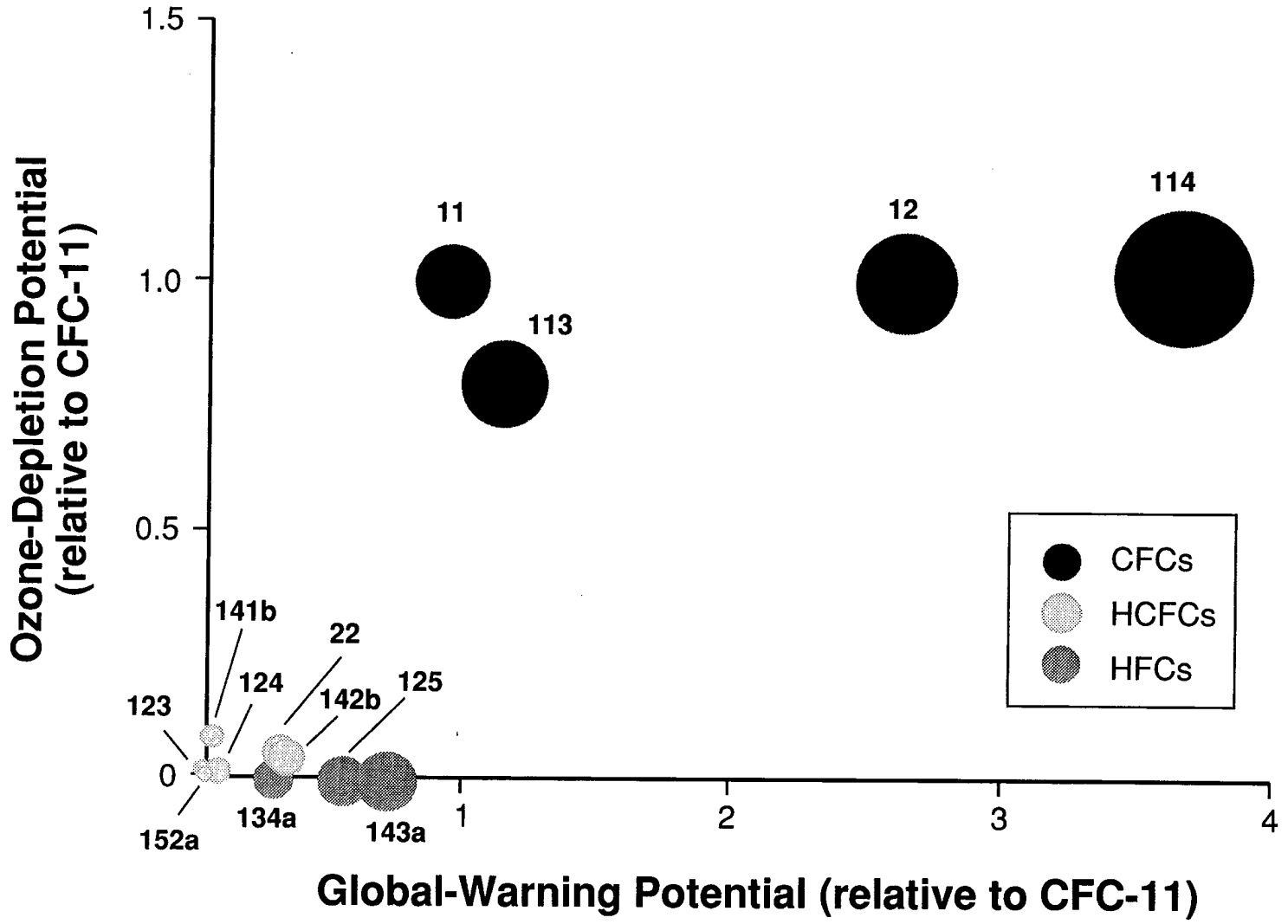


Figure 6. Ozone Depletion Potential (ODP) and Global Warming Potential (GWP) of Various Chemicals Relative to CFC-11. The circles are proportional in area to the atmospheric lifetimes of the compounds (9).

The PAFT consists of three committees: management, toxicology, and product quality(13,14). Direction was provided by the management committee, composed of senior representatives of participating companies. This committee controlled the budget and decided what chemicals would be produced. The management committee also chartered the toxicology committee, which was charged with evaluating the existing toxicology data on the first two candidate chemicals, HCFC-123 and HFC-134a. The toxicology committee, consisting of 10 senior toxicologists from the member companies, then used that information as the basis for the preparation of a toxicity testing program for the substitutes. Subsequently, four other groups have been formed to evaluate candidate chemicals as shown in Table 5. The unique cooperation engendered by the PAFT is illustrated by Table 6, which shows some of the inhalation laboratories participating in the testing.

TABLE 5. PAFT: PROGRAMME FOR ALTERNATIVE FLUOROCARBON TOXICITY TESTING

- Compounds Currently Under Evaluation
  - PAFT I: HFC-134a and HCFC-123
  - PAFT II: HCFC-141b
  - PAFT III: HCFC-124 and HFC-125
  - PAFT IV: HCFC-225ca and HCFC-225cb
  - PAFT V: HCF-32
- Estimated Programme Costs
  - $3,000,000 to $5,000,000 per Chemical
  - $25,000,000 to $35,000,000 Total Programme

TABLE 6. PAFT: SOME CONTRIBUTING LABORATORIES

AG Pharma Forschung (Hoechst)	Germany
Bio/Dynamics	USA
Central Toxicology Laboratory (ICI)	United Kingdom
CIVO-TNO Institute	Netherlands
Duphar (Solvay)	Belgium
Haskell Laboratory (Du Pont)	USA
Hita Research Laboratory	Japan
Huntingdon Research	United Kingdom
IRCHA	France
Japan Bioassay Laboratory	Japan
Kurume Research Laboratory	Japan
Life Sciences Research	United Kingdom
RCC-NOTOX BV	Netherlands
Toxicology Research Laboratory (Dow)	USA
University of Rochester	USA
University of Würzburg	Germany

The management committee also established a product quality committee to provide support to the testing effort. Due to the fact that the compounds were not in commercial production, decisions had to be made on which compounds to test, what purity and impurities were likely, and which compounds and composition were of relevance to the final product. The analytical chemists comprising this committee also developed the analytical and monitoring methods necessary to guarantee the purity of the test compound and to assure that established exposure levels were achieved.

The PAFT program conducted the toxicity testing in a tiered format (phases), with the screening and short-term tests first, followed by subchronic tests, and ending with lifetime studies. This design allowed the test data of one phase to be evaluated to determine if another parameter required further investigation in the next phase. The tiered testing approach to an individual candidate chemical is provided in Table 7.

TABLE 7. PAFT TOXICOLOGY TESTING PROGRAM TIER APPROACH

- PHASE I (6-8 months)
  - Acute Oral Limit Test (rat)
  - Acute Inhalation Toxicity (rat)
  - Dermal Irritation (rabbit)
  - Dermal Toxicity (rabbit, rat)
  - Repeat Insult Patch (human)
  - Eye Irritation (rabbit)
  - Sensitization (Guinea pig)
  - Cardiac Sensitization (dog)
  - Ames Assay
  - *In Vitro* cytogenetics (human lymphocyte, CHO cells)
  - *In Vivo* Micronucleus Assay (mice)
- PHASE IIA (6-8 months)
  - Sub-Acute Study (4 weeks: 3 doses + control)
  - Preliminary Pharmacokinetics (blood levels, uptake and elimination rates)
  - Teratology Probes (rat, rabbit)
  - Environmental Toxicology
- PHASE IIB (1 year)
  - Teratology (rat, rabbit)
- PHASE III (1 year)
  - 3-month Toxicity Study (rat)
  - Metabolism
  - CNS Effects
- PHASE IV (4-5 years)
  - Chronic Toxicity/Carcinogenicity Study (rat)
  - Reproduction (optional)

The tests incorporated in the tiers also reflect an attempt to address areas of previously observed toxicity associated with fluorine-containing organic compounds. Although introduced as "inert" refrigerants, reports of fatalities associated with abuse and misuse of aerosol products in general and of bronchodilator aerosols in particular pointed to potential cardiopulmonary toxicity(16). Following these reports, experimental procedures were developed to evaluate the potential toxicity of the inhalants in humans. Comparative studies showed that the dog was the preferred species for evaluating this potential toxicity[7]. A standard protocol was developed to evaluate "cardiac sensitization" (CS), defined as an increased susceptibility of the heart to catecholamines (principally epinephrine) that can potentially result in fatal cardiac arrhythmias, usually manifested as ventricular tachycardia and/or fibrillation(17) [8]. The CS protocol is included as part of the Phase I testing protocol used by PAFT (see Table 7).

The subchronic toxicity study by inhalation is the most important single component of the program and is included with every compound in order to ensure current protocols(13). The 90-day study would be used to evaluate hepatotoxicity (suggested by the presence of constituent chlorine in these compounds). Because of relatively short half-lives, little metabolic activity was anticipated with these compounds. Thus, the initial pharmacokinetic tests consisted of a study of uptake versus elimination in the rat and determination of serum and urinary fluoride levels in the subchronic study together with careful review of the clinical results. Developmental toxicity was an important component of the program because data available in the literature were equivocal and typically available only in the rat. In those cases, the PAFT program called for testing in a nonrodent species, usually the rabbit. If no information was available on a particular compound, teratology investigations might be performed in two species. Reproductive toxicity evaluation was not included unless triggered by morphologic effects on reproductive organs in either sex or on the hormonal axes during the subchronic and chronic testing or by significant findings in the developmental studies.

Table 8 summarizes the data compiled by the PAFT by approximately mid-1989. Many of the studies in progress will provide the only data with which to quantitatively evaluate the dose–response of these chemicals. The program should be commended for integrating past and present toxicological information to perform a comprehensive risk assessment with efficiency of time and resources in order to accommodate the uncertain economic future and regulatory schedules imposed on these chemicals. These attributes are summarized in Table 9.

TABLE 8. COMPARATIVE TOXICITY OF HYDROFLUOROCARBONS (HFCS) AND HYDROCHLOROFLUOROCARBONS (HCFCS) AS COMPILED BY PAFT

Structure	HCFC-22	HCFC-123	HCFC-124	HFC-134a	HCFC-141b	HCFC-142b
Formula	$HCClF_2$	$HCCl_2CF_3$	CF_3CClFH	H_2CFCF_3	H_3CCCl_2F	H_3CCClF_2
LC_{50}	220,000	35,000	207,000	500,000	62,000	400,000
Ames	Positive	Negative	Negative	Negative	+/-	Positive
Cardiac Sensitization	5%	1%	2.5%	5%	1%	5%
Subchronic toxicity NOEL (ppm)	50,000	5,000	20,000	50,000	8,000	20,000
Chronic toxicity NOEL (ppm)	10,000	IP	NT	IP	IP	20,000
Carcinogenicity	Negative	IP	NT	Positive	NT	Negative
Teratology	Negative	Negative	Negative	Negative	Negative	Negative
Male Fertility	Negative	NT	NT	NT	NT	Negative

IP = In progress; NT = Not tested.

TABLE 9. ADVANTAGES TO PAFT CONSORTIUM

- Pooled existing data
- Pooled scientific expertise
- Conserved resources
- Conserved limited quantities of research-grade test chemicals not in commercial production
- Reduced the time for obtaining results
- Effectively used the limited toxicology testing laboratory space
- Collaborated on the development of test data
- Shared results with all member companies
- Ensured the rapid publication of results

Risk Characterization of the Substitutes — The SNAP Program

Section 612 of the CAAA requires the EPA to develop a program to evaluate the risks to human health and the environment posed by the substitute chemicals to the ODS. Due to the accelerated phaseout schedules, review of these risks was expedited to avoid delay in industry's effort to replace the ODS. The EPA is referring to this program as the SNAP program. In its May 12, 1993 NPRM, EPA introduced its plan for administering the program and issued its preliminary decisions on the acceptability of certain substitutes(10). The Advance Notice of Proposed Rulemaking and Request for Data (57 FR 1984; January 16, 1992) solicited industry to submit information on substitutes and to identify additional alternative chemicals to be considered in the SNAP program. The data received as a result of this call-in, together with data submitted by the PAFT, served as the basis for assessments in the risk characterization approach to decisions listing the substitutes as either acceptable or unacceptable. This section describes the general principles used for the risk characterization process.

The EPA examined the risks of the substitutes in a comparative framework using risks from continued use of the ODS or the use of substitutes as reference points. The evaluation considered factors such as effects due to ODP (e.g., skin cancer and cataracts) as well as effects due to direct toxicity. Other risk factors considered included direct and indirect (e.g., conversion to hydrofluoric acid) effects on air and water quality, direct and indirect contributions to global warming, and occupational health and safety. Because the key goal of the SNAP program is to promote the use of substitutes that minimize risks to human health and the environment relative to other alternatives, some substitutes designated as acceptable may pose some toxic or other environmental risk. Some substitutes, such as the volatile organic compounds (VOCs) or hazardous air pollutants are already regulated under other sections of the CAAA, and determinations of the SNAP program will take these existing regulations into account.

Environmental and human health exposures can vary significantly depending on the particular application of a substitute so that the risk characterization analysis was directed at eight different sectors determined to represent the principal industrial sectors that historically consume large volumes of ODS. These sectors are: refrigeration; foam blowing; solvent cleaning; fire extinguishing; tobacco puffing; adhesives, coatings and inks; aerosols; and sterilants(10). Further, substitutes are evaluated in the context of particular end uses within each sector.

Evaluation of each substitute by end use is based on the following types of information and analyses. Atmospheric effects are assessed by predicting ODP and GWP using models. Ozone depletion potential is measured in terms of cumulative chlorine loadings and increased incidences of skin cancer cases and mortalities. Exposure assessments are used to estimate the concentrations to which workers, consumers, the general population, and environmental receptors may be exposed and over what period of time. Personal or area data monitoring are used if available. Otherwise, exposures are assessed using measured or estimated releases as input to mathematical models. Toxicity data are used to develop dose—response estimates to assess the possible health and environmental effects from exposure to substitutes. Flammability is examined as a possible safety concern for workers and consumers. Data evaluated include flash points and flammability limits (e.g., Occupational Safety and Health Administration [OSHA] flammability/combustibility classifications), test data on flammability in consumer applications conducted by independent laboratories (e.g., Underwriters Laboratories), and information on flammability risk minimization techniques. Some of the proposed substitutes in several sectors are VOCs that increase tropospheric air pollution by contributing to ground-level ozone formation. Local and nationwide increases in VOC loadings from the substitutes are also evaluated for these sectors.

DOSE–RESPONSE[9] ANALYSIS FOR EVALUATION OF HUMAN HEALTH RISK

Within each sector, candidate substitutes are evaluated in the context of particular end uses. These may include both short-term (e.g., occupational) and long-term scenarios. In order to assess the potential toxicity of an estimated exposure for one of these scenarios, appropriate toxicity reference values must be determined. In general, the EPA uses data obtained in short-term studies to evaluate potential risk of acute effects at high concentrations (e.g., episodic emissions in the workplace or accidental ambient exposures). Data obtained in chronic bioassays are used to assess potential adverse effects from continued exposure to low-level ambient concentrations(18). Reproductive and developmental hazards are also evaluated and are considered potential targets for either scenario.

The strategies used by other agencies for short-term risk evaluation were adapted under the SNAP to develop procedures for deriving reference values for potential acute toxicity. Although beyond the scope of this paper, the differences in underlying assumptions and intended applications of these various short-term exposure limit values are addressed elsewhere(19). These short-term exposure limit values were evaluated for applicability to the context to be addressed for short-term exposure to the candidate substitutes. For the purposes of evaluations required under the SNAP, two levels are generally estimated:

(1) the workplace guidance level (WGL), and (2) the emergency guidance level (EGL). These levels are intended as management screening tools.

The WGL is essentially analogous to an OSHA-permissible exposure level (PEL). When available, the WGL is estimated as an order of magnitude greater than the inhalation reference concentration (RfC) (see below) because the uncertainty factor generally applied in using the RfC methodology to protect sensitive populations was not deemed applicable to a risk estimate intended for a healthy worker population. When inhalation RfC data are not available, an estimate may be derived using the OSHA PEL, the National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, the American Conference of Governmental Industrial Hygienists threshold limit value or the American Industrial Health Association (AIHA) workplace environmental exposure level.

The EGL is similar in intent to the NIOSH immediately dangerous to life and health value. The latter is defined as "the maximum concentration from which, in the event of respirator failure, one could escape within 30 min without experiencing any escape-impairing or irreversible health effects". The EGL also resembles the National Research Council Emergency Exposure Guidance Level and the AIHA emergency response planning guidance level in intent. Given that the risk to individuals from exposure to the candidate substitutes will generally be from discharges that occur infrequently (e.g., the discharge of a fire extinguisher at high concentrations for a short period of time), the EGL derivation is based on analysis of acute toxicity associated with exposure to these compounds such as cardiotoxicity or developmental toxicity(18). The EGL is thus based on the no-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level for cardiotoxicity in the dog. This EGL value based on cardiotoxicity was also selected as the basis for short-term evaluation of potential toxicity to the general population.

Long-term exposure scenarios were developed for the general population to evaluate the risk of ambient emissions. Inhalation RfCs and cancer slope factors (SFs) are used as dose–response estimates of potential human health toxicity for noncancer and cancer end points, respectively.

Noncancer toxicity refers to adverse health effects or toxic end points, other than cancer and gene mutations, that are due to the effects of environmental agents on the structure or function of various organ systems. Generally, based on understanding homeostatic and adaptive mechanisms, most dose—response

assessment procedures operationally approach noncancer health effects as though there is an identifiable threshold (both for the individual and for the population) below which effects are not observable. An inhalation RfC is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of adverse noncancer effects during a lifetime(20). The methodology for deriving these dose–response estimates is described elsewhere(20). Generally, a NOAEL and the LOAEL are determined for the specified adverse effect from the exposure levels of individual studies. These effect levels observed in laboratory animal experiments or in human epidemiological or occupational studies are then dosimetrically adjusted to human equivalent concentrations (HECs) for ambient exposure conditions. These conditions are currently assumed to be 24 h/day for a lifetime of 70 years. Default equations are used to perform these dosimetric adjustments when more sophisticated modeling approaches are not available(20). Physiologically based pharmacokinetic and dosimetry models are considered the optimal approach for these conversions. The critical toxic effect used in the dose–response assessment is usually characterized by the lowest NOAEL(HEC) that is also representative of the threshold region (the region where toxicity is apparent from the available data) for the entire toxicity profile or data array of the chemical. Uncertainty factors (UFs) are then applied to the NOAEL(HEC) to account for recognized uncertainties in the extrapolations from the experimental data conditions to an estimate appropriate to the assumed human scenario. An additional modifying factor may also be applied when the scientific uncertainties (e.g., small sample size or poor exposure characterization) in the study chosen for operational derivation are not explicitly addressed by the standard UFs.

When RfC values are not available from the EPA's Integrated Risk Information System (IRIS), surrogate values are estimated from the available data (including oral administration) using analogous methods or based on structure–activity analysis of analogous chemicals with well-documented dose–response data. Table 10 provides the status of the ongoing RfC dose–response analyses available for the candidate substitutes. It can be seen from these data that, as anticipated, these compounds are relatively nontoxic with minimal systemic toxicity observed. Exceptions are HCFC-141b that demonstrated reproductive toxicity, and HCFC-123 which was shown to be metabolized and to be hepatotoxic in a PAFT 2-year bioassay. A dose–response evaluation for the effects of HCFC-123 will be described in greater detail below.

TABLE 10. NONCANCER INHALATION TOXICITY DOSE—RESPONSE ASSESSMENT

Halocarbon	Study Duration	Critical Effect	LOAEL (ppm)	NOAEL (ppm)	RfC Status
HCFC-22	Chronic	Increased Liver, Kidney, Adrenal and Pituitary Weights	50,000	10,000	Verified†
HCFC-123		Under Review			Under Review
HCFC-124	90-Days	Transient CNS Effects (narcosis)	50,000	15,000	Verified
HFC-134a		Under Review			Under Review
HCFC-141b	2-Gen Repro	Decreased Reproductive Performance	20,000	8,000	Verified
HCFC-142b	2-years	None	N/A	20,000	Verified
HCFC-152a	2-years	None	N/A	25,000	Verified

† Indicates that concensus agreement has been reached on dose—response evaluation by EPA RfD/RfC Work Group.

In the absence of data to the contrary, chemicals that produce cancer are assumed to have no threshold for their effects (i.e., no NOAEL) and the cancer SF is derived using the linearized multistage model(21). This model expresses upper confidence limits on cancer risk as a linear function of dose in the low-dose range. The SF is estimated by fitting the model to experimental carcinogenicity data using the maximum likelihood method. The 95% statistical upper bound on the linear term is used to reflect uncertainty in the extrapolation. Alternative models, (e.g., biologically based dose—response models) are considered when mechanistic data suggest that the LMS approach is inappropriate for a particular chemical. As for the RfC, the preferred SF values for the dose—response analyses under the SNAP are the values provided on IRIS. When these EPA consensus values are not available, SF values have been estimated on an interim basis according to the described methods using the recently completed PAFT 2-year bioassay data.

HCFC-123 as a Model for Dose—Response Analysis

There are no human toxicity data for HCFC-123, because, as with most of the candidate compounds, the chemical has only recently been produced and introduced into commerce. This requires

extrapolation from the available laboratory animal data in order to derive a dose–response estimate for potential human toxicity. Unlike some of the other replacement candidates, HCFC-123 does undergo considerable metabolism and this may contribute to its observed chronic toxicity. HCFC-123 is a candidate replacement for Halon-1211 and for CFC-11, -12, and -113, making it of interest to risk evaluation in a number of sectors, including fire-fighting. Thus, it requires both a short- and long-term dose–response estimate. A dose–response analysis for each is provided below.

Acute Toxicity of HCFC-123

The acute toxicity of HCFC-123 is not remarkable other than observed central nervous system (CNS) depression and cardiotoxicity at high exposure concentrations. All laboratory animal species exhibit CNS depression; whereas death in rodent LC_{50} (lethal concentration to 50% of exposed population) studies is attributable to CNS depression. The rodent LC_{50} values are comparable across species and average 3.7% (37,000 ppm) for exposures of 4 to 6 h(22,23,24,25). The lowest HCFC-123 concentration reported to cause CNS depression (as measured by observed inactivity or an altered response to auditory stimuli) is 0.5% (5,000 ppm) in rats(26). A concentration of 0.1% (1,000 ppm) does not produce narcosis in rats or dogs(27).

The potential for HCFC-123 to produce developmental and reproductive toxicity has not been evaluated conclusively in two species due to experimental design deficits. No consistent evidence for developmental toxicity was observed in the litters of pregnant New Zealand rabbits exposed to 0, 500, 1,500, or 5,000 ppm on Gestation Days 6 through 18, but maternal toxicity was manifest at all concentrations with decreased food consumption and body weight gain(28). The data of Culik and Kelly(29) suggest a fetotoxic effect in offspring of female rats exposed to 10,000 ppm; but the results were not statistically significant, and no other concentration was tested. No two-generation reproductive studies have been conducted. In the 4-week study of Kelly(30), degenerative changes were noted in the testes of rats exposed to 20,000 ppm, but these effects have not been observed in rats exposed to lower concentrations for longer durations.

As discussed above, CS is considered to be an appropriate end point by the SNAP program for basing the short-term dose–response estimate. Although the phenomenon of CS has been established, and despite its potential significance to human health, the causative mechanisms are not yet defined. Several investigators have attempted unsuccessfully to correlate potency to induce CS with

physicochemical parameters such as degree of saturation; molecular size and shape; and degree, type, and pattern of halogenation(31). HCFCs and HFCs also act like anesthetic gases and cause CNS depression at high concentrations. Noting that the vapor pressure and narcotic potency of anesthetics are directly related, a correlation was established between the partial pressure at the EC_{50} (concentration causing a 50% response rate) for CS and the saturated vapor pressure (P_s) of halogenated and unsubstituted hydrocarbons(31). It was concluded that because these compounds were relatively stable, lipid soluble compounds with high vapor pressures, the effect on the CNS and heart were probably structurally nonspecific actions due to "physical toxicity" (simply being present in some part of the cell to disorganize its function temporarily rather than specific combination with targets or receptors)(32). Clark and Tinston further stated that based on physical rather than specific receptor-based toxicity, the CNS and CS effects should disappear without permanent damage to the heart or brain cells when the chemical has been eliminated(32). The reversibility of CNS effects by CFCs and the HCFCs has been noted in the literature.

A potential occupational scenario for short-term exposures to HCFC-123 is as a fire-fighting agent. The exposure duration of concern involves a 1-min period to simulate an employee discharging either the entire contents of a small (1- to 3-lb) extinguisher or the partial contents of a large (150-lb) extinguisher while attempting to put out a fire, and subsequently immediately leaving the exposure area(33). An HCFC-123 concentration of 5% (50,000 ppm) has been proposed as a dose—response estimate for this scenario based on CS(30). Pharmacokinetic studies in the dog are under way which will investigate whether this "physical toxicity" is more directly related to chemical concentration or to the product of concentration and time (Haber's Law). Beck et al.(34) suggested that CS induced by bromochlorodifluoromethane as a prototype HCFC followed Haber's Law. The EC_{50} (95% confidence interval) in dogs was determined to be 1.9% (1.29 to 2.82%) for a 5-min exposure. If one applies Haber's Law to the low end of the 95% confidence interval, the concentration (ppm) multiplied by time (minute) product is 64,500 (12,900 x 5 min). Thus, the selection of 50,000 ppm for a 1-min exposure level is below the low end of the 95% confidence interval of the EC_{50} value in epinephrine-challenged dogs.

Chronic Toxicity of HCFC-123

For evaluation of chronic toxicity, the only data available are from a PAFT 2-year bioassay in rats(35). Crl:CD BR rats were exposed to 0, 300, 1000, and 5000 ppm. Exposure-related changes

consistent with hepatotoxicity included select serum chemistry values (triglyceride, glucose, and cholesterol levels), increased hepatic beta-oxidation enzyme activity, and degenerative changes (centrilobular fatty change, altered cellular foci, biliary hyperplasia, and focal necrosis). These effects were consistent with data from the 90-day study in the same laboratory showing increased relative liver weights and lipid metabolism perturbations(36) and with effects observed in a 90-day study on both dogs and rats(37). Compound-related decreases in mean body weight that were statistically significant occurred in both sexes exposed at the highest concentration and the increased survival also seen in these exposure groups was attributed to the weight loss. The incidence data for the hepatic lesions suggest a NOAEL at 300 ppm because the majority of histopathology is not statistically significant until the 1000 ppm exposure level, but unequivocal effect level designation awaits additional analyses evaluating the response function of the hepatic lesions in concert with consideration of the hepatic tumors. The data also identify significant diffuse retinal atrophy as a potential critical effect at the highest concentration, but it has been proposed to discount this effect as both an artifact of artificial lighting environments and the increased survival because the type of atrophy shown appeared to be age-related(35).

Three types of tumors were increased in incidence at the end of the 2-year bioassay(35): (1) hepatocellular adenomas and/or cholangiofibroma, (2) pancreatic acinar cell adenoma, and (3) interstitial cell adenoma in the testes. The analysis for SF for HCFC-123 will require determination of the appropriate tumor type to model based on statistical and biological considerations. Recent evidence suggests that HCFC-123 may be a peroxisome proliferator that may confound the quantitative extrapolation of the rat hepatic tumors to human risk(38). Whether the noncancer hepatic end points can be biologically related as preneoplastic events or should be evaluated independently remains to be elucidated (see below). There is also evidence that suggests Leydig cell adenomas in the testes may be hormonally mediated, and this mechanism warrants consideration when evaluating the dose−response for the testicular tumors(39,40).

Comparative Metabolism and Toxicity of Halothane

Increased urinary excretion of fluoride was noted in the Malley investigations, indicating that HCFC-123 undergoes metabolism(35,36). HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) is a structural analogue of the anesthetic gas halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), differing only by substitution of chlorine for a bromine. Brashear et al.(41) have shown that HCFC-123 and halothane follow very similar pathways of metabolic degradation. The pathways for HCFC-123 are presented in

Figure 7(41). The dominant pathway (approximately 90%) for both chemicals is via oxidation catalyzed by cytochrome P-450 which produces a dichlorogeminal halohydrin that is unstable and releases HCl to form trifluoroacetylchloride(41). The acetylchloride is rapidly hydrolyzed to trifluoroacetic acid (TFA). The reductive pathway begins with reductive dehalogenation to produce a radical intermediate that can either accept a hydrogen atom from a protein or phospholipid to form HCFC-133a or lose a fluorine to yield 2-chloro-1,1-difluoroethylene(41).

Figure 8 depicts potential mechanisms for halothane hepatotoxicity originally proposed in the late 1970s. These remain the target of active research to elucidate the toxic mechanisms of action of halothane(42,43). Support for the hypothesis that the metabolism of halothane is responsible for its hepatotoxicity has been developing ever since the first reports of unexplained postanesthetic jaundice appeared with the introduction of halothane into clinical practice. Lunam et al.(44) showed that liver damage, indexed by both severity grade of histopathology and amount of serum alanine aminotransferase (ALT) released, correlates with the amount of oxidative metabolism. SKF-525A, an inhibitor of the P-450 isotype enzyme IE1, was also shown to decrease the effect of halothane on each of these liver damage indices. Lind et al.(45) have shown that deuterium-substituted halothane decreases the resultant hepatic necrosis and the organic fluoride bound to protein. Plasma TFA, determined as an index of oxidative metabolism, was reduced in guinea pigs exposed to deuterium-substituted halothane. Serum ALT, an indicator of cellular damage, was also shown in the same study to correlate inversely with deuterium substitution. The carbon-deuterium bond strengthens the carbon-hydrogen bond and decreases the lability of the halogen leaving group.

Support for the importance of the oxidative metabolic pathway in producing toxicity comes from demonstration that the reactive intermediate, the trifluoroacetyl lysinyl moiety, can act as an epitope to alter protein antigenicity. Brown et al.(46) have shown that covalent binding of ^{14}C-labeled halothane to guinea pig liver slice proteins increases linearly with the increase in trifluoroacetylated proteins and deuterated halothane in the same study did not develop any neoantigens. Harris et al.(47) postulated that the hepatotoxicity observed with HCFC-123 exposure may be secondary to its similar metabolism because they identified a trifluoroacetylated lysine protein adduct for both halothane and HCFC-123 using ^{19}F-NMR (nuclear magnetic resonance) spectroscopy(44). Recent data from the Toxicology Division at Wright-Patterson Air Force Base, OH, support this hypothesis(48). The location, incidence, and severity of liver degeneration as well as two biochemical indices of damage, isocitrate dehydrogenase and ALT, demonstrated a dose−response relationship with compound concentration(48).

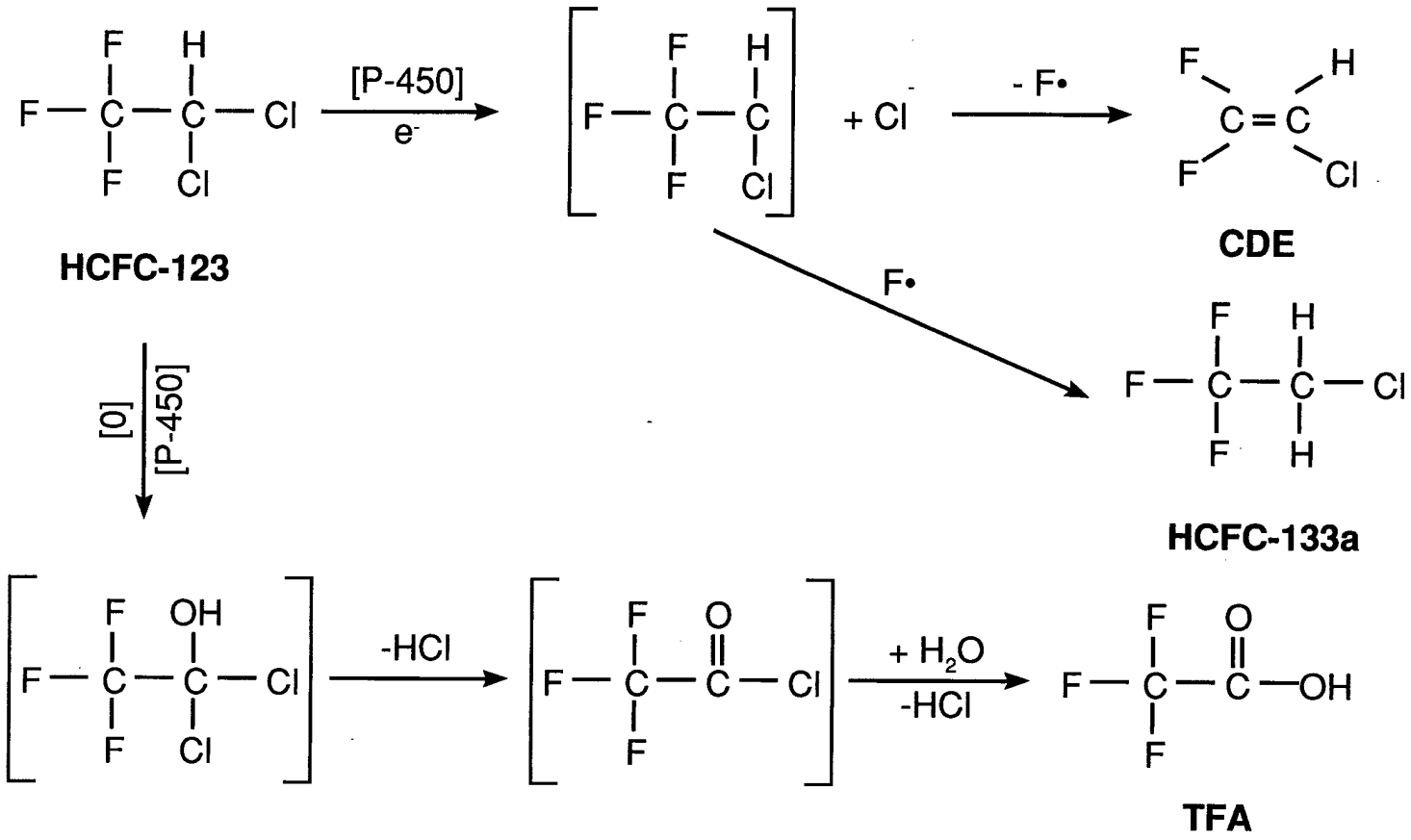


Figure 7. Proposed Metabolic Schema for HCFC-123 Showing Reductive and Oxidative Pathways. Metabolism for structural analog halothane is postulated via same pathways (41).

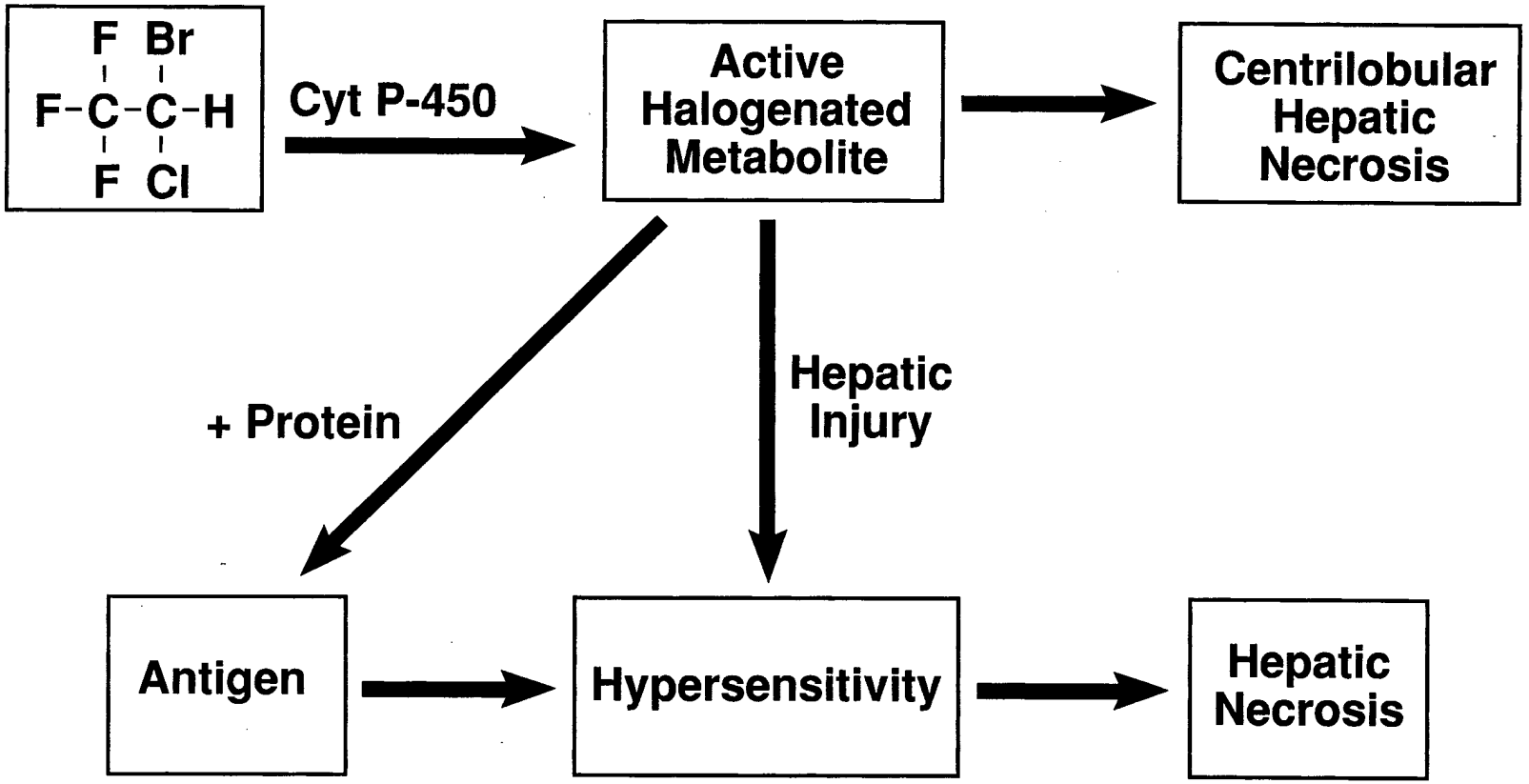


Figure 8. Schematic of Mechanisms of Action for Halothane Hepatotoxicity.

Parallelogram Approach for PBPK Modeling of Dose—Response

Based on the considerable evidence that HCFC-123 toxicity is likely to be mechanistically similar to that induced by its structural analogue halothane, a parallelogram approach for the dose—response analysis of HCFC-123 is proposed as shown in Figure 9. The approach relies on development and validation of a PBPK model in the relevant laboratory species. This PBPK model structure is then extrapolated to humans using available human data and scaling assumptions. Due to the fact that no human data exist on HCFC-123, the human PBPK model is indirectly validated against human exposure data for halothane based on its similarity.

Figure 10 shows the schematic of the model structure developed to simulate the pharmacokinetics (absorption, distribution, metabolism and elimination) of both HCFC-123 and halothane. Because oxidative metabolism is hypothesized as the key mechanism for hepatotoxicity, production of metabolite in the liver is explicitly incorporated. The structure is a six-compartment PBPK model with a hybrid, one-compartment classical description for the oxidative metabolite TFA, a structure previously used successfully to model trichloroethylene and its principal metabolite, trichloroacetic acid(49). HCFC-123 equilibrates with lung blood and is distributed in the systemic compartments as described by flow-limited conditions. Systemic clearance of HCFC-123 is described by metabolism and exhalation. The production of TFA is described as a proportion of the rate of HCFC-123 metabolism and this stoichiometric yield distributes into the one-compartment volume. Systemic clearance is described as a first-order rate loss from that compartment. Details of the PBPK model development and parameter values are provided elsewhere(50), but the approach is described here to illustrate the usefulness of using template structures to aid development and validation of models for chemicals that are similar in structure and mechanism of action.

It should be emphasized that many of the key parameters for the model have been developed directly using empiric data for each of the compounds. As outlined in Table 11, development of the rat model was based on the general PBPK model structure of Ramsey and Andersen(51) with the modification to account for TFA distribution and excretion(49). The blood:air and tissue partition coefficients, metabolism, and excretion values were each measured in the laboratory and incorporated directly into the model structure. Figure 11 depicts data used to derive the metabolic parameters for model development in the Fischer 344 (F-344) rat. Gas-uptake studies at various concentrations were conducted to characterize metabolism of HCFC-123 in rats(50). In the figure, the squares represent data points, and the solid line represents model simulation. The PBPK model predictions were optimized against these data points by adjusting the maximum rate of oxidation (V_{max}) and the Michaelis-Menten constant (K_m).

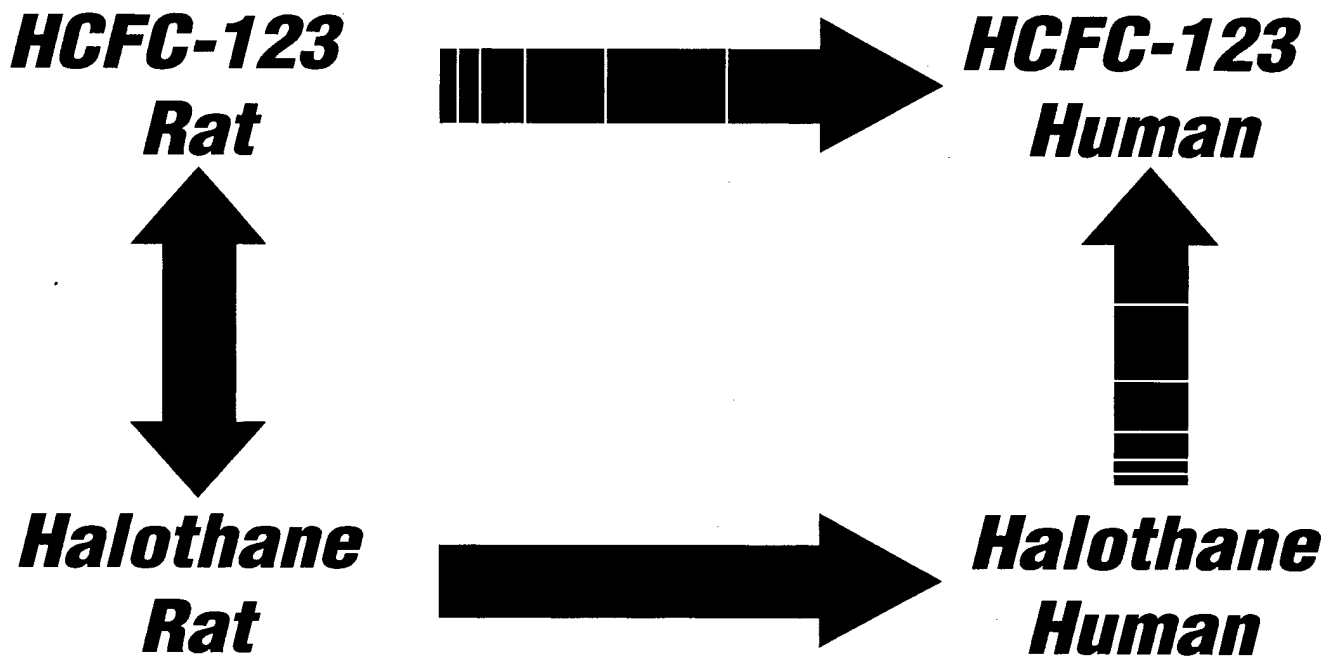


Figure 9. **Parallelogram for Interspecies Extrapolation of Hepatotoxicity Based on Structural Similarity of Structure and Mechanisms of Action Between Halothane and HCFC-123.**

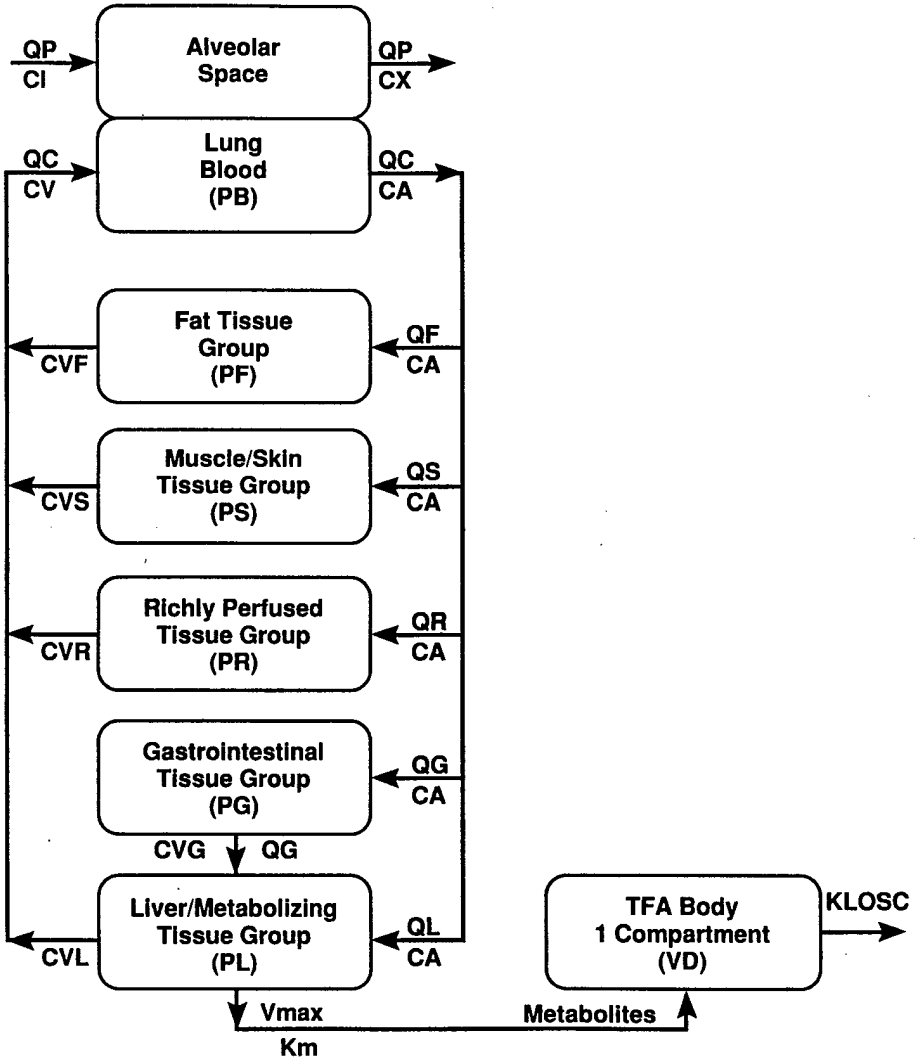


Figure 10. Physiologically Based Pharmacokinetic Model Structure for HCFC-123 and Halothane in Rats and Humans.

TABLE 11. TYPES OF DATA FOR DEVELOPMENT OF PBPK RAT MODEL FOR PARALLELOGRAM EXTRAPOLATION OF HCFC-123 AND HALOTHANE

- VOC Template with Chemical-Specific Modifications
  - Structure
  - Scaling Assumptions

- Empiric Data for Physiological Constants and Metabolic Parameters
  - Parent Compound (HCFC-123 or Halothane)
    - Partition Coefficients
    - Gas Uptake Experiments to determine V_{max}, K_m (HCFC-123 at 7 concentrations)
  - Oxidative Metabolism Marker (TFA)
    - TFA Cumulative 180 h Urinary Excretion after inhaled Halothane at .78%
    - TFA in blood after intravenous dosing with HCFC-123 at .01, 0.1 and 1.0%

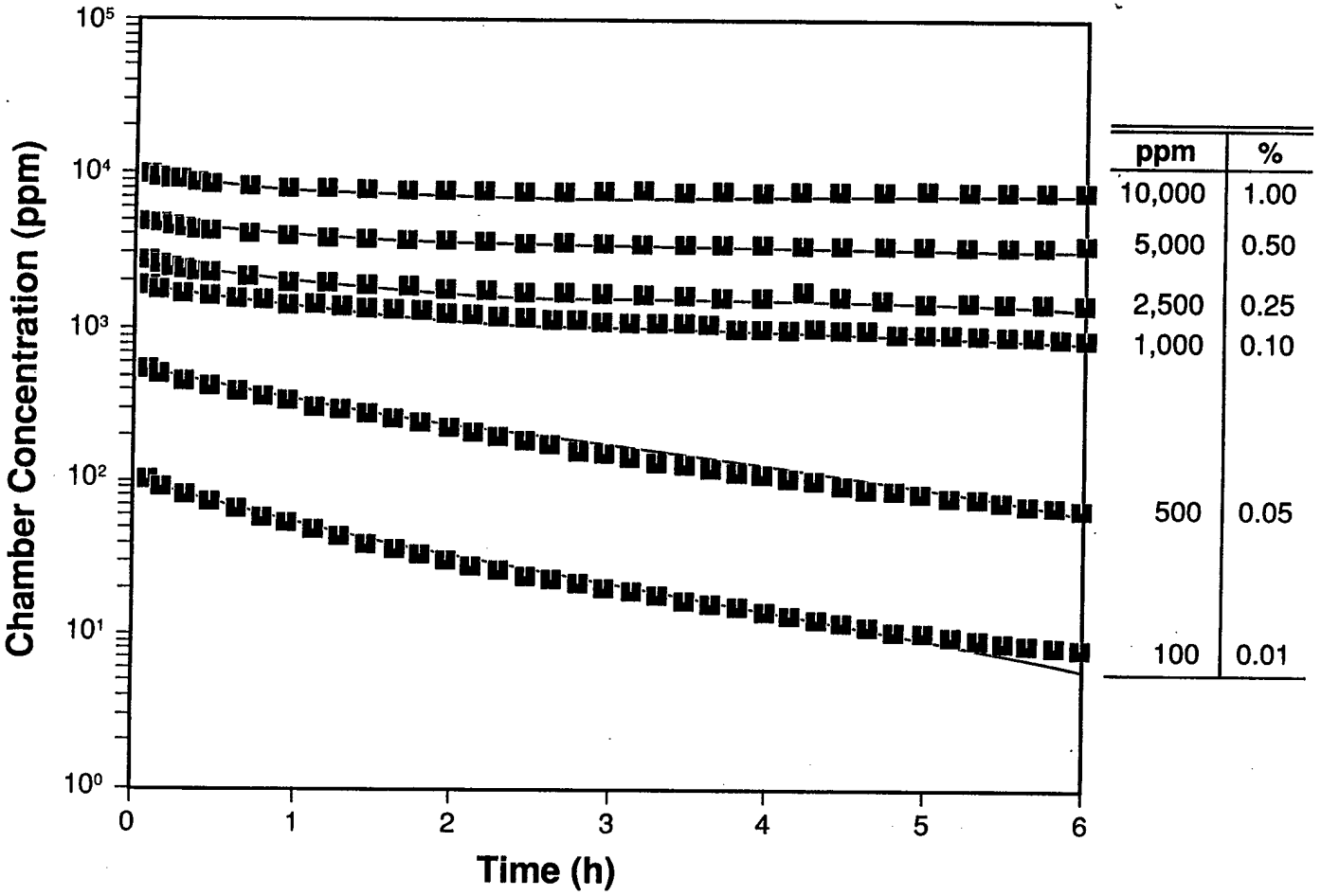


Figure 11. Gas Uptake Data (Squares) for HCFC-123 at Various Exposure Concentrations in the Rat. Model predictions (solid lines) were optimized against these data points by adjusting the V_{max} and K_m for model development.

Validation of the rat model for each compound, HCFC-123 and halothane, was then performed by comparing model predictions against other experimental data not used for model development. Utilization of data for both compounds increased the confidence that the model structure was sound and decreased the need for additional testing for one or the other compound. The types of available validation data are summarized in Table 12. HCFC-123 model output for a given measurement were compared against experimental data for the same measurement. For example, model predictions were in close agreement with measurements of exhaled breath HCFC-123 concentrations during and post a 2-h exposure at various concentrations tested(50).

TABLE 12. TYPES OF DATA FOR VALIDATION OF RAT PBPK MODELS FOR PARALLELOGRAM EXTRAPOLATION OF HCFC-123 AND HALOTHANE

- HCFC-123
  - Parent Compound
    - Venous Blood Concentration Time Course
    - Exhaled Breath at 0.01, 0.1, and 1.0% Exposures (anesthetized)
  - Oxidative Metabolism Marker (TFA)
    - TFA Venous Blood Concentration Time Course of 0.01, 0.1, and 1.0% Exposures
- HALOTHANE
  - Parent Compound—None
  - Oxidative Metabolism Marker (TFA)
    - TFA Venous Blood Concentration Time Course after Halothane at .78%

Model estimates for production of TFA, utilized as a marker for oxidative metabolism, were also shown to match well against experimental data for TFA blood concentrations in rats during and after a 4-h constant exposure to various concentrations HCFC-123(50). Thus, the model also closely predicts the experimental data for the marker of oxidative metabolism considered key to the hepatotoxicity of interest for the dose−response assessment.

Human PBPK models for HCFC-123 and halothane were then developed in a similar fashion(52). Data from human halothane exposures outlined in Table 13 were used to validate the model. The human PBPK model adequately predicts halothane kinetics in humans. The model simulation agrees closely with

experimental data, for example, of halothane concentration exhaled by a human subject during a ½-h exposure to 0.2% (2,000 ppm)(52). By structural and metabolic analogy, the model is likely to adequately simulate human HCFC-123 kinetics also.

TABLE 13. TYPES OF DATA FOR VALIDATION OF HUMAN PBPK HALOTHANE MODEL FOR PARALLELOGRAM EXTRAPOLATION OF HCFC-123 AND HALOTHANE

- HALOTHANE
  - Parent
    - Exhaled Breath at 2% Exposure Concentration
    - *In Vitro* Rates of TFA Formation
  - Oxidative Metabolism Marker (TFA)
    - Urinary TFA Excretion after Intravenous Halothane Administration

Given that the PBPK model structures discussed adequately describe the kinetics and the hypothesized critical factors (oxidative metabolism) responsible for the observed hepatotoxicity of halothane and HCFC-123 in both rats and humans, use of the PBPK models is proposed as a more accurate approach to dosimetrically adjust the observed effect levels in the rats to an HEC rather than the use of the default equations. In order to extrapolate laboratory animal data using a PBPK model, the laboratory animal exposure regimen (e.g., 6 h/day, 5 days/week) is simulated and the resultant appropriate dose metric (e.g., the area under the blood concentration curve [AUCB] of the parent compound) is calculated. This is done assuming steady-state conditions for chronic studies if it is determined that these conditions were met for 90% of the study duration or the entire exposure can be simulated with the model(53). The model is then exercised with the human parameters under the human exposure scenario (e.g., 24 h/day) to ascertain the exposure concentration that results in an equivalent dose metric. This exposure concentration back-extrapolated from the equivalent dose metric is the HEC. Human equivalent concentration values calculated using this PBPK model approach and based on two different dose metrics, total TFA (mg) produced per gram of liver and the AUCB of the parent compound, are provided in Table 14. Because TFA is the marker for the proposed oxidative metabolism hypothesized to be the key mechanism of toxicity, adjustment based on this dose metric is considered more relevant to the hepatic lesions under consideration as the critical effect than adjustment based on the parent AUCB. Because PBPK modeling to accomplish interspecies extrapolation is considered the

optimal approach in the RfC methodology, a decrease in the standard UF applied is also reasonable to propose.

TABLE 14. HUMAN EQUIVALENT CONCENTRATIONS (MG/M^3) CALCULATED WITH PBPK MODEL PARALLELOGRAM APPROACH AND BASED ON VARIOUS INTERNAL DOSE METRICS

Exposure Concentration	Exposure Concentration (mg/m^3)	Dose Metric		
		Default	Total TFA (mg) per (g) liver	AUC Parent in Blood
300	1,880	335	812	390
1,000	6,260	1,118	1,520	1,454
5,000	31,300	5,587	3,307	7,270

SUMMARY

Against the background of the theory of ozone depletion and the legislative framework for phaseout of chemicals contributing to this problem of global importance, expedited development of dose–response estimates for health risk assessment required to help characterize the use of proposed replacement candidates for the important CFC chemicals has been described. This development required consideration of structure–activity relationship when designing the replacement compounds and when designing tests to evaluate potential toxicity. Structure–activity considerations were also shown to be a useful component of a parallelogram approach for development and validation of PBPK models in rats and humans for dose–response analysis of the observed hepatotoxicity data. Such considerations can be critical when time is of a premium and can be used to decrease the need for extensive laboratory animal testing when coupled with judicious experimental design.

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DISCLAIMER

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FOOTNOTES

[1]The GWP is formally defined as the time-integrated change in radiative forcing[1] due to the instantaneous release of 1 kg of a trace gas expressed relative to that from the release of 1 kg of CO_2(5,6). A positive radiative forcing results in a net increase in radiation energy being retained below the troposphere and thus, contributes to a potential warming effect. Because the GWP is defined as a time integral, different time horizons yield different future concentration estimates. Future concentration estimates are calculated using a combination of factors that include the residence time (lifetime) of the greenhouse gas in the atmosphere and its chemical reactivity with other atmospheric constituents. The term radiative forcing refers to any change imposed on the climate system that modifies the radiative balance of the climate system. Quantitatively, it is the net amount of energy change per unit area caused by a particular gas at the tropopause, the boundary between the troposphere and the stratosphere, and is expressed in Watts/m^2.

[2]Public Law 101-549.

[3]A more detailed set of use categories and replacement candidates can be found in the EPA's Notice of Proposed Rulemaking (NPRM) for the Significant New Alternatives Policy (SNAP) Program(10).

[4]Halon 1211 is used primarily in streaming applications in which it is manually dispensed through a nozzle from a hand-held or portable extinguisher. Halon 1301 is used in total flooding and explosion protection applications in which a predetermined quantity of the gas is dispensed into a fixed location in order to achieve a specific extinguishing concentration of gas.

[5]The leading nonindustry research toward alternatives for these agents is being funded by the Air Force Wright Laboratory at the New Mexico Engineering Research Institute and at the National Institute of Standards and Technology.

[6]The "Halocarbon Numbering System" was developed by Du Pont for Freon chemicals in the late 1930s. The system was later expanded and formalized into a standard by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) and the American National Standards Institute (ANSI): "Number Designation and Safety Classification of Refrigerants", ANSI/ASHRAE Standard 34-1992, 1992)(12). Many of these chemicals were originally given numbers and called "Freons" when first introduced but prefixes consisting of a series of letters denoting the constituents in the compound are now usually used when naming these chemicals (as has been the convention used in this paper) because this is a trade name. The refrigeration industry will often precede the halocarbon number with an "R".

[7]Dogs in general were affected at lower concentrations than rodent species (rat and mouse), are similar to humans in catecholamine release mechanisms, and are easier to train and handle.

[8]The procedure exposes healthy male beagle dogs, 1 to 2 years old, to a vapor concentration of the chemical in question following a "priming" dose of epinephrine (0.008 mg/kg administered intravenously in 1 mL saline over 9 sec = 50 μg/kg/min). After 5 min of exposure to the test chemical, a "challenge" dose of epinephrine is administered under the same conditions as the priming dose. Exposure to the test chemical continues for an additional 5 min after the second epinephrine dose. Cardiac activity is followed by electrocardiography throughout the 17 min test period. A "marked" response is defined as "those arrhythmias considered to pose a serious threat to life (multiple consecutive ventricular beats) or which ended in cardiac arrest (ventricular fibrillation)"(17).

[9]Although strict definitions that distinguish "response" and "effect" are necessary in order to determine appropriate mathematical or statistical models for analysis, the conventions of the NAS paradigm are used in this paper. Therefore, in the qualitative sense, the term "dose" may encompass administered dose (i.e., exposure concentration), delivered dose, or target tissue dose. Likewise, "response" in the qualitative sense, is an indication of an adverse influence regardless of whether the data were measured as quantal, count, continuous, or ordered categorical.

REFERENCES

1. M. J. Molina and F. S. Rowland, "Stratospheric Sink for Chlorofluoromethanes – Chlorine Atom-Catalyzed Destruction of Ozone," *Nature* 810-812 (1974).

2. U. S. Environmental Protection Agency, "Assessing the Risks of Trace Gases that can Modify the Stratosphere," Report No. EPA 400/1-87/001 (December 1987).

3. U. S. Environmental Protection Agency, "CFCs and Stratospheric Ozone," (Office of Public Affairs, Washington, D.C., December 1987).

4. *Federal Register*, "Protection of Stratospheric Ozone," Final Rule, FR (August 12), 53(156): 30566-30602 (1988).

5. Intergovernmental Panel on Climate Change, "Scientific Assessment" (University Press, Cambridge, 1990).

6. Intergovernmental Panel on Climate Change, "Scientific Assessment Supplement" (University Press, Cambridge, 1992).

7. U.S. Code, "Clean Air Act", §§601-618, Title VI – Stratospheric Ozone Protection, U.S.C. 42:§§7661-7678.

8. *Federal Register*, "Protection of Stratospheric Ozone, Accelerated Phaseout Schedule" Proposed Rule, FR (March 18) 58(51): 15014-15049 (1993).

9. T. Moore, "CFCs: The Challenge of Doing Without," *EPRI J.* **14(6),** 4-13 (1989).

10. *Federal Register*, "Protection of Stratospheric Ozone, Significant New Alternatives Policy (SNAP) Program," Notice of Proposed Rulemaking, FR (May 12) 58(90): 28093-28159 (1993).

11. J. R. Floden and R. E. Tapscott, "The Quest for Chemical Alternatives to Halon-1211," *The Milit. Eng.* **No. 537,** 13-15 (August 1990).

12. R. E. Tapscott, "Definitive Rules for Naming and Numbering Haloalkanes," Center for Global Environmental Technologies, New Mexico Engineering Research Institute, University of New Mexico, Albuquerque, NM (September 1992).

13. G. M. Rusch, "Description of the Programme for Alternate Fluorocarbon Toxicology Testing Activities," Allied Signal, Morristown, NJ (July 24, 1989).

14. G. M. Rusch, "Overview of PAFT Toxicology," Presented at the 10th Annual Meeting of the American College of Toxicology, Williamsburg, VA (October 30-November 1, 1989).

15. H. J. Trochimowicz, "Industrial Research on Alternative Fluorocarbons," *Toxicol. Lett.* **68**, 25-30 (1993).

16. D. M. Aviado and M. S. Micozzi, "Fluorine-Containing Organic Compounds," in *Patty's Industrial Hygiene and Toxicology (3rd ed.)* Revised. Volume IIB. Toxicology.

17. C. F. Reinhardt, A. Azar, M. E. Maxfield, P. E. Smith, and L. S. Mullin, "Cardiac Arrhythmias and Aerosol 'Sniffing'," *Arch. Environ. Health* **22**, 265-279 (1971).

18. R. Rubenstein, "Human Health and Environmental Toxicity Issues for Evaluation of Halon Replacements," *Toxicol. Lett.* **68**, 21-24 (1993).

19. A. M. Jarabek and S. A. Segal, "Noncancer Toxicity of Inhaled Air Toxics: Available Approaches for Risk Assessment and Risk Management," in D. R. Patrick (ed.), *Toxic Air Pollution Handbook* (Van Nostrand Reinhold, NY 1993).

20. A. M. Jarabek, M. G. Menache, J. H. Overton, Jr., M. L. Dourson, and F. J. Miller, "The U.S. Environmental Protection Agency's Inhalation RfD Methodology: Risk Assessment for Air Toxics," *Toxicol. Ind. Health* **6**, 279-301 (1990).

21. U. S. Environmental Protection Agency, "The Risk Assessment Guidelines of 1986," Office of Research and Development, Office of Health and Environmental Assessment. EPA Report No. EPA 600/J-88-310.

22. G. T. Hall and B. L. Moore, "1,1-Dichloro-2,2,2-trifluoroethane. Acute Inhalation Toxicity," Haskell Laboratory Report No. 426-75 (1975).

23. R. W. Darr, "An Acute Inhalation Toxicity Study of Fluorocarbon 123 in the Chinese Hamster," Allied Corporation Report No. MA-25-78-15 (1981).

24. W. B. Coate, "LC_{50} of G123 in Rats," Final Report, Hazleton Laboratories America, Inc., Project No. M165-162 (1976).

25. R. S. Waritz and J. W. Clayton, "Acute Inhalation Toxicity (FC-123)," Haskell Laboratory, Report No. 16-66 (1966).

26. L. S. Mullin, "Behavioral Toxicity Testing. Fluorocarbon 123," Haskell Laboratory, Report No. 941-76 (1976).

27. H. J. Trochimowicz and L. S. Mullin, "Cardiac Sensitization Potential (EC_{50}) of Trifluorodicholoroethane," Haskell Laboratory, Report No. 132-73 (1973).

28. Bio/Dynamics, "An Inhalation Developmental Toxicity Study in Rabbits with HCFC-123," Project No. 88-3304 (1989).

29. R. Culik and D. P. Kelly, "Embryotoxicity and Teratogenic Studies in Rats with Inhaled Dichlorofluorethane (FC-123)," E. I. Du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Haskell Laboratory, Report No. 227-76 (1976).

30. D. P. Kelly, "Four-Week Inhalation Study with HCFC-123 in Rats," E. I. Du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Haskell Laboratory, Report No. 229-89 (1989).

31. D. G. Clark and D. J. Tinson, "Correlation of the Cardiac Sensitizing Potential of Halogenated Hydrocarbons with Their Physicochemical Properties," *Brit. J. Pharmacol.* **49**, 355-357 (1973).

32. D. G. Clark and D. J. Tinson, "Acute Inhalation Toxicity of some Halogenated and Non-halogenated Hydrocarbons," *Human Toxicol.* **1**, 239-247 (1982).

33. J. N. McDougal, A. M. Jarabek, and J. W. Fisher, "Dose-Response Assessment of 2,2-Dichloro-1,1,1-Trifluoroethane (HCFC-123): Dosimetrics at Different Durations," (in preparation).

34. P. S. Beck, D. G. Clark, and D. J. Tinson, "The Pharmacologic Actions of Bromochlorodifluoromethane (BCF)," *Toxicol. Appl. Pharmacol.* **24**, 20-29 (1973).

35. L. A. Malley, "Combined Chronic Toxicity/Oncogenicity Study with HCFC-123. Two-Year Inhalation Toxicity Study in Rats," Haskell Laboratory, Report No. 669-91 (1992).

36. L. A. Malley, "Subchronic Inhalation Toxicity: 90-Day Study with HCFC-123 in Rats," E. I. Du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Haskell Laboratory, Report No. 594-89 (1990).

37. C. D. Crowe, "Ninety-Day Inhalation Exposure of Rats and Dogs of Vapors of 2,2-Dichloro-1,1,1-Trifluoroethane (FC-123)," Haskell Laboratory, Report No. 229-78 (1978).

38. P. H. Leider, J. C. Cook, and D. A. Keller, "Similarities in Peroxisome Proliferation and Biochemical Effects Between HCFC-123 and Halothane (HCFC-123b1) in Rats," *The Toxicologist,* **396**, Abstract No. 1552 (1993).

39. J. C. Cook, S. M. Murray, S. R. Frame, and M. E. Hurtt, "Induction of Leydig Cell Adenomas by Ammonium Perfluorooctanoate: A Possible Endocrine-Related Mechanism," *Toxicol. Appl. Pharmacol.* **113**, 209-217 (1992).

40. L. B. Biegel, M. E. Hurtt, S. R. Frame, M. Applegate, J. C. O'Connor, and J. C. Cook, "Comparison of the Effects of Wyeth-14,643 in Crl:CD BR and Fisher-344 Rats," *Fund. Appl. Toxicol.* **19**, 590-597 (1992).

41. W. T. Brashear, M. M. Ketcha, D. L. Pollard, C. S. Godin, H. F. Leahy, P. P. Lu, E.R. Kinkead, and R. E. Wolfe, "Metabolite Identification of Halon Replacement Compounds," ManTech Environmental Technology, Inc., Toxic Hazards Research Unit, in *Air Force Systems Command, Armstrong Laboratory Report No. AL-TR-1992-0078* (Wright-Patterson Air Force Base, OH, June 1992).

42. A. J. Gandolfi, "Biotransformation of Inhalation Anesthetics: Role in Producing Toxicity," (1992).

43. H. J. Zimmerman, "Vulnerability of the Liver to Toxic Injury," in *Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver* (Appleton-Century-Crofts, NY, 1978, pp. 32-46).

44. C. A. Lunam, P. de la M. Hall, and M. J. Cousins, "The Pathology of Halothane Hepatoxicity in a Guinea-Pig Model: A Comparison with Human Halothane Hepatitis," *Br. J. Exp. Path.* **70**, 533-541 (1989).

45. R. C. Lind, A. J. Gandolfi, and P. de la M. Hall, "Covalent Binding of Oxidative Biotransformation Intermediates is Associated with Halothane Hepatoxicity in Guinea Pigs," *Anesthesiology* **73**, 1208-1213 (1990).

46. A. P. Brown, K. L. Hastings, and A. J. Gandolfi, "Generation and Detection of Neoantigens in Guinea Pig Liver Slices Incubated with Halothane," *Int. J. Immunopharmacol.* **13**, 429-435 (1991).

47. J. W. Harris, L. R. Pohl, J. L. Martin and M. W. Anders, "Tissue Acylation by the Chlorofluorocarbon Substitute 2,2-Dichloro-1,1,1-Trifluoroethane," *Proc. Natl. Acad. Sci.* **88**, 1407-1410 (1991).

48. G. B. Marit, M. E. George, D. E. Dodd, and A. Vinegar, "Hepatoxicity in Guinea Pigs Following Acute Inhalation Exposure to HCFC-123," *Toxicol. Pathol.* (Submitted).

49. J. W. Fisher, T. A. Whittaker, D. H. Taylor, H. J. Clewell III, and M. E. Andersen, "Physiologically-Based Pharmacokinetic Modeling of the Pregnant Rat: A Multiroute Exposure Model for Trichloroethylene and Its Metabolite, Trichloroacetic Acid," *Toxicol. Appl. Pharmacol.* **99**, 395-414 (1989).

50. A. Vinegar, R. J. Williams, J. W. Fisher and J. N. McDougal, "Dose-Dependent Metabolism 2,2-Dichloro-1,1,1-trifluoroethane(HCFC-123): A Physiologically-Based Pharmacokinetic Model in the Male Fisher 344 Rat," *Toxicol. Appl. Pharmacol.* (Submitted).

51. J. C. Ramsey and M. E. Andersen, "A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Rats and Humans," *Toxicol. Appl. Pharmacol.* **73**, 159-175 (1984).

52. R. J. Williams, A. Vinegar, J. N. McDougal, A. M. Jarabek, and J. W. Fisher, "Species Extrapolation of 2,2-Dichloro-1,1,1-trifluoroethane: A Corollary Approach to Physiologically Based Pharmacokinetic Modeling," (in preparation).

53. A. M. Jarabek and V. Hasselblad, "Inhalation Reference Concentration Methodology: Impact of Dosimetric Adjustments and Future Directions Using the Confidence Profile Method," Presented at the 84th Annual Meeting and Exhibition, June, Vancouver, British Columbia. Air and Waste Management Association, Pittsburgh, Paper No. 91-173.3 (1991).

SESSION III

WHERE THE PARADIGM NEEDS CHANGE

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Issues in the Development of a Risk Assessment for Fire Hazards

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ABSTRACT

In general terms, the risk assessment process determines information on the nature and extent of a hazard, as differentiated from the risk management process that encompasses judgements of acceptability and determination of degree to which the risks should or can be controlled. Risk assessment is primarily scientific, whereas risk management takes into consideration other factors such as cost, technical feasibility, public opinion/policy and timing.

To adequately assess the predominant hazards to humans resulting from exposure to fire conditions, the direct effects from heat and flames, visual obscuration due to the smoke density or eye irritation, and narcosis or irritation from inhalation of the products of combustion must be addressed. These effects may prevent escape and lead to subsequent injury or death. Although the toxic hazard is only one element of total risk due to fire, factors that influence smoke production must receive emphasis during the risk assessment because 80% of fire fatalities are due to smoke inhalation.

Assessing the effects of exposure of humans to smoke is extremely difficult because smoke is a continuously changing mixture of airborne solid and liquid particles and gases whose production is dependent on the burning conditions of the fire. The toxicity of the smoke produced in a fire is therefore time-dependent. This dictates that the inherent material properties that influence flammability and flame spread and the time course of the fire be examined in detail. To minimize risk from fire hazards, emphasis must be placed on SURVIVABLE smoke exposures in which the survival time is measurable and dependent upon two variables: potency of each smoke toxicant evolved as well as its rapidity of action. Fire conditions influence both the evolution of smoke and performance of a material in the fire. Thus, the inherent chemical/physical properties that determine flammability also influence the toxicity of smoke produced by a material and the resulting hazard. A methodology was developed to predict the hazard due to smoke exposure, which also allows an evaluation of the risk of the fire hazard produced.

Research on an OTA Assessment of Federal Research in Health Risk Assessment

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INTRODUCTION

Federal agencies conduct and support a diverse array of research to carry out their missions. The burgeoning use of risk assessments for decisionmaking and priority setting in many administrative agencies makes research to improve risk assessments an increasingly important agency function.

At the request of Congress, the Office of Technology Assessment (OTA) surveyed the federal programs conducting research to improve health risk assessments to describe the agency research efforts and examine how research priorities are established. The scope of the survey was narrowed to research programs dealing with environmental and occupational exposures to chemicals and radiation and to contaminants in food with a focus on cancer risks because the great controversies exist about environmental risks for cancer.

This paper describes the existing federal research activities designed to improve health risk assessments. The first part provides the scope of the agency risk assessment research activities. The second section addresses the direction of the federal research effort and the needs for improving the process or risk assessment.

FEDERAL RESEARCH ACTIVITIES IN HEALTH RISK ASSESSMENT

The OTA, for the assessment of agency research activities, divided health risk assessment research into three key areas, based on the research objectives. Methodological research is specifically aimed at improving the process of assessing risks by developing quantitative risk assessment methods. Basic research contributes to the understanding of how environmental agents perturb normal biological functioning. The last category involves research that expands the database of information about specific chemicals for use in risk assessments. The results of all three types of research are crucial; inadequate development in any one area could impede the progress of the overarching research objectives to make risk assessment more credible. Methodological research, for instance, develops models based on the biological parameters established in basic research but is driven by chemical-specific data.

These three areas were used because OTA could not fully categorize the research according to the process of risk assessment, as outlined by the National Research Council of the National Academy of Sciences (NRC, 1983). The NRC's sequential four-step process begins with hazard identification, progresses to dose–response and exposure assessments, and ends in risk characterization. Whereas the NRC "paradigm" has successfully laid out and formalized the process and made it transparent for decisionmakers and the public (Paustenbach, 1991; Rosenthal, Graham, and Gray, 1992), it does not delineate the role of research and the relationship between research and risk-based decisionmaking as rigorously as it did the risk assessment process. Thus, OTA's research led to its defining the following three distinct objectives of risk assessment research: (1) research to improve health risk assessment methodologies, (2) research to fill in chemical-specific data gaps, and (3) basic research to understand how environmental agents produce their adverse effects (Table 15).

TABLE 15. HEALTH RISK RESEARCH

A. METHODS DEVELOPMENT

1. **Methods and model development**
   The development of model tests and structure-activity analysis for identifying toxicants. The development of models for predicting human exposures. The development of methods for extrapolating effects, dose, and dose–response from laboratory study results to humans. Activities include:
   i) For effects identification and extrapolation
   ii) For exposure extrapolations
   iii) For dose–response extrapolations
   iv) Uncertainty analysis

2. **Methods Evaluation and Validation**
   The iterative process of integrating knowledge from the various disciplines and design experiments for acquiring new ones to determine the applicability of testing or extrapolation models from learned to new cases. Validated methods are applied to data collection or risk assessments.

(continued)

TABLE 15. Continued

B. BASIC RESEARCH

1. **Toxicity Mechanisms**
   Research for determining the sequence/combination of events (consequences of the interactions/effects), The concentration of the toxicant/its metabolite reaching the site of action and the rates of the reactions with its target(s) causally linked to disease/toxic effects development.

2. **Basic Research**
   Biological and biomedical sciences
   Biological and biomedical research on the structure and function of molecules, cells, organs, physiological systems, and organism. Knowledge on comparative genetics, biochemistry, physiology, will reduce uncertainty in effects, dose, and dose–response extrapolations.

   Chemical and physical sciences
   Research on physical and chemical properties that govern absorption, distribution, and transformation in the environment and in biological systems.

C. CHEMICAL-SPECIFIC DATA DEVELOPMENT

1. **Toxic Effects**
   Research design to identify the nature of the toxic effect of agents and the nature of dose−response under defined conditions of exposure.

   i) Human studies
   ii) Whole-animal studies using various conditions of exposure such as:
   iii) Mammalian tissue, organ and cellular studies
   iv) Microorganism and others

2. **Exposure Assessment**
   Environmental monitoring
   Measuring toxicant levels in different media

   Biological monitoring
   Measuring concentration/amount of toxicants in biological tissue

Research to Improve Risk Assessment Methodology

The OTA defines methodological research as research that develops models and methods to identify effects, to extrapolate dose−response or exposure relationships, or to measure uncertainty. Methodological research is difficult to define, but it can be characterized as research directed at devising approaches for extrapolating results from animal models to human estimates of risk, from high- to

low-dose exposure, from emissions to population and individual exposure, for estimating uncertainty and developing new assay systems. Some scientists expect the results of such research to be incorporated into decisionmaking within a relatively short time frame, about 3 to 5 years, and they should improve risk estimates by reducing the uncertainty in the ultimate risk numbers. An important and often overlooked part of methods research is evaluating and validating these methods with experimental data.

Arguably, methodological research holds the most immediate promise for providing substantive changes in the risk assessment process. To begin with, this research is generic; its results can have a large impact on many chemicals. Moreover, these methods are directed at the most uncertain aspects of risk assessments, especially extrapolations from high to low dose, extrapolations from animal models to human population risk estimates and methods for predicting toxicity of chemicals for which little or no toxicity data exist.

Basic Research in Support of Risk Assessment

Basic research requires the most time for incorporation into decisionmaking, and it has the lowest probability of success, but it also has the potential for broadly impacting the risk assessment process (OTA, 1991; Smith, 1991). In fact, the movement of basic research findings into technical fields important to risk assessment can sometimes be surprisingly rapid. Within the last several years, many molecular biological techniques and perspectives have proliferated throughout the field of toxicology (Marshall, 1993; DHHS, 1992).

Basic research, for the purposes of this report, is separable into two types: basic health risk research and basic sciences. Basic health risk research involves investigating the mechanisms of disease associated with exposure to possible toxic agents and the experimental tools for use in risk assessment research. Further removed from health risk assessment research, basic biological and biomedical sciences investigate the structure and function of molecules, cells, organs, physiological systems, and their relationship to the functioning organism. Basic chemical and physical scientists investigate chemical and physical agents and their reactivities and interactions with the environment.

Chemical-Specific Data Development

Chemical-specific data development is designed to identify the nature of the toxic effects of agents and to characterize the dose–response under defined exposure conditions. Hazard identification probably

represents the broadest, largest, and most diverse category of data development research and involve testing of agents relevant to the agency mission. Furthermore, collection of data on exposure to environmental agents is included in this research. Some scientists dismiss "data collection" or "data gathering" as less important than research, but OTA considers such work to be research. Its accuracy is a critical consideration because exposure data and basic toxicologic information usually form the basis of agency rulemaking. More important to this report, data collection activities provide essential input for both research into risk assessment models and basic research into the mechanisms of research.

The distinction between methods research and data collection can be blurry. Whereas OTA was unable to apply these criteria in every case, in general, "data collection" involves the use of established methods, and methods development focuses on changing or substantially refining methods.

Health Risk Assessment Research Portfolio

The OTA found that health risk assessment research could not be solely defined by the risk assessment paradigm, as originally outlined by the NRC (NRC, 1983). Instead, OTA further classified health risk assessment research into three distinct categories: (1) research to improve the method of risk assessment; (2) basic research that may provide information to improve risk assessment; and (3) chemical-specific data development. The OTA believes that all three areas must progress for a substantial improvement in the process of risk assessment and reduction in the uncertainty of risk estimates.

Taken together, the Federal research effort to improve risk assessment appears incomplete and imbalanced. In particular, given the promise of methodology research, resources allotted to it appear disproportionately small relative to data gathering and basic research efforts, as it receives only 11% of the estimated $639 million spent on health risk assessment research (Table 16). As a result, methodological research is a secondary priority for both research and regulatory agencies. In times of restricted resources and in the wake of congressional imperatives, the agencies must maintain the core programs that support their primary program objectives. Methodological research becomes marginalized in the process. Currently, this is evidenced by the shrinking resources allocated to the Environmental Protection Agency's (EPA's) Research to Improve Health Risk Assessment (RIHRA) program in the current proposed administration budget.

TABLE 16. 1993 R & D (ENVIRONMENTAL, OCCUPATIONAL HEALTH AND FOOD SAFETY) ESTIMATES

Agency	(Dollars in Millions)	Methods	Percent Methods
NIEHS	129	14	
DoE	90*	12[a]	
DoD	18	2.3[a]	
USDA	11	1.4[a]	
ATSDR	10[b]	–	
EPA	40[b]	21.3[c]	
FDA (other than NCTR)	10[b]	1.3[a]	
NCTR	33.6	7.6	
NIOSH	34**	4.6	
NCI	85[b]	4.3[b]	
other NIH	136[b]	2.4[b]	
ADAMHA	43[b]	–	
Total	639	71.2	11

[a] Calculated as 13% of Agency R & D on health.
[b] Estimate based on previous agency trend in *Research on Toxicology*.
[c] RIHRA estimated to be $5 million, $21.3 is sum of funding for human exposure, health effects and risk assessment methods.
* 1991 Research in Toxicology 7.8 millions.
** 1991 Research in Toxicology 4.5 millions.

Expanding methodological research is not simply redirecting funds at the expense of either basic or data collection research. Instead, methodological research should be considered complementary with the other types of research and integrated into the research being conducted on them. For methodological research, the results of basic mechanistic research provides the biological framework for many of the methods and models being developed. Dose–response and pharmacokinetic models, for example, are based on the physiologic parameters and metabolic routes obtained from basic research. Similarly, data collection research is also required because, for each chemical, risk assessments will need toxicity, dose–response, and exposure data. Furthermore, methodological research, especially extrapolation models, and basic research are both driven by chemical-specific data. The past decade witnessed nearly revolutionary developments in the biological sciences. Researchers are poised to incorporate these advances into the field of environmental health, especially into improving health risk assessments (Science, 1993; Olden, 1993). Despite the potential for advances, the present federal risk assessment research and development infrastructure remains a source of controversy. Many scientists interviewed by OTA claim the research system "is broke". Resources, they argue, are squandered on a system that is incapable of setting priorities. Consequently, the perception exists that the areas of highest priority

research, those most likely to improve the process of risk assessment, are not being funded or conducted at the expense of lower priority or even irrelevant research. The nature of the "right" research, however, remains problematic and the source of active debate on how the agencies determine their research priorities.

STRUCTURING THE FUTURE OF RESEARCH ON HEALTH RISK ASSESSMENT

If policymakers wanted to create the ideal climate to advance health risk assessment research of the highest quality, how would the research environment be structured, what scientific areas would be nurtured, and what types of research linkages would be pursued to assure necessary funding?

The OTA has observed some characteristics that are common to high quality research programs: leadership, defined objectives, investigator-initiated research, competitive awards and peer review, criteria for success, collaboration and coordination, training opportunities, and advisory input. Of course, some high quality research programs lack some or most of these characteristics, but these are features which many in the scientific community believe to contribute to scientific excellence.

Research Opportunities

The current health risk assessment research milieu is different from that of the past 20 years. Breakthroughs and rapid developments in the biological sciences – especially molecular biology and genetics – coupled with improved microelectronics and high-speed computers provide scientists with tools to study environmental health and toxicology that did not exist before.

One area in which these new techniques have had a significant impact is in stimulating new thinking about the role of toxins in the development of diseases. Mechanistic understanding of toxicity now calls into question certain accepted practices in health risk assessments. For example, some assumptions used in inferring and estimating risk can be examined for validity. The new knowledge, techniques, and examination promise methods to enhance our ability in predicting human health risks from exposure to toxicants and to reduce the associated uncertainties.

The need for improved risk assessments to support regulation creates the opportunity for more focused research for policy development. Unlike basic research, research in support of policy development has to be designed to address the needs of the policy maker, in addition to multidisciplinary

collaborations and integration of research efforts feedback from decisionmakers critical to the development of such science.

Methodological Research

Methods for identifying toxicants, exposed individuals and populations; models for inferring human health effects from animal studies; techniques for estimating risks and predicting health effects with few data are all in need of improvement or development. Although toxicological and biomedical research in the last decade has produced a large volume of information, the most immediate impact on risk assessment is expected from evaluating existing data to determine the credibility of current methods and to guide the development of alternative approaches. This evaluation can also identify specific short-term and long-term research activities to improve health risk assessment. Opportunities for methodological research exist in new methods for toxicity studies, biochemical and molecular epidemiology, mechanistically based effects and dose–response extrapolations, and human exposure methods.

Basic Toxicological Studies and Data Development

The results of ongoing basic biological research have long-term implications for future health risk assessment research. The application of knowledge from basic biology to basic toxicological research may be immediate, usually, however, it requires a considerable amount of time and resources. Scientists and decisionmakers interviewed by OTA stressed the importance of the relationship between conducting basic research and improving risk assessment methodology. Of, arguably, the greatest long-term significance for the environmental health sciences, is the study of the interaction between genetic susceptibility and the environment. Molecular techniques give scientists the capacity to focus on specific genetic damage associated with environmentally related diseases and to monitor DNA damage following exposure to environmental toxicants. These studies can identify genes susceptible to damage by toxicants, as well as sensitive subpopulations for adverse health effects of environmental exposures. Areas of opportunity in this type of research include basic biomedical research and data development of toxicological information on specific chemicals.

Risk Characterization, Communication, and Information Management

The process of risk characterization produces a summary and interpretation of the available information needed by risk managers to make decisions and to communicate results to the public. There is general agreement that methods to improve risk characterizations are needed. Research on risk characterization is directed towards more completely characterizing uncertainties, assumptions, alternative choices of analysis, and expressing the full range of plausible risk estimates. For addressing issues of uncertainty, statistical decision theory is being used by scientists to estimate the value of new information (Finkel and Evans, 1987). This approach could possibly be used by decisionmakers in establishing research priorities.

The explosion of research results useful for risk assessments, arising from many different fields, has created a need for improved access to relevant information. More advanced computational tools will be developed to store and analyze this information; ultimately, scientists will provide ways to use the available information for predicting the toxicity of other chemicals for which limited data or data only from related chemicals exist. The broad tasks of data synthesis will play an increasingly important role in the characterization and comparison of risk posed by different environmental problems. Scientists are seeking ways to improve the size and reliability of the toxicological database on environmental agents.

Fostering Research Linkages

Research linkages and collaborations offer enduring benefits to all partners. They bring together researchers with different strengths and expertise, they foster the dissemination of knowledge, and they permit sharing of resources. Research linkages also permit researchers to undertake projects which might otherwise not be possible.

Linkages can occur within and between federal agencies, as well as between federal and non-federal institutions. Linkages in health risk assessment research have traditionally taken place between government and universities. Research linkages are fewer between government and industry. The paucity of those linkages stems from publicly funded research being primarily devoted to identifying hazards and calculating risks, some of which were associated with industry products. This created a conflict-of-interest between public and industry concerns.

Health risk assessment research must expand into other broader areas of research to foster the linkages necessary to develop the field. By building bridges between fields of different research disciplines, new perspectives and insights can be encouraged. Furthermore, health risk research provides a link to societal needs for more basic research. As represented in Figure 12, health risk research is inextricably linked to the basic biological, chemical, and physical sciences, all of which are connected to decisionmaking. In addition to the invaluable expansion of the scientific knowledge base, basic science, in this perspective, can also be directed towards the "risk sciences" and, by extension, risk decisions. Analogous to the Human Genome Project in which collaborations form among scientists working to sequence the human genome, scientists from a plethora of disciplines can work together to improve health risk assessments as a desirable social and scientific goal.

By necessity, risk assessment research is increasingly multidisciplinary. No one field of academic training or disiplinary focus covers the research needs for risk assessments, which range from basic biomedical research to computer models simulating experimental conditions. Inevitably, no single focus of research, whether it is epidemiologic or toxicologic studies, will sufficiently provide the information necessary for chemical-specific risk assessments. In particular, the larger issues of risk assessment, that cut across several topics and are not chemical-specific, require research results from the spectrum of public health research disciplines. However, multidisciplinary interactions in most scientific endeavors require tremendous resources, from intellectual, to personal and financial; because the requirements are so great and barriers so high, many collaborations across disciplines do not succeed (Chubin, et al., 1986; Klein, 1990). Nevertheless, the benefits often include establishing new, even revolutionary, frontiers of science, arising from the exchange of information across disciplines (Kuhn, 1964). Given the extraordinary advances in the biological sciences and the unprecedented collaborative environment, advances in the process of assessing risk promise a new era in public health strategies and protection.

Linking Health Risk Research to Decision Making

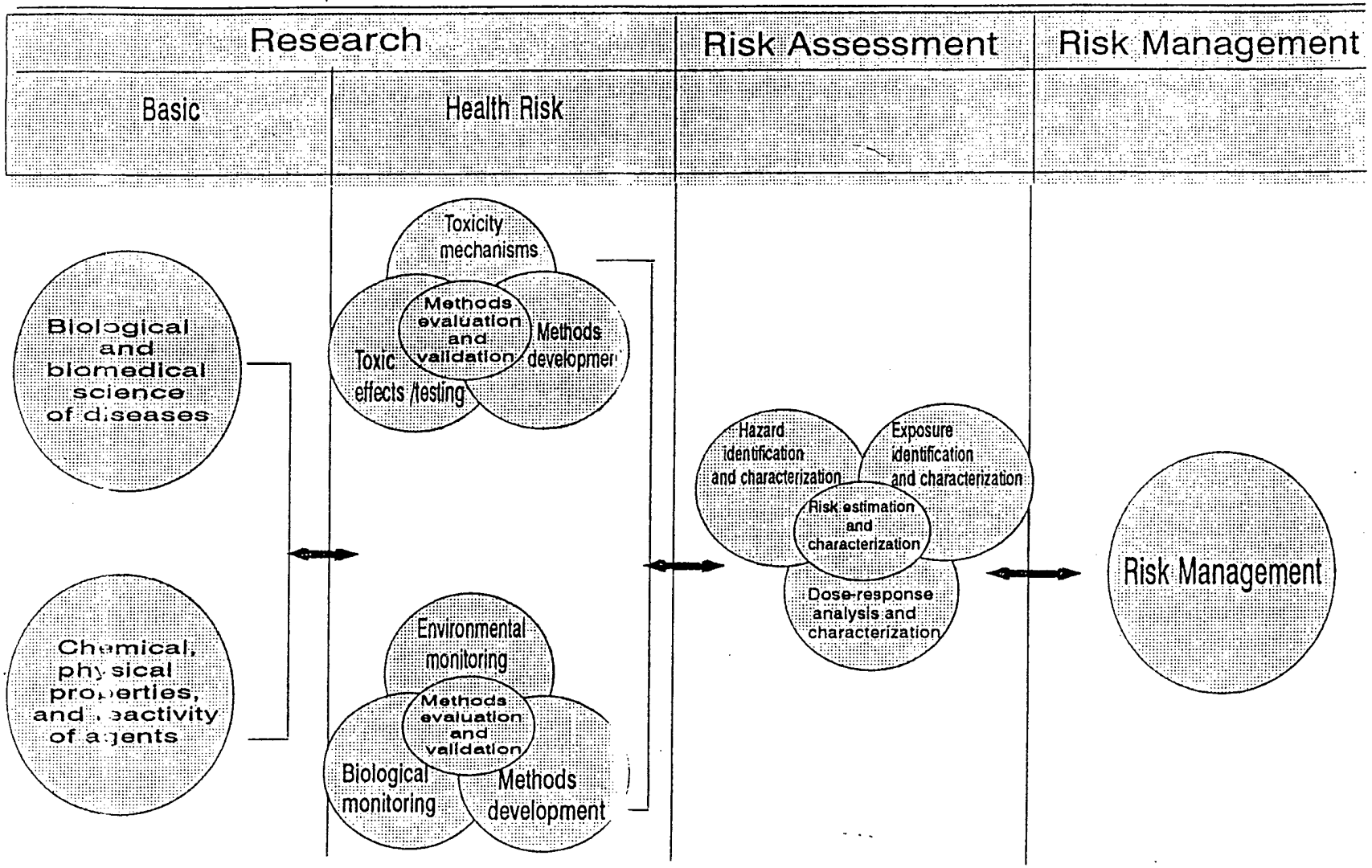


Figure 12. Linking Health Risk Research to Decision Making.

An example of the benefits of multidisciplinary research includes the new tools and advances in cellular and molecular biology that present nearly revolutionary opportunities for many disciplines in the environmental health sciences and are becoming increasingly evident in agency research (Olden, 1993; Marshall, 1993). These developments are shaping and refining our understanding of the processes involved in the pathogenesis of diseases, down to the cellular and molecular levels. Combining these molecular approaches, which can serve as indicators of genetic predisposing traits, with our expanding knowledge of nongenetic host factors, such as lifestyle or nutrition, provides powerful information for studying disease mechanisms and designing prevention strategies (Perera, 1990; NRC, 1991).

In addition to scientists collaborating to improve risk assessments, health risk researchers can transfer knowledge to the private sector to foster economic growth and competitiveness, which has become a vital part of the mission of many research agencies. In particular, legislation enacted during the 1980s provides federal agencies with incentives to promote technology transfer. This legislation encourages commercialization of research by permitting federal grantee institutions, contractors, and laboratories to retain the rights to inventions developed with federal funding. Scientists at these institutions can collect a portion of the royalties. The legislation also authorizes federal agencies to enter into research with the private sector through Cooperative Research and Development Agreements. These research agreements can be in place very early in the development process – well before an invention has been developed.

REFERENCES

1. G. Brown, "Science's Real Role in Policy-Making," *Chem. Eng. News*, pgs. 9-11 (May 31, 1993).

2. D. Chubin, et al., "Interdisciplinary Analysis and Research" (Lomond, MD, 1986).

3. Department of Health and Human Services (DHHS), "Human Health and the Environment - Some Research Needs," (National Institutes of Health, National Institute of Environmental Health Sciences, Draft Report of Task Force IV, National Advisory Environmental Health Sciences Council, 1991).

4. A. Finkel and J. Evans, "Evaluating the Benefits of Uncertainty Reduction in Environmental Health Risk Assessment," *J. Air Pollut. Control Assoc.* **37**, 1164-1171 (1987).

5. J.T. Klein, "Interdisciplinarity: History, Theory & Practice," (Wayne State University Press, MI, 1990).

6. T. Kuhn, "The Structure of Scientific Revolutions," (University of Chicago Press, Chicago, IL, 1962).

7. E. Marshall, "Toxicology Goes Molecular," *Science*, Vol. 259 (March 5, 1993).

8. NRC, Commission on Life Sciences, Committee on the Institutional Means for Assessment of Risks to Public Health, *Risk Assessment in the Federal Government: Managing the Process* (National Academy Press, Washington, D.C., 1983).

9. K. Olden, "Environmental Health Science Research and Human Risk Assessment," *Reg. Toxicol. Pharmacol.* **17**, 230-233 (1993).

10. OTA, *Federally Funded Research: Decisions For a Decade*, OTA-SET-490 (1991).

11. D.J. Paustenbach, "Important Recent Advances in the Practice of Health Risk Assessment: Implications For the 1990s," *Reg. Toxicol. Pharmacol.* **10**, 204-243 (1989).

12. F.P. Perera, "Molecular Epidemiology: A New Tool in Assessing Risks of Environmental Carcinogens," *CA-A Cancer J. Clinic.* **40(5)**, 277-288. (September/October 1990).

13. A. Rosenthal, G.M. Gray, and J.D. Graham, "Legislating Acceptable Cancer Risk From Exposure to Toxic Chemicals," *Ecol. Law Quarterly* **19(2)**, 269-362 (1992).

14. "Academy Splits On Risk," *Science* 259, 759 (1993).

15. B. Smith, "American Science Policy Since World War II" (The Brookings Institutions, Washington, D.C., 1990).

A Computer-Aided Approach to Quantification of Human Intake for Risk Assessments

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ABSTRACT

A risk assessment was conducted by The Earth Technology Corporation to evaluate risks to human health resulting from soil contamination found at an industrial facility. This paper describes the use of several dBASE programs that were developed by The Earth Technology Corporation that greatly facilitated rapid review of a large quantity of data.

A total of 858 soil samples were collected and analyzed for 25 inorganic and 159 organic chemicals. Soil analytical data derived from the sampling effort were available in a set of related databases in the Air Force Installation Restoration Program Information Management System (IRPIMS) format.

A methodical approach was used to select chemicals of potential concern for risk calculations. Unusable data were deleted from the IRPIMS databases with the aid of several dBASE programs. A dBASE program, SITESTAT.PRG, was used to calculate statistical data. This program was executed separately on individual site databases for all depths sampled and for specific depths-of-concern. Data generated by SITESTAT.PRG included maximum concentration detected, arithmetic mean concentration, the number of valid detections, and the total number of samples analyzed. Analytical data were evaluated with a variety of criteria, including frequency and magnitude of detection and consideration of the depth-of-concern. A dBASE program, ABOVEMAX.PRG, was developed to greatly facilitate comparisons of site inorganic data to background site data. The collective result of these analyses is a table in which a summary is provided to justify retention or deletion of a chemical of concern for further evaluation for each site.

Human receptors who could be impacted by migration of site contaminants or by direct contact with site soil were identified. Soil contact exposure pathways were identified for these receptors. Two dBASE programs, AIREXPS.PRG and SOILEXP.PRG, were developed by The Earth Technology Corporation to estimate average exposure and reasonable maximum exposure for identified air and soil exposure pathways.

INTRODUCTION

A baseline risk assessment was conducted as part of a remedial soil investigation to evaluate baseline risks to human health resulting from soil contamination found at an industrial facility. A total of 858 soil samples were collected from 10 potential hazardous waste sites and analyzed for 25 inorganic and 159 organic chemicals. A risk assessment was not conducted for groundwater contamination because:

- The regional aquifer that supplies drinking water for off-site residents has been identified as contaminated, and an operating remediation system is in effect.
- The remediation system has impeded migration of contaminated groundwater off the industrial facility property.
- Current and future offsite residents will not be impacted by the contaminated plume contained on the facility property.
- Uncontaminated drinking water is supplied to facility workers by a municipal water system.

Several references provided guidance for the baseline risk assessment. These references included:

- Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A), Interim Final, U.S. Environmental Protection Agency (U.S. EPA), Office of Emergency and Remedial Response, Washington, DC, December 1989
- Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual, Supplemental Guidance, "Standard Superfund Default Exposure Factors", Interim Final, U.S. EPA, Office of Emergency and Remedial Response, Washington, DC, March 1991.

Soil analytical data derived from the sampling effort were available in a set of related databases in the Installation Restoration Program Information Management System (IRPIMS) format. These databases were constructed as .DBF files and were managed using dBASE III Plus version 1.0. dBASE was chosen for its applicability and cost effectiveness.

This paper describes the use of several dBASE programs to calculate statistics used for the selection of chemicals of potential concern, to calculate the average and 95% upper confidence limit (UCL) concentrations for chemicals of potential concern at identified human receptors, and to estimate human daily intake values for identified pathways. Statistical results from these programs greatly facilitated rapid review of a large quantity of data.

Although a full, baseline risk assessment was conducted, only the following risk assessment procedures will be discussed:

- Selection of Chemicals of Potential Concern
- Identification of Human Receptors
- Identification of Exposure Pathways
- Estimation of Concentrations of chemicals of potential concern at human receptors
- Estimation of human intake values.

OVERVIEW OF DATABASE MANAGEMENT

The IRPIMS format required the use of several databases; BCHRES.DBF (batch result database) was the main database used. It contained the analytical soil results for each analysis performed on all samples. Specifically, each BCHRES.DBF record contained the analytical results for a single chemical by sample; a trip, equipment, ambient air, or laboratory blank; or for a surrogate spike.

BCHRES.DBF was used to construct an individual site database for each site evaluated. BCHRES.DBF is composed of many fields; only a subset of these fields were used for the risk assessment. Table 17 displays the BCHRES.DBF fields used for the risk assessment.

TABLE 17. BCHRES.DBF FIELDS USED FOR THE RISK ASSESSMENT

LOCXREF	-	A unique identifier for the location of the sample; typically, the borehole ID
ANMCODE	-	The analytical method code
EXMCODE	-	The extraction method code
SBD	-	The sample beginning depth
SED	-	The sample ending depth
PARLABEL	-	Chemical label
PARVQ	-	Indicator of sample detection
PARVALDLUN	-	Dry weight soil concentration (mg/kg)
LABDL	-	Laboratory detection limit
SACODE	-	Type of sample (blank, field replicate)
SITEID	-	Number designating the site from which the sample was obtained
RAQUALIFY	-	Validation qualifier (U,UJ,J,UR,R)
HTFLAG	-	Indicates the sample exceeded a holding time
NDBLANKC	-	Indicates the sample did not exceed 10 or 5 times, depending on chemical, the maximum detection of any blank associated with the sample batch

SELECTION OF CHEMICALS OF POTENTIAL CONCERN

The following criteria were used to initially screen organic and inorganic soil data:

1. **Data Validation.** Ten percent of the data were validated in accordance with U.S. EPA guidelines (1)(2). All qualified data were reviewed. Table 18 presents validation qualifiers used for soil data.

TABLE 18. SOIL DATA VALIDATION QUALIFIERS

J	-	The associated numerical value is an estimated quantity
R	-	The data are unusable (compound may or may not be present); Resampling and reanalysis is necessary for verification
UJ	-	The material was analyzed, but the analyte was not detected; The sample quantitation limit is an estimated quantity
UR	-	The material was analyzed, but the analyte was not detected; The data are unusable and is rejected
U	-	The material was analyzed, but the analyte was not detected; The associated value is the sample quantitation limit

A dBASE program inserted "R" or "UR" qualifiers in the RAQUALIFY field in BCHRES.DBF. Any records qualified with a "R" or "UR" were considered unusable and were not added to any individual site database. A data qualified with "J", "U", or "UJ" were considered valid and were retained in site databases.

2. **Quality Control Analysis.** A dBASE program was developed to evaluate the analytical results for all quality control blanks in accordance with U.S. EPA Contract Laboratory Program (CLP) guidelines. These blanks included trip blanks, equipment blanks, ambient condition blanks, laboratory calibration blanks, and laboratory method blanks. The dBASE program performed two tasks in the evaluation of quality control blanks:

   A. Quality control blanks were analyzed for the presence of common laboratory contaminants, including acetone, 2-butanone, toluene, methylene chloride, and phthalate esters. Contaminant detections were considered valid only if the analyte concentration exceeded 10 times the maximum analyte concentration in any blanks associated with the sample batch. If an analyte concentration did not exceed 10 times the maximum concentration for an analyte found in any blanks, the dBASE program inserted a "B" in the NDBLANKC field of the BCHRES.DBF analyte record. If a "B" was assigned to the NDBLANKC field, the analyte concentration was not considered valid and was deleted from further consideration.

   B. For any other contaminant, an analyte detection was considered valid only if the analyte concentration exceeded five times the maximum analyte concentration detected in any blanks associated with the sample batch. If an analyte concentration did not exceed five times the maximum analyte concentration in any blanks associated with the sample batch, the dBASE program inserted a "B" in the NDBLANKC field of the BCHRES.DBF analyte record. If a "B" was assigned to the NDBLANKC field, the sample concentration was not considered valid and was deleted from further consideration.

3. **Laboratory Holding Time Analysis.** Laboratory holding times are time constraints for stages in laboratory sample analysis. Laboratory holding times were evaluated to identify all samples which exceeded any laboratory holding time with respect to extraction and analysis. Analytical results based on missed holding times were rejected from further consideration.

To accomplish this task, a dBASE program was developed to evaluate missed holding times by analyzing one of the IRPIMS-formatted databases, BCHTEST.DBF (batch test database). In BCHTEST.DBF, each record contained a sample collection date, an extraction date, and an analysis date. The dBASE program evaluated each record in BCHTEST.DBF to determine if the analysis for a sample exceeded a laboratory holding time. Each BCHTEST.DBF record was associated with a set of records in BCHRES.DBF which contained the analytical results for the set of chemicals run for the analysis. For each record in BCHRES.DBF containing analytical results for an analysis which exceeded a holding time, the dBASE program inserted the qualifier "OUT" in the record's HTFLAG field. During the construction

of individual site databases, any records qualified with "OUT" in the HTFLAG field were considered unusable.

A dBASE program, SITESTAT.PRG, was used to calculate statistics required for the selection of organics and inorganics of potential concern for each site. This program was run separately on each individual site database for the entire depth sampled and for a specific soil sample depth-of-concern. A depth-of-concern is defined as the soil interval which a receptor could contact. If two receptors could contact site soil at different depths (e.g., a current worker contacting surface soil and a future excavation worker contacting subsurface soil), the greatest depth-of-concern was used to generated statistical data. Statistics included: the maximum and minimum concentration, the average concentration, the 95% UCL of the arithmetic mean of the analyte's concentrations, the number of samples evaluated, and the number of detections for an analyte. R&R Report Writer was used to print the calculated statistics from the statistics databases produced by SITESTAT.PRG and an additional dBASE program, ABOVEMAX.PRG, in table form for each site.

The criteria for retaining or deleting inorganic constituents involved comparing inorganic concentrations at each site to inorganic concentrations at a selected background site. To accomplish this, output from SITESTAT.PRG was evaluated using the following approach. First, SITESTAT.PRG was executed for each site database for the entire depth sampled. An inorganic chemical that was not detected in any site samples was deleted from further consideration for the site. Second, comparisons were made using the maximum concentration and arithmetic mean for each inorganic chemical at the background site and at each individual site for all collected samples. Table 19 provides an example of statistics calculated by SITESTAT.PRG for inorganics (i.e., arsenic and magnesium) for a site. Third, the frequency of detections above maximum background concentrations for each inorganic chemical within a sample depth-of-concern was evaluated. This frequency was obtained by using output from SITESTAT.PRG and ABOVEMAX.PRG. Specifically, SITESTAT.PRG identified the number of samples analyzed for each inorganic chemical within a sample depth-of-concern. ABOVEMAX.PRG identified the detections above maximum background concentrations for each inorganic chemical for all depths. The frequency of detections above maximum background concentrations for each inorganic chemical was manually calculated by dividing the number of detections above maximum background concentrations within the depth-of-concern by the total number of samples within the depth-of-concern. Fourth, the magnitude of detections above maximum background concentrations within the depth-of-concern was evaluated using output from ABOVEMAX.PRG. Table 20 provides an example of magnesium detections above maximum background concentrations for a site.

TABLE 19. STATISTICS DATA

PARLABEL	MAX	MAX SITEID	MAX UNITS	MAX SBD	MAX SED	MAX QUAL	AR MEAN	ARMUC L 95	SP SIZE	DETECTS
Inorganics										
Arsenic	14.4	1	mg/kg	10.0	10.5		5.1	5.2	173	2
Magnesium	21765	1	mg/kg	10.5	11.0		8083	8623	158	158
Organics										
Bis(2-ethyl-hexypthalate)	5.5	1	mg/kg	11.0	11.5		0.7	0.8	108	37
Xylenes	0.108	1	mg/kg	10.5	11.0		0.003	0.004	191	5

Key:

PARLABEL	-	Analyte label
MAX	-	Maximum concentration
MAXSITEID	-	Number designating the site from which the maximum sample was obtained.
MAXUNITS	-	Units of MAX
MAXSBD & MAXSED	-	Max's sample beginning depth and ending depth, respectively
MAXQUAL	-	Qualifier for the maximum concentration; if the MAX value was a nondetect, an "ND" would appear in this field
ARMEAN	-	Arithmetic mean
ARMUCL95	-	95% UCL of the ARMEAN
SPSIZE	-	Number of samples analyzed
DETECTS	-	Number of valid detections

Note: This table was created from a SITESTAT.PRG database.

TABLE 20. DETECTION BY DEPTH

Inorganics[1]:
(only concentrations above the background level are given)

Site ID	PAR LABEL	LOCXREF	ANMCODE	SBD	SED	PARVQ	LABDL	PARVAL-DLUM
1	Mg	MWA1	SW6010	10.5	11.0	=	50	21,765
1	Mg	MWA1	SW6010	90.5	91.0	=	50	16,230
1	Mg	MWA1	SW6010	120.5	121.0	=	50	16,216
1	Mg	MWA1	SW6010	215.5	216.0	=	50	18,003

Organics[2]

Site ID	PAR LABEL	LOCXREF	ANMCODE	SBD	SED	PARVQ	LABDL	PARVAL-DLUM
1	Xylenes	MWA1	SW8240	10.5	11.0	=	0.005	0.108
1	Xylenes	MWA1	SW8240	70.0	70.5	=	0.005	0.018
1	Xylenes	MWA1	SW8240	80.5	81.0	=	0.005	0.013
1	Xylenes	MWA1	SW8240	90.0	90.5	=	0.005	0.027
1	Xylenes	MWA1	SW8240	100.0	100.5	=	0.005	0.017

Mg = Magnesium
Xylenes = Total Xylenes
[1]Inorganic data table was created from an ABOVEMAX.PRG database.
[2]Organic data table was created using dBASE.

For selection of organic chemicals of potential concern, a similar evaluation process was utilized with the aid of SITESTAT.PRG output. For organic chemicals, however, the background site was not used as a comparison. Consequently, ABOVEMAX.PRG was not used. The primary analysis was made using frequency and magnitude of detections. First, as previously described, SITESTAT.PRG was executed for the entire depth sampled. All organic compounds that were detected in less than 5% of the site samples were eliminated from further consideration. Second, SITESTAT.PRG was executed for each site database for the depth-of-concern. The frequency of detections within the depth-of-concern was evaluated. Third, dBASE was used to construct a database of detections for all depths. The magnitude of each detection within the site depth-of-concern was evaluated. In general, compounds which were not detected at levels significantly greater than the laboratory detection limit were eliminated from further consideration for the site. Tables 19 and 20 provide examples of SITESTAT.PRG results used to justify retention or deletion of organics of potential concern.

Table 21 provides an example of justification for retention or deletion of inorganic and organic chemicals for a site, using criteria previously discussed.

Figures 13 and 14 provide a general description of the steps performed by SITESTAT.PRG. It is beyond the scope of this paper to provide a detailed discussion of SITESTAT.PRG. Note that the program requires creation of an index file for analysis of a site database. The index file must group all samples for an analyte in ascending order according to the sample beginning depth. This is accomplished by indexing the site database using the concatenated fields PARLABEL+SBD.

IDENTIFICATION OF HUMAN RECEPTORS

Conceptual site models were developed for each site. Each conceptual site model includes a description of previous or current activities, including chemical sources and amounts, identification of chemicals of potential concern which can be attributed to site activities, identification of migration pathways, and identification of current or future human receptors who could be impacted by contact with contaminated site soil or chemicals migrating from a site.

TABLE 21. EXAMPLES OF JUSTIFICATION FOR SELECTION OF CHEMICALS OF POTENTIAL CONCERN AT A SITE

Inorganics	Explanation	Status
Silver (Ag)	Ag is detected in only 1 of 131 sample concentrations (3.2 mg/kg at 10.5 feet). Concentrations within the depth-of-concern (0 to 58 feet) are considered to be at background levels.	Deleted
Arsenic (As)	The maximum concentration at the site is less than the background maximum concentration. Also, the arithmetic mean for the site is less than the background arithmetic mean. Concentrations are considered to be at background levels.	Deleted
Magnesium (Mg)	Only 4 of 158 sample concentrations exceed the maximum background level. Three of the samples were taken from soil depths which are not of exposure concern (90.5 to 215.5 feet). Only one sample was taken from a soil depth-of-concern (21,765 mg/kg at 10.5 feet). The site arithmetic mean only slightly exceeds the background arithmetic mean. Concentrations are considered to be within background at the soil depth-of-concern.	Deleted
Mercury (Hg)	All site sample concentrations are below the laboratory detection limit. Concentrations are considered to be at background levels.	Deleted
Zinc (Zn)	Five of 158 sample concentrations exceed the maximum background level. Four of these samples were taken from soil depths of exposure concern (146 mg/kg and 375 mg/kg at 0.0 feet; 264 mg/kg at 1.0 foot; 117 mg/kg at 20.5 feet). Because two sample concentrations greatly exceed the maximum background concentration (3.6 times and 2.5 times the maximum background), concentrations are considered above background.	Retained
Bis(2-ethylhexyl) phthalate (BEHP)	BEHP was detected in 37 of 108 samples (0.38 to 4.8 mg/kg at 0.0 to 30.5 feet). Several of these sample concentrations significantly exceed the laboratory detection limit. Because of the significant concentrations in the soil depth-of-concern, and because of the relatively high sample detection frequency (34%), this analyte is retained for further consideration.	Retained
Chlorobenzene	Chlorobenzene was detected in 8 of 126 samples (0.006 to 0.012 mg/kg at 1.0 to 3.0 feet) for a detection frequency of 6%. Three of the detections are very low, only slightly exceeding the detection limit. Consequently, the relative detection frequency is 4%. Chlorobenzene is deleted from further consideration.	Deleted
Total Xylenes	Total xylenes were detected in 5 of 191 samples (0.013 to 0.0108 mg/kg at 10.5 to 100.0 feet). Because of low sample detection frequency (3%) and because there is only one detection within the soil depth-of-concern, total xylenes is deleted from further consideration.	Deleted

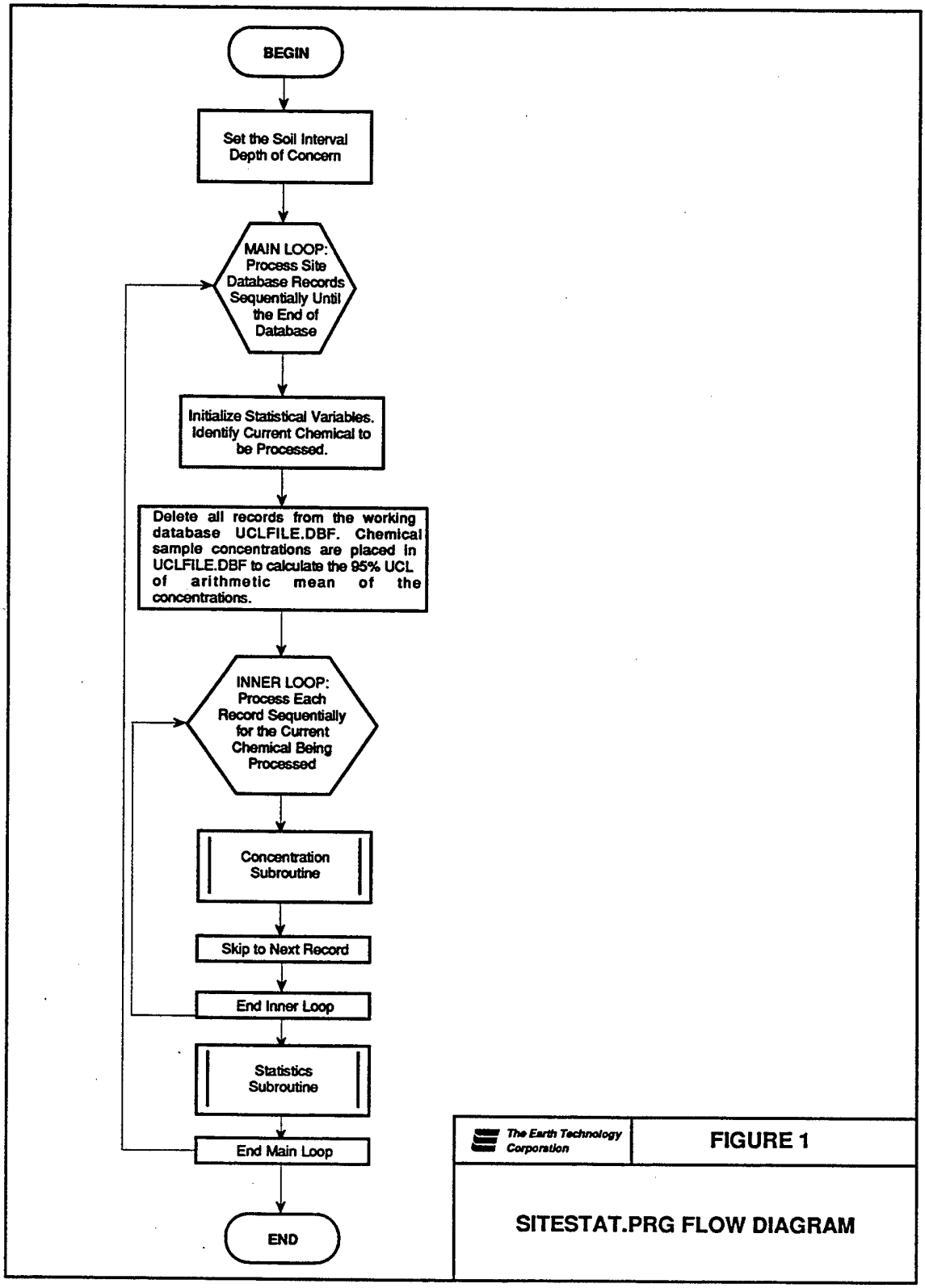


Figure 13. SITESTAT.PRG Flow Diagram.

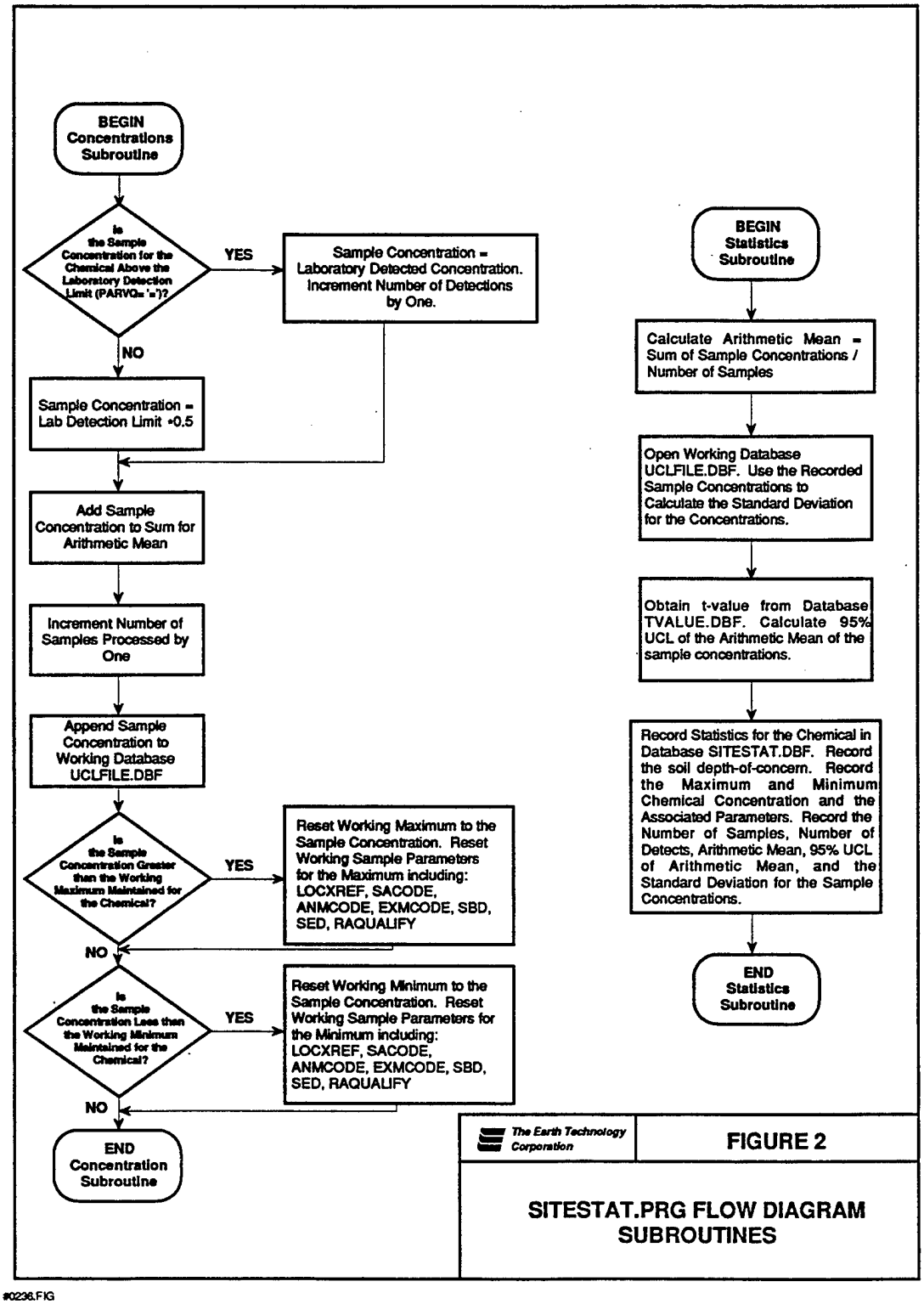


Figure 14. SITESTAT.PRG Flow Diagram Subroutines.

Three migration pathways were identified whereby chemicals of potential concern could potentially migrate toward human receptors. These migration pathways are:

- Migration of volatile organic compounds (VOCs) from the soil saturated and unsaturated zone to air
- Migration of dust containing particulate-bound contaminants to air
- Transport of surface soil contaminants by ephemeral surface water runoff.

Human receptors who could be impacted by migration of site contaminants or by direct contact with site soil were identified. These receptors are current workers, current off-site resident children, and future excavation workers.

First, current workers were identified who could contact contaminated site soil directly. These receptors were workers in buildings located on sites with contaminated soil or workers whose activities brought them in contact with site soil, such as workers who jog across a site with contaminated soil. Second, on-site current workers were identified who could be impacted by migration of VOCs from the site soil saturated and unsaturated zone or migration of contaminated dust to air. Third, off-site current workers were identified who would be maximally impacted by VOCs migrating off site from soil or by dust containing particulate-bound contaminants migrating off site. Fourth, current resident children (i.e., 6 years of age) were identified as a sensitive subpopulation who could be impacted by contaminants migrating from a site in ephemeral surface water runoff through drainage channels and ditches. Finally, future receptors were identified who could be impacted by on-site excavation activities. Investigations revealed that excavation activities could occur in the future at three sites. Therefore, future excavation workers were identified at these sites who could be impacted by airborne VOCs or contaminant-bound dust, or who could contact the contaminated soil directly.

IDENTIFICATION OF EXPOSURE PATHWAYS

Exposure pathways were identified for human receptors who could be exposed to a chemical through a migration pathway or by direct contact with the chemical in soil.

The following exposure pathways were identified for current worker receptors who could be impacted by soil migration pathways or who could contact soil directly:

- Incidental ingestion of contaminated surface soil
- Dermal absorption of chemicals from surface soil
- Inhalation of contaminated dust
- Inhalation of VOCs.

Exposure pathways for off-site resident children who could contact chemicals in soil transported from a site in the drainage channels and ditches are:

- Incidental ingestion of contaminated surface soil
- Dermal absorption of chemicals from surface soil.

The existence of surface water in the drainage channels and ditches is temporary (i.e., on the order of hours), and only 11 in. per year of precipitation falls at the facility. Consequently, the exposure of off-site children to contaminated surface water is negligible, and resultant surface water exposure pathways are considered to be incomplete.

The following exposure pathways were identified for future excavation workers:

- Incidental ingestion of contaminated subsurface soil
- Dermal absorption of chemicals from subsurface soil
- Inhalation of contaminated dust
- Inhalation of VOCs.

DETERMINATION OF EXPOSURE CONCENTRATIONS AT RECEPTORS

Both the arithmetic mean and 95% UCL of the arithmetic mean contaminant concentrations at receptors were quantified per federal and regional U.S. EPA guidance. Because data were not collected in an unbiased manner and goodness-of-fit statistical tests rejected log-normal distribution of a subset of soil data, data were assumed to be normally distributed. Consequently, arithmetic concentrations, rather than geometric concentrations, were quantified at receptor intake locations.

Table 22 presents the formulas used to quantify the arithmetic mean and 95% UCL concentration at a receptor for each chemical of potential concern:

TABLE 22. FORMULAS USED TO CALCULATE THE ARITHMETIC MEAN AND 95% UCL OF THE ARITHMETIC MEAN CONCENTRATIONS AT HUMAN RECEPTORS

Arithmetic Mean:

$$\bar{x} = \frac{1}{n} \sum_{i=1}^{n} C_i$$

where: $\bar{x}$ =The arithmetic mean concentration
n =Number of contaminant samples
C_i =Contaminant concentration.

95% UCL:

$$95\%\ UCL\ of\ the\ arithmetic\ mean = \bar{x} + (t_{0.95,\, n-1}) \left(\frac{s}{\sqrt{n}} \right)$$

where: $\bar{x}$ = The arithmetic mean concentration
$t_{0.95,\, n-1}$ = The 95% t distribution value for n-1 degrees of freedom
s = Standard deviation
n = Number of contaminant samples.

Soil Contaminants

The SITESTAT.PRG program was used to calculate site soil mean and 95% UCL exposure concentrations at receptors for varying exposure depths-of-concern. As stated previously, if a "B" was assigned to the NDBLANKC field or an "OUT" was assigned to the HTFLAG field, reported analyte concentrations were considered unusable. Nondetectable concentrations were represented by an "ND" in the PARVQ field of BCHRES.DBF. In accordance with USEPA guidance, for all concentrations of chemicals of potential concern which were reported as nondetectable concentrations (i.e., the chemical concentration does not exceed the laboratory detection limit), a value of one-half the laboratory detection limit was used for calculating exposure concentrations by SITESTAT.PRG (3). All valid analyte detections were represented by "=" in the PARVQ field in BCHRES.DBF. If an "=" was assigned to the PARVQ field, the dry weight soil concentration in the PARVALDLUN field was used by SITESTAT.PRG to calculate exposure concentrations. In those cases where the 95% UCL of the mean concentration exceeded the maximum detected chemical concentration, the maximum detected

concentration was used as the exposure concentration. Examples of arithmetic mean and 95% UCL exposure concentrations have been presented in Table 19.

For current worker soil pathway exposures, sample data collected from the surface interval were used to calculate surface soil concentrations. For future excavation worker exposure, sample data collected from assumed subsurface excavation depths (e.g., 20 ft depths for two sites, 10 ft depth for one site) were used to calculate soil exposure concentrations.

For the current resident child exposure, the resident child was assumed to be exposed directly to chemical concentrations detected in site surface soil. It was conservatively assumed that site chemicals were totally transported to resident child receptors by surface water runoff, without any chemical dilution (e.g., adsorption to soil). Consequently, the site surface soil chemical concentrations calculated are assumed to be the surface soil exposure concentrations for the resident child.

Air Contaminants

Soil gas data were used to calculate VOC concentrations in air for current worker and future excavation worker exposure. The Farmer Model was used to determine emission rates of VOCs from each site to determine exposure concentrations for current workers. The Farmer Model is a modified Fick's First Law for steady-state diffusion. Fick's First Law assumes that transport of a VOC through the soil cover layer is controlled by molecular diffusion. It does not account for the effects of atmospheric conditions, such as temperature, wind speed, and barometric pressure, upon emission rates.

Using chemical air diffusion coefficients for each VOC and assuming the total soil porosity and air-filled soil porosity of the site-specific soil to be 30% and 10%, respectively, the Farmer Model was applied to the average soil gas concentration and 95% UCL of each compound. The VOC emission rates were determined in units of milligrams per square meter-second (mg/m^2-s). The total mass of VOCs emitted per second was determined by multiplying the VOC emission rate by the area (in square meters) of each site.

To determine the ambient air concentration of VOCs for on-site or off-site worker receptors, the Industrial Source Complex-Long Term (ISC-LT) dispersion model program was used. The ISC-LT is a U.S. EPA-approved Gaussian dispersion model. The model operates in both long-term and short-term

modes. The model uses meteorological data, including wind speed, wind direction, and atmospheric stability class, and area or point-source chemical airborne concentrations, to determine impact at a receptor location. The model program was used in the area source and long-term modes to estimate worst-case airborne VOC concentrations at maximally impacted worker receptors. The location of the nearest (on-site or off-site) suitable receptor was used to determine the maximum exposure for that site.

The ISC-LT dispersion model was used to estimate exposure concentrations for off-site, maximally exposed workers. These workers were identified by selecting the building at which the model predicted a maximum concentration. The ISC-LT dispersion model was also used for on-site worker receptors because it predicts long-term exposure concentrations needed to quantify chronic worker exposure.

The average and 95% UCL on-site or off-site worker exposure concentrations, in milligrams of VOC per cubic meter of air, were determined for each detected VOC.

For future exposure, an assumption was made that all VOCs in the soil would be released to the atmospheric excavation volume. The VOC concentration in the air per hour can be calculated using the box model method. The box model method assumes steady-state, hourly emission rates and uniform dispersion conditions so that VOC emissions are uniformly distributed throughout a "box" which is defined by the area of the source and the mixing height. The box model is applicable for estimating airborne VOC exposure concentrations for excavation workers because it uses short-term conditions (e.g., average annual windspeed, hourly emission rates) to estimate exposure concentrations for short-term excavation tasks.

Applying the box model method to the average and 95% UCL concentration of VOCs in the soil gas yielded the average and 95% UCL air concentration of VOCs which may be inhaled by excavation workers.

Inhalation of contaminated dust by current workers was also investigated. Particulate matter (PM-10) data for the site vicinity were obtained, including the 24-h maximum concentrations and the quarterly averages for 1990 and 1991.

The average and 95% UCL concentrations for chemicals of potential concern were determined from surface soil sampling results. Chemical concentrations in airborne dust were calculated by multiplying the chemical surface soil concentration by the appropriate PM-10 air concentration. The average PM-10 value and an average surface soil concentration were used to determine an average dust concentration. The maximum PM-10 value and the 95% UCL surface soil concentration were used to determine 95% UCL dust concentrations.

For future exposure pathways, dust concentrations inhaled by excavation workers were determined using similar parameters and assumptions used in the determination of VOC exposure concentrations. Using assumed excavation parameters, a particulate emission rate for backhoe excavation work was obtained from the U.S. EPA Air/Superfund National Technical Guidance Study Series, Volume III, January, 1989 (5).

After determining a mixing volume using the box model method, the total amount (in metric tons) of soil to be removed was multiplied by the particulate emission rate, yielding the mass of particulates emitted per hour of excavation. Assuming an equal dispersion throughout the atmospheric excavation volume, the particulate concentration in air per hour can be calculated. Applying this value to the calculated concentrations of chemicals in the soil in mg/kg yielded air concentrations of chemicals which may be inhaled by excavation workers.

ESTIMATION OF DAILY INTAKE VALUES

Intake values were estimated for identified exposure pathways. Human intake (i.e., the magnitude of exposure) is expressed as the amount of chemical at an exchange boundary (e.g., skin, lungs, gut) which is available for absorption. Chronic Daily Intake (CDIs) were estimated for current worker exposure; Subchronic Daily Intakes (SDIs) were estimated for current resident child and future excavation worker exposure. In accordance with U.S. EPA Guidance, CDIs were estimated for an exposure of 7 years to a lifetime and SDIs were estimated for exposure of 2 weeks to 7 years (3).

The basic formula used to estimate CDI or SDI is presented in Table 23.

TABLE 23. BASIC FORMULA USED TO ESTIMATE INTAKE VALUES

$$CDI \text{ or } SDI\ (mg/kg\text{-}day) = C \times \frac{CR \times EF \times ED}{BW} \times \frac{1}{AT},$$

Where:

CDI or SDI	=	CDI or SDI by the receptor in mg/kg body weight-dry
C	=	Chemical concentration; the arithmetic mean or 95% UCL of the mean concentration contacted over the exposure period
CR	=	Contact Rate; the amount of contaminated media contacted per unit time or event
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
BW	=	Body Weight of receptor; the average body weight over the exposure period (kg)
AT	=	Averaging Time; period over which the exposure is averaged (days).

As previously described, SITESTAT.PRG was used to calculate the mean and 95% UCL of the mean soil chemical concentrations at receptors for each site. These data were established in statistical databases by SITESTAT.PRG. Concentrations of VOCs in air at receptors were estimated using the Farmer Model and air dispersion modeling. PM-10 data and surface soil concentrations were used to determine dust concentrations at receptors. The arithmetic mean chemical concentration was used to quantify average intake; the 95% UCL of the arithmetic mean chemical concentration was used to quantify the reasonable maximum exposure (RME), in accordance with U.S. EPA (3). As stated previously, if the 95% UCL concentration exceeded the maximum concentration for a chemical, the maximum concentration was used.

Standard default exposure factors were used to estimate intake where applicable (3)(4); reasonable assumptions were made to quantify site-specific exposure factors.

The exposure frequency for all permanent current workers and future excavation workers was assumed to be 250 days/year (4). The assumption is made that an adult is at work 5 days/week for 50 weeks/years. Workers were assumed to jog on a contaminated site for 30 minutes/day, 5 days/week, and 50 weeks/year.

Resident children were assumed to play off site in the drainage channel for 4 h/day (exposure time) at 1 day/week for 50 weeks/year, or 50 days/year (exposure frequency).

The exposure duration for future excavation at two sites was assumed to be 150 h (i.e., 0.075 year); the exposure duration for future excavation at the third site was assumed to be 500 h (i.e., 0.25 year).

Two dBASE programs, AIREXPS.PRG and SOILEXPS.PRG, were developed to estimate average exposure and RME for identified air and soil exposure pathways, respectively. These programs implemented standard default and site-specific exposure factors for each identified exposure pathway. For each site with an identified VOC or dust exposure pathway(s), AIREXPS.PRG used the arithmetic mean and 95% UCL of the mean chemical concentrations for VOCs or dust at site receptor(s) to quantify the average and RME for each chemical.

For each site with an identified soil exposure pathway(s), SOILEXPS.PRG used the arithmetic mean and 95% UCL of the mean chemical concentration at site receptor(s) to quantify the average and RME for each chemical.

CONCLUSIONS

dBASE was used to manage several IRPMIS-formatted databases containing analytical soil data for several hazardous waste sites. Several dBASE programs were developed to automate portions of the risk assessment process, including the calculation of statistics used for the selection of chemicals of potential concern; the calculation of soil concentrations at receptors; and the calculation of human daily intakes for identified exposure pathways. An automated approach provided several advantages:

- Analytical soil data were evaluated for consistency and completeness.
- A large quantity of data were evaluated within a short timeframe.
- Accuracy of the risk assessment calculations was improved.
- A large quantity of data were evaluated in a cost-effective manner.

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REFERENCES

1. U.S. Environmental Protection Agency, "Region I Laboratory Data Validation -- Functional Guidelines for Evaluating Inorganics Analyses" (Washington, D.C., June, 1988).

2. U.S. Environmental Protection Agency, "Region I Laboratory Data Validation -- Functional Guidelines for Evaluating Organics Analyses" (Washington, D.C., February, 1988).

3. U.S. Environmental Protection Agency, "Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A), Interim Final" (Washington, D.C., December, 1989).

4. U.S. Environmental Protection Agency, "Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual Supplemental Guidance, 'Standard Default Exposure Factors,' Interim Final" (Washington, D.C., March, 1989).

5. U.S. Environmental Protection Agency, "Air/Superfund National Technical Guidance Study Series," Vol. III (Research Triangle Park, NC, January 1989).

Issues in Ecological Risk Assessment: The CRAM Perspective

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ABSTRACT

In 1989, a Committee on Risk Assessment Methodology (CRAM) was convened by the National Research Council (NRC) to identify and investigate important scientific issues in risk assessment. One of the first issues considered by the Committee was the development of a conceptual framework for ecological risk assessment, defined as "*the characterization of the adverse ecological effects of environmental exposures to hazards imposed by human activities....*" Adverse ecological effects include all biological and nonbiological environmental changes that society perceives as undesirable. The Committee's opinion was that a general framework is needed to define the relationship of ecological risk assessment to environmental management and to facilitate the development of uniform technical guidelines. The framework for human health risk assessment proposed by the NRC in 1983 was adopted as a starting point for discussion.

The CRAM concluded that, although ecological risk assessment and human health risk assessment differ substantially in scientific disciplines and technical problems, the underlying decision process is the same for both. The CRAM, therefore, recommended that the 1983 risk assessment framework be modified to accommodate both human health and ecological risk assessment. The CRAM defined an integrated health/ecological risk assessment framework consisting of the following four components: (1) Hazard Identification, (2) Exposure Assessment, (3) Dose−Response Assessment, and (4) Risk Characterization. The CRAM further provided recommendations on the scope of issues to be addressed in ecological risk assessment, critical research needs, and mechanisms for providing more detailed guidance on the scientific content of ecological risk assessments.

INTRODUCTION

In 1983, the National Research Council's (NRC) Committee on the Institutional Means for Assessment of Risks to Public Health published a landmark report on human health risk assessment. The report, *Risk Assessment in the Federal Government: Managing the Process* (1), proposed a conceptual framework for risk assessment that incorporates research, risk assessment, and risk management. Risk

assessment was defined as "... the characterization of the potential adverse health effects of human exposures to environmental hazards." The report proposed a conceptual scheme for risk assessment consisting of the following four components: (1) Hazard Identification, (2) Dose–Response Assessment, (3) Exposure Assessment, and (4) Risk Characterization. However, the report did not include in-depth discussion of scientific issues in health risk assessment. The 1983 Committee's objectives were limited to addressing institutional and procedural issues such as whether the analytic process of risk assessment should be cleanly separated from the regulatory process of risk management, whether single organization could be designated to perform risk assessments for all regulatory agencies, and whether uniform risk assessment guidelines could be developed for use by all regulatory agencies. Detailed development of technical guidelines was left to the agencies themselves.

In 1989, a new Committee on Risk Assessment Methodology (CRAM) was convened within the Board on Environmental Studies and Toxicology of the NRC's Commission on Life Sciences to identify and investigate important scientific issues in risk assessment. The Committee was asked to consider changes in the scientific foundation of risk assessment that have occurred since the 1983 report and to consider applications of risk assessment to noncancer end points. The first three issues considered by CRAM were (1) the use of the maximum tolerated dose in animal bioassays, (2) the use of the two-stage model of carcinogenesis, and (3) the development of a conceptual framework for ecological risk assessment. The Committee has recently issued a report on these three issues (2). In addition to describing an integrated framework for human health and ecological risk assessment, CRAM's report discusses the scope of applicability of ecological risk assessment and identifies major categories of scientific uncertainty for which additional research is needed. The purpose of this paper is to briefly describe the framework recommended by the Committee and compare it to the Environmental Protection Agency's (EPA's) recently published Framework for Ecological Risk Assessment (3, 4).

Ecological risk assessment was defined by CRAM as the "characterization of the adverse ecological effects of environmental exposures to hazards imposed by human activities." Adverse ecological effects include all biological and nonbiological environmental changes that society perceives as undesirable. Hazards include both unintentional hazards such as pollution and soil erosion and deliberate management activities such as forestry and fishing that are often hazardous either to the managed resource itself or to other components of the environment. The Committee's opinion was that a general framework analogous to the 1983 human health risk assessment framework is needed to define

the relationship of ecological risk assessment to environmental management and to facilitate the development of uniform technical guidelines. For example, a framework for ecological risk assessment could be used to do the following:

- Evaluate the consistency and adequacy of individual assessments,
- Compare assessments for related environmental problems,
- Identify explicitly the connections between risk assessment and risk management, and
- Identify environmental research topics and data needs common to many ecological risk assessment problems.

Like the health risk assessment framework, an ecological risk assessment framework would define the boundaries between risk assessment and risk management and identify general categories of scientific information relevant to risk assessment, but would not provide specific technical guidance. In addition, a general framework applicable to a wide variety of environmental problems would facilitate the development of truly interdisciplinary approaches to ecological risk assessment. In the past, environmental science has been fragmented into different groups of professionals (e.g., ecotoxicologists, fisheries biologists, and foresters) who perform similar work but who rarely interact. This fragmentation has impeded the transfer of new knowledge and methods between fields and resulted in significant duplication of efforts.

The Committee chose to investigate the feasibility issue by conducting a workshop in which six case studies representing different types of current assessments would be examined with respect to their consistency with a common framework. The six case studies were as follows.

- Assessing the effects of tributyltin on Chesapeake Bay shellfish populations,
- Testing agricultural chemicals for ecological effects,
- Predicting the fate and effects of polychlorinated biphenyls,
- Assessing responses of populations to habitat change,

- Regulating species introductions, and
- Harvesting the Georges Bank multispecies fishery.

A Workshop on Ecological Risk Assessment was held on February 26 through March 1, 1991, at Airlie House, Warrenton, VA. In addition to presentation and discussion of the case study papers, the workshop included breakout sessions to discuss conceptual and technical aspects of ecological risk assessment. A summary of the workshop presentations and discussion is included as an appendix to the CRAM report (2); three of the case study papers have been independently published (5-7). A general consensus emerged at the workshop that an ecological version of the 1983 framework is desirable and feasible, but no specific endorsement of a particular framework was sought or obtained. On reviewing the written materials produced at the workshop, the Committee concluded that the 1983 human health framework could be expanded to accommodate both human health and ecological risk assessment. For general applicability to ecological assessments, the 1983 scheme requires augmentation to address some of the interfaces between science and management, primarily because of the need to focus on appropriate questions relevant to applicable environmental law and policy under different circumstances. Specifically, the scheme needs modification to address (1) the influence of legal and regulatory considerations on the initial stages of ecological risk assessment and (2) the importance of characterizing ecological risks in terms that are intelligible to risk managers. The Committee's opinion was that these augmentations are as important for human health risk assessment as they are for ecological risk assessment.

THE INTEGRATED FRAMEWORK

The CRAM concluded that integration of ecological risks into the 1983 risk assessment framework is preferable to developing a *de novo* ecological risk assessment framework. Like health risk assessment, ecological risk assessment must be defined in broad terms if it is to be applicable to the full array of environmental problems that regulatory and resource management agencies must address. Moreover, any framework chosen for ecological risk assessment must be simple, flexible, and general, so that it will be understood by both scientists and the risk managers with whom scientists must communicate. The 1983 framework, by any measure, has been extraordinarily successful in communicating the broad features of health risk assessment throughout the scientific and regulatory communities. Although ecological risk assessment and human health risk assessment differ substantially in terms of scientific disciplines and technical problems, CRAM concluded that the underlying decision process is the same for both. The function of risk assessment is to link science to decision making, and

that basic function is essentially the same whether it is risks to humans or risks to the environment that are being considered.

The 1983 report defined Hazard Identification as "... the process of determining whether exposure to an agent can cause an increase in the incidence of a health condition," including "... characterizing the nature and strength of the evidence of causation." Dose–Response Assessment was defined as "... the process of characterizing the relation between the dose of an agent administered or received and the incidence of an adverse health effect as a function of human exposure to the agent," accounting for exposure intensity, age, sex, lifestyle, and other variables affecting human health responses to hazardous agents. Exposure Assessment was defined as "... the process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent currently present in the environment or of estimating hypothetical exposures that might arise from the release of new chemicals into the environment." Risk Characterization was defined as "... the process of estimating the incidence of a health effect under the various conditions of human exposure described in exposure assessment. It is performed by combining the exposure and dose–response assessments. The summary of effects of the uncertainties in the preceding steps are described in this step."

On evaluating the consistency of the six case studies presented at the workshop with the 1983 framework, CRAM concluded that most of the case studies fit reasonably well. The most obvious common deficiency related to Risk Characterization. Only one of the case studies, the Georges Bank study, included any quantification of risks in terms that could be used for risk-benefit calculations, valuation studies, or other quantitative comparisons applicable to decision making. Even in this case, the value of the assessment to decision making is uncertain. During plenary discussion, the study author emphasized that communication between scientists and managers is still inadequate and that fisheries management actions are often only marginally influenced by quantitative assessments. Approaches to hazard identification exemplified in the case studies were, on the other hand, substantially more diverse and in some cases more sophisticated than envisioned in the 1983 framework. Ecological hazard identifications often include identifications of specific species or ecosystems of interest, delineation of study areas, and determination of types of laboratory or field data on which an assessment will be based. These decisions reflect both scientific considerations (Which systems are vulnerable? What kinds of effects are possible?) and management considerations (Which species or ecosystems are to be protected? must costs be weighed against benefits? Is the objective to protect the resource or to optimize exploitation

of the resource?). The workshop consensus was that definitions of Hazard Identification and Risk Characterization proposed in the 1983 report are inadequate for the purposes of ecological risk assessment.

The CRAM agreed with the consensus at the workshop that the 1983 framework is inadequate as written for application to ecological problems, because (1) it does not account for legal mandates and other policy considerations that influence the initial stages and focus of ecological risk assessments, and (2) it pays insufficient attention to the critical problem of effective communication with risk managers and the public. The opinion of the Committee, however, is that these deficiencies are not unique to ecological risk assessment. Differences in the functions of different regulatory agencies clearly influence the types of data and inference guidelines used in health risk assessments, and effective risk communication is as important (and often as inadequately performed) in health as in ecological risk assessment.

The above finding does not contradict the 1983 recommendation that risk management and risk assessment must be separated. This recommendation was intended to prevent risk managers from attempting to force scientific results to conform with preferred management decisions and to prevent scientists from embedding their own risk management preferences within their technical analyses. Members of CRAM perceived that the recommendation to separate science and management has often been interpreted as requiring minimization of communication between managers and scientists. On the contrary, scientists and decision makers must work together to ensure that the technical scope of the analysis is relevant to the important management questions and that the results are communicated to decision makers in a form that can be readily understood by nonscientists. The need for communication is especially acute in ecological risk assessment, because few risk managers have any ecological training and because the end points of interest in ecological assessment (e.g., loss of biodiversity) are much less intuitive than are typical health risk assessment end points (e.g., cancer and birth defects).

Hazard Identification was redefined by CRAM to be the determination of whether a particular hazardous agent is associated with health or ecological effects of sufficient importance to warrant further scientific study or immediate management action. Exposure-Response Assessment was defined as the determination of the relation between the magnitude of exposure and the probability of occurrence of the effects in question. Replacement of the term "dose" with a more general term is required, because "dose" has a distinctly medical connotation and cannot be effectively applied to nonchemical stresses,

such as habitat change or harvesting. The "responses" addressed in ecological risk assessments include both direct effects of exposure and the much broader indirect effects, such as secondary poisoning of raptors due to accumulation of pesticide residues in their prey and effects of harvesting on fish-community structure. Exposure assessment was defined by CRAM as the determination of the extent of exposure to the hazardous agent in question before or after application of regulatory controls. In the Committee's view, the term "exposure" can be applied legitimately to nonchemical stresses, including both physical stresses (such as habitat change and ultraviolet radiation) and biological stresses (such as species introductions). Alternative terms (e.g., stress or stressor) were deemed unsuitable because of conflicts with medical uses of the same or similar terms. Risk Characterization was defined as the description of the nature and often the magnitude of risk, including attendant uncertainty, expressed in terms that are comprehensible to decision makers and the public.

The revised framework is summarized in Figure 15. In addition to the four basic components, Figure 15 depicts two key aspects of risk assessment. As noted above, it is essential to recognize the influence of policy considerations on hazard identification. The CRAM also wanted to emphasize the need to create a connection between the results of today's risk assessments and the science base for future risk assessments. The risk assessment process should not end when a regulatory decision is made. Follow-up in the form of monitoring (where measurable effects have been predicted), validation studies, and basic research are needed to improve the data and models available to technical risk assessors whenever the same or a similar problem is encountered in the future.

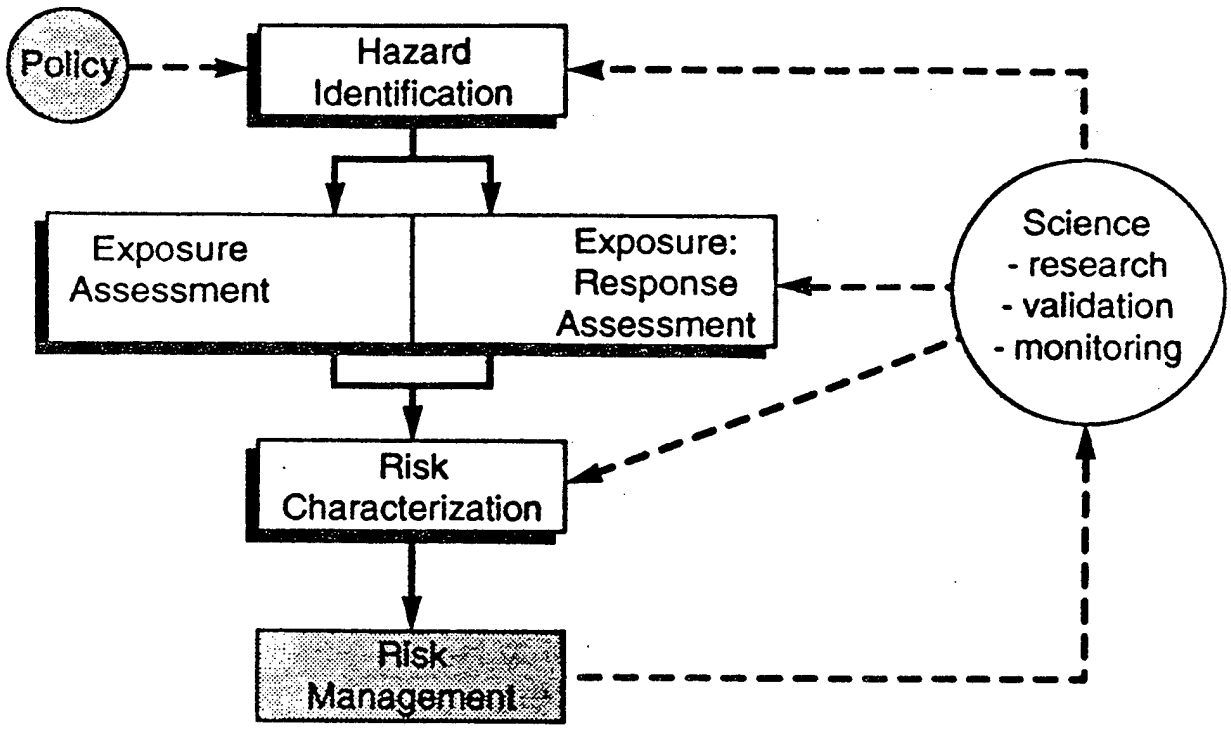


Figure 15. The CRAM Integrated Human Health/Ecological Risk Assessment Framework.

COMPARISON TO EPA'S FRAMEWORK FOR ECOLOGICAL RISK ASSESSMENT

The EPA's recently published *Framework for Ecological Risk Assessment* (3, 4) is quite similar to CRAM's integrated framework, and the similarity is not accidental. The EPA consciously modeled its framework on the 1983 NRC health risk assessment framework. Moreover, several of the authors of EPA's framework document participated in the CRAM ecological risk assessment workshop and a CRAM member served on a review panel that evaluated EPA's framework (8). The CRAM's "Hazard Identification" is replaced by "Problem Formulation" in EPA's version. The CRAM's "Exposure Assessment" and "Exposure-Response Assessment" are subsumed by EPA in a step called "Analysis," which is in turn subdivided into "Characterization of Exposure" and "Characterization of Effects." Definitions of the components are more specifically ecological and somewhat more explicit than are the definitions in the CRAM framework. Problem formulation, for example, is described as a "systematic planning step" that includes discussions with risk managers, a preliminary description of the potential ecological effects of the stressor, identification of the specific effects (termed "assessment end points") to be addressed in the assessment, and development of a conceptual model to guide the assessment.

The relationship between assessment and management in the EPA framework reflects EPA's specifically regulatory mission and might be approached differently by another agency or a private-sector organization involved in ecological risk assessment. Policy input is provided by a risk manager who discusses the assessment with the technical staff during the problem formulation phase. When the assessment is complete, the results are discussed with the risk manager, who then is responsible for making a decision and communicating the results to the public at large. In more general kinds of assessments, such as environmental impact assessments performed to satisfy the National Environmental Policy Act (NEPA), the planning phase (termed "scoping" in NEPA regulations) includes substantial public involvement. In others, such as assessments performed during development of environmentally safe products, frequent iterative interactions between design engineers, marketing staff, and risk assessors might be expected.

THE FUTURE OF ECOLOGICAL RISK ASSESSMENT

Neither the CRAM framework nor the EPA framework were intended to provide an explicit recipe for the scientific content of ecological risk assessment. The EPA expects the process of technical guidance development to implement its framework to take several years (4). The CRAM report recommends that expert committees be convened to discuss the major scientific issues in ecological risk

assessment. The report identifies four major areas in which scientific consensus is lacking: (1) extrapolation across scales of time, space, and ecological organization; (2) quantification of uncertainty; (3) validation of predictive tools; and (4) economic valuation of ecological resources. The principal objective of both frameworks is to provide a common conceptual foundation that can enhance the consistency and credibility of ecological risk assessments.

The CRAM made five specific recommendations concerning the future development and use of ecological risk assessment.

- Risk assessors, risk managers, and regulatory agencies should adopt a uniform framework for ecological risk assessment. The extension of the 1983 NRC human health risk assessment framework described in the CRAM report and depicted in Figure 1 is general enough to apply to most assessment problems and emphasizes the common elements of health risk and ecological risk assessment.

- State and federal agencies should expand the issue of risk assessment in strategic planning and priority-setting as a means of focusing their resources on critical environmental problems and uncertainties.

- Agencies should support the development of improved methods of risk characterization and consistent guidelines for applying them. Specific areas where current approaches are inadequate include extrapolation of population and ecosystem effects, expression of risks in terms that are useful for decision making and understood by the public at large, and evaluation and communication of both quantitative and qualitative uncertainties.

- To improve the science base for future risk assessments, agencies should institute systematic follow-up of risk assessments with research and monitoring to determine the accuracy of predictions and resolve remaining uncertainties.

- The EPA and other agencies should support systematic research programs to improve the credibility and utility of ecological risk assessments, and should draw on scientific expertise available outside the agencies themselves to develop technical guidance on the scientific content of ecological risk assessments.

The intent of CRAM's recommendations is to facilitate understanding of ecological assessment principles by nontechnical decision makers and the public at large and to ensure consistent improvement in the science supporting ecological risk assessment.

The Committee does not mean to imply that all organizations will perform ecological risk assessments exactly the same way. Clearly, different agencies and stakeholders have different interests, and specific technical guidelines consistent with the framework will be developed by each organization

performing ecological risk assessments. The past few years have seen a major increase in public interest in the environment. The adoption of "sustainable development" a as general environmental goal implies that economic development strategies should strive to simultaneously maximize both human welfare and environmental quality. An integrated framework for risk assessment of the kind recommended by CRAM can facilitate achievement of this goal.

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REFERENCES

1. National Research Council (NRC), *Risk Assessment in the Federal Government: Managing the Process* (National Academy Press, Washington, DC, 1983).

2. National Research Council (NRC), *Issues in Risk Assessment* (National Academy Press, Washington, DC, 1993).

3. Environmental Protection Agency (EPA), *Framework for Ecological Risk Assessment*, Report No. EPA/630/R-02/001 (U.S. Environmental Protection Agency, Washington, DC, 1992).

4. S. B. Norton, D. J. Rodier, J. H. Gentile, W. H. van der Schalie, W. P. Wood, and M. W. Slimak, "A Framework for Ecological Risk Assessment at the EPA," *Environ. Toxicol. Chem.* **11**, 1663-1672 (1992).

5. M. J. Fogarty, A. A. Rosenberg, and M. P. Sissenwine, "Fisheries Risk Assessment: Sources of Uncertainty," *Environ. Sci. Technol.* **26**, 440-447 (1992).

6. R. J. Huggett, M. A. Unger, P. F. Seligman, and A. O. Valkirs, "The Marine Biocide Tributyltin," *Environ. Sci. Technol.* **26**, 232-237 (1992).

7. R. J. Kendall, "Farming with Agrochemicals: The Response of Wildlife," *Environ. Sci. Technol.* **26**, 239-245 (1992).

8. Environmental Protection Agency (EPA), Peer-Review Workshop Report on *A Framework for Ecological Risk Assessment*, Report No. EPA/625/3-91/022 (U.S. Environmental Protection Agency, Washington, DC, 1992).

An EPA Perspective on Ecological Risk Assessment

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ABSTRACT

The U.S. Environmental Protection Agency (EPA) is placing increasing emphasis on using a risk-based approach to a wide range of ecological problems. To begin the process of developing Agency-wide guidance in this area, EPA's Risk Assessment Forum has developed a simple, flexible approach, or framework, for ecological risk assessment.

There are three major elements in EPA's ecological risk assessment framework. The first phase, problem formulation, is a planning and scoping process that establishes the goals, breadth, and focus of the risk assessment. Available information on the stressors, ecosystems potentially at risk, and ecological effects are used to select end points and to develop a conceptual model of the assessment. The analysis phase uses scientific information to develop exposure and effects profiles for the stressor. In risk characterization, these profiles are compared to determine the expected risk, and the uncertainties and limitations of the assessment are discussed.

Ideally, this framework can be used to evaluate different types of stressors, ecosystems, levels of ecological organization, and spatial and temporal scales, but the applicability of the framework in some areas (such as for physical and biological stressors) needs further evaluation. Other important issues include determining ecological significance (e.g., natural versus anthropogenic change and the potential for recovery) and evaluating uncertainties.

(For additional information on this subject, please refer to the article entitled "A Framework for Ecological Risk Assessment at the EPA," by S.B. Norton, D.J. Rodier, J.H. Gentile, W.H. van der Schalie, W.P. Wood, and M.W. Slimak. *Environmental Toxicology and Chemistry*, 1992, Vol. 11:1663-1672.)

SESSION IV

ADVANCING THE SCIENCE OF RISK ASSESSMENT – PART 1

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Analysis of the Multistep Process of Carcinogenesis Using Human Fibroblasts

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ABSTRACT

Normal human cells in culture have never been neoplastically transformed by carcinogen exposure. One possible explanation is that the life span of such cells is too short for them to acquire the necessary changes. To test this hypothesis, we needed normal human cells with a greatly extended or an infinite life span. We transfected the v-*myc* gene and a selectable marker into normal human fibroblasts, identified a drug resistant clone expressing v-*myc* protein, and passaged the progeny of the clone until they senesced. A few cells continued to proliferate and gave rise to a diploid, infinite life span cell strain, MSU-1.0, that has normal growth control and is nontumorigenic in athymic mice. Analysis showed that one more genetic change, in addition to unregulated expression of the v-*myc* gene, was involved in generating MSU-1.0 cells. They spontaneously gave rise to a variant strain, designated MSU-1.1, that grows more rapidly and is less dependent on exogenous growth factors. Analysis showed that at least two additional changes were involved in generating this cell strain. It has a stable karyotype composed of 45 chromosomes, including two markers. Transfection of specific oncogenes was used to determine the numbers and nature of additional changes required to transform MSU-1.1 cells into malignant cells. Analysis indicated that two changes were involved, but no change in karyotype. Exposure of MSU-1.1 cells to a single carcinogen treatment, followed by selection for cells with the characteristics of oncogene-transformed MSU-1.1 cells also yielded malignant human cells. We conclude that malignant transformation of normal human fibroblasts requires six or seven genetic changes, some of which involve suppressor genes.

INTRODUCTION

The recent identification of proto-oncogenes and tumor suppressor genes in the mammalian genome, including humans, along with many other recent insights, has led to a new understanding of how cancer develops. Specific activating mutations in proto-oncogenes cause such normal genes to produce protein in an inappropriate cell type, or to synthesize protein at a higher than normal level in a cell type

in which such proteins are ordinarily expressed at a lower level, or to synthesize a protein that functions abnormally. On the other hand, for suppressor genes to participate causally in the carcinogenesis process, they must lose the ability to produce functional protein. This ordinarily requires inactivating genetic changes, deletions or point mutations, in both copies of such genes.

Mutations in these cancer-related genes (i.e., proto-oncogenes and suppressor genes), can occur as a result of exposure to mutagenic carcinogens or, at a much lower frequency, as a result of spontaneous DNA replication errors. Such mutations frequently give cells a proliferative advantage (see below), which results in the clonal expansion of mutant cells. Obviously, clonal expansion increases the chance that a second rare mutational change could occur in one of the progeny cells that has already acquired one cancer-related mutational change. According to this hypothesis of carcinogenesis, the process is repeated until a cell that has acquired all the appropriate genetic changes needed for tumorigenicity arises. Clonal expansion of this cell gives rise to the tumor.

Studies, such as those of Knudson (1) on retinoblastoma (*Rb*), demonstrate that the mutations in cancer-related genes need not occur only in somatic cells, but can be inherited. In the case of the *Rb* tumor suppressor genes, rare individuals inherit one mutant copy of the *Rb* gene and one wild type copy. These individuals have a 100% risk of *Rb*, a tumor of the retinal cells that always arises in early childhood, and typically the tumors arise independently in both eyes. The common interpretation of these data is that independent inactivating mutations arise in the wild type *Rb* gene in a retinoblast cell in each eye, and that this is sufficient to cause this cell to become a tumor cell. This interpretation has practical, as well as theoretical implications. For example, at least one major hospital now uses less aggressive tumor treatment protocols involving ionizing radiation and/or chemotherapy with mutagenic agents for all individuals assumed to have inherited any of the mutant cancer-related genes, because it is feared that such individuals need only a second mutation for a cell to become tumorigenic (2).

Studies such as those of Knudson contrast with those by Vogelstein and colleagues (3) who analyzed colon tumors in adult humans to determine what genetic changes were responsible. The latter group found that genes located on chromosomes 5q, 12p, 18q, and 17p, as well as additional genetic changes not yet identified, are involved in colon carcinogenesis. Recent studies (4) indicate that although

the genetic alterations often occur in a preferred sequence, it is the total accumulation of changes, rather than the chronological order of appearance, that is responsible for the tumors' biological properties. Renan (5) has recently addressed the question of the number of mutational changes required for 28 common human tumors by plotting the log of the age-specific death rate against the log of age in years of the person affected, and deducing the number of changes (m) from the slope of the straight line (m-1), in the same manner as Armitage and Doll (6) had done earlier for colon and pancreatic tumors. However, because Renan had a much more extensive data set than Armitage and Doll (i.e., data from the Atlas of Cancer Mortality for U.S. Counties, 1950-1969), he was able to determine the contribution of various subpopulations. This type of analysis revealed that early onset (childhood) tumors of the nose required only three mutational changes after birth, whereas late onset (adult) tumors of the nose required six mutational changes. The common adult human tumors of the stomach, pancreas, kidney, skin, and colorectum required seven or eight mutational changes, and tumors of very late onset, such as prostate cancer, required 12 changes. Retinoblastoma was not analyzed in this study because of the paucity of the data. However, bone tumors were analyzed. Individuals that inherit a defective *Rb* gene are unusually susceptible to this tumor. The result was a biphasic curve, with a population of individuals that were unusually sensitive to such tumors at a young age (presumably *Rb* gene heterozygotes), and an older population that was much less sensitive. The younger individuals required only three mutational events after birth to form a tumor, the older individuals required six mutational events. Renan (5) interprets the linearity of the two curves for bone tumors, one with a slope of 2 (early onset) and the other with a slope of 5 (late onset) as indicating that the early onset tumors result because such individuals already have undergone three mutational changes before birth, one in the germ line (a defect in one copy of the *Rb* gene) and two *in utero*, whereas persons developing bone tumors after age 35 require six mutational changes after birth.

Obviously, mathematical modeling studies, such as those of Renan, have their limits. They cannot identify what genes are involved. Furthermore, they are based on several assumptions, some of which are clearly oversimplifications (e.g., that mortality rates provide a true indication of incidence rates for particular tumor types, and that the proliferation rate of a particular tissue remains constant throughout life). Nevertheless, they are important because they demonstrate that there are real differences in the

apparent number of genetic changes required for various adult tumors to form, and that childhood tumors, in general, represent a special subclass that results from fewer post-birth mutations.

Ultimately, mathematical analyses of the number of genetic changes required for tumorigenicity, such as those of Renan, or analysis of cells from tissues exhibiting various degrees of tumorigenicity to determine the various types of mutations that are present in specific genes, such as those of Vogelstein and colleagues, cannot answer the question of whether these events, by themselves, are causal of cancer. Such a demonstration requires that one take normal cells and convert them to cancer cells by a stepwise process in which each genetic change is known. We have undertaken just such studies, using normal human fibroblasts of foreskin origin. We have not yet identified each gene involved, but the results clearly demonstrate that six or more genetic changes are required. Our data fit well with the study of Renan (5), as well as those of Vogelstein and his colleagues (3).

STRATEGY

Because the process we and our colleagues wished to study involved the transformation of human fibroblasts from nontumorigenic cells to tumorigenic cells, we first carried out detailed studies to demonstrate that we could identify tumorigenic cells. The most rigorous criterion is that the transformed cells form a tumor in a suitable animal host. We, therefore, injected normal human skin fibroblasts or cells derived from human fibrosarcomas subcutaneously into the flank or shoulder of Balb C athymic mice (10^7 cells/site). The normal fibroblasts did not produce a growth of any sort; and extensive further testing since then has confirmed this. Five human fibrosarcoma cell lines that were tested formed sarcomas with a short latency (1 to 2 months). The tumor-derived cells were shown to have a human karyotype, which demonstrated that the tumors developed from the injected cells. Recent studies indicate that subcutaneous injection of 10^7 fibroblasts derived from human fibromas (benign tumors) produce fibromas in athymic mice (J.J. McCormick, unpublished studies). Therefore, we concluded that if human cells transformed in culture are equivalent to the human tumor-derived cells, athymic mice are a suitable test system for detecting them.

Because human fibroblasts in culture have never been observed to undergo spontaneous malignant transformation, we considered three possible ways to cause such transformation in culture. One method was to infect normal cells with SV40 or similar DNA viruses or to transfect them with the DNA of such viruses. One problem with this procedure is that although such agents cause transformation of cells in

culture and on very rare occasions such transformed cells can make tumors (7), there is no evidence that such viruses play a causal role in human fibroblastic cancers. Therefore, we did not use this approach. A second possibility was to expose normal fibroblasts to repeated carcinogen treatment. Carcinogen treatment of rodent fibroblasts in culture can transform them into tumorigenic cells (see 8, for example) and can induce mutations in human fibroblasts (see 9, for example). Repeated carcinogen treatments is known to lead to immortalization of human fibroblasts, but such results occur very rarely, and the infinite life span cells are not tumorigenic (10). An important problem with such studies is that there is no straightforward way to determine how many genetic changes are involved or which genetic change leads to which phenotypic change. Because there are 50,000 to 100,000 genes in the mammalian genome, it is a formidable task to sort out these changes. Therefore, we did not use this method. A third approach was to transfect oncogenes into cells. Because oncogenes are dominant-acting, a single copy expressed in a cell is sufficient to confer a distinctive phenotype. Because this approach offered us the promise of identifying the specific genetic changes involved, it was used for our studies.

Obviously, success with this strategy is determined by the insight with which one chooses the various oncogenes to be transfected into cells, and also the choice of the recipient cells. Our choice of particular oncogenes to be transfected into human fibroblasts was based on the following criteria: they have been reported to transform human or animal fibroblasts in culture, are found activated in human sarcoma-derived cells, are found activated in cells from persons with an inherited predisposition to develop sarcomas, or are the transforming genes of acute transforming viruses that induce sarcomas in animals. Even though we realized that transfer of activated oncogenes is not the way human cells become tumorigenic, the rationale for using this approach was that each of these genes has a homolog in the human genome that could be activated by appropriate carcinogen treatment, and each has a proven role in sarcoma induction in animals or humans. So far, we have had positive results using the H-*ras*, K-*ras*, N-*ras*, v-K-*ras*, v-H-*ras*, v-*fes*, v-*sis*, and v-*myc* oncogenes (11-19, and J. J. McCormick, unpublished studies).

Our ultimate choice of recipient cells was based on the following two principles: (1) our failure to obtain malignant cells using normal diploid foreskin-derived fibroblasts as recipients (12, 14), and (2) a realization that it might be necessary to sequentially introduce more than one oncogene into the cells in order to obtain a fully transformed malignant cell. Normal human cells in culture can only undergo two sequential clonal selections before they enter crisis and senesce (20). We considered it important to try

to develop cell strains with an infinite life span, rather than merely a greatly extended life span, because unlike cells derived from normal human tissue, cells derived from human tumors frequently exhibit an infinite life span when placed in culture, suggesting that the infinite life span characteristic plays an essential role in the tumor process *in vivo*.

ADVANTAGE OF USING THE MSU-1 LINEAGE OF CELLS

We succeeded in generating the infinite life span MSU-1 lineage (family) of human fibroblasts (17). The parental cell line of the lineage, LG1, was a nontumorigenic, diploid cell line with normal growth control derived in our laboratory from foreskin tissue of a normal newborn. We transfected a plasmid carrying a v-*myc* gene and selectable marker (*neo*) into a population of LG1 fibroblasts, selected for drug resistant colonies, and identified a clonal population that expressed the v-*myc* protein (17). The progeny cells of this clonally derived cell strain were carried in culture for many population doublings until the cells entered crisis and senesced. When they did so, a small group of replicating cells (presumably a clone) was found among the senescing cells, and these eventually gave rise to a diploid cell strain that is indistinguishable from the LG1 cells, except for expression of the v-*myc* gene and *neo* gene, and an infinite life span in culture. This diploid strain was designated MSU-1.0. From MSU-1.0 cells, a spontaneous variant strain with a growth advantage arose and overgrew the culture. This strain was designated MSU-1.1. The cells have a stable karyotype composed of 45 chromosomes, including two unique marker chromosomes (17).

MSU-1.1 cells are not tumorigenic but have a alteration in growth control because, unlike MSU-1.0 cells or LG1 cells, they grow moderately well in culture medium without exogenous growth factors. When we and our colleagues transfected MSU-1.1 cells with the H-*ras* (13) or N-*ras* oncogene (15) in a high expression vector, the transfected cells were found to express high levels of mutant *ras* protein. The *ras* transfectants were morphologically altered, formed large colonies in 0.33% agarose, grew rapidly in medium without exogenous growth factors, and formed sarcomas in athymic mice at the site of injection with a short latency period (see Table 24). However, they did not exhibit any change in karyotype beyond the two markers that are seen in the MSU-1.1 cells. This was also true of the cells derived from the sarcomas.

From the successful malignant transformation of MSU-1.1 cells using overexpressed H-*ras* and N-*ras* oncogenes (13, 15), and our failure to obtain tumorigenic cells by transfection of the same plasmids

into diploid, finite life span human fibroblasts, including LG1 (12, 14), or into MSU-1.0 cells (J.J. McCormick, unpublished studies), we conclude that the MSU-1.1 cells have acquired sufficient changes so that they only require two additional changes to become fully malignant. A judgment that two additional changes are needed is based on the fact that only *ras* oncogene-containing vectors engineered to synthesize high levels of mutant *ras* oncoprotein are able to malignantly transform these cells. For carcinogen treatment of MSU-1.1 cells to accomplish this would require two independent DNA changes, the introduction of a point mutation into the *ras* proto-oncogene, and the introduction of some change that results in a significant increase in the level of expression of the oncoprotein.

TABLE 24. GROWTH CHARACTERISTICS OF VARIOUS CELL STRAINS IN THE MSU-1 LINEAGE

Cell Strain	Colonies in Agarose per 104 Cells Plated (Diameter ≥80 μm)	Colonies in Agarose per 104 Cells Plated (Diameter ≥120 μm)	Growth Factor Independence	Animals with Tumors per Animals Injected	Mean No. of Days for Tumors to reach 1 cm in Diameter	Malignancy
LG1	0	0	--	0/30	--	--
MSU-1.0	0	0	--	0/20	--	--
MSU-1.1	2	0	+	0/40	--	--
MSU-1.1 H-*ras*	250	68	+++	30/38	32	High Grade
MSU-1.1 N-*ras*	270	70	+++	13/13	30	High Grade

If this analysis is correct, it predicts that *ras* oncogenes with enhanced transforming ability will be able to transform MSU-1.1 cells, even in the absence of high levels of *ras* expression. One example of such a *ras* oncogene is the v-K-*ras* gene, which contains two activating mutations, a point mutation in codon 12 and one in codon 59. To test this hypothesis, we and our colleagues (16) transfected MSU-1.1 cells with a plasmid carrying the v-K-*ras* oncogene and found that it readily transforms the cells and that the malignant cells do not exhibit elevated levels of v-K-*ras* protein. They have relatively low expression levels, as predicted.

An important finding from our transfection studies with the H-*ras*, N-*ras*, and v-K-*ras* genes is that each of the oncogene-transformed cell strains derived from MSU-1.1 cells that formed malignant tumors exhibit certain common characteristics in culture. They are morphologically altered, grow to high saturation densities, form large colonies in 0.33% agarose (>120 μm in diameter) at a high frequency ($\geq$5%), and proliferate in growth factor-free medium as rapidly as normal fibroblasts replicate in medium supplemented with 10% serum (Table 24). In addition, they maintain the same stable karyotype seen in nontransfected MSU-1.1 cells (13,15).

TRANSFORMATION OF MUS-1.1 CELLS BY CARCINOGEN TREATMENT

If MSU-1.1 cells are only two steps removed from being malignantly transformed, one should be able to transform them into malignant cells by carcinogen treatment and application of suitable selection techniques. Such experiments have recently been carried out (21). We and our colleagues exposed the cells to a single dose of carcinogen and selected them for focus formation (i.e., cells able to continue multiplying on a monolayer of cells in medium with a reduced level of serum [growth factors]). We observed distinct focal areas of overgrowth, and cells isolated from these foci grew to a higher final density than the parental cells. Only those focus-derived cells that formed large colonies in agarose (>120 μm in diameter) at a high frequency (5 to 19%) and proliferated rapidly in medium without growth factors formed malignant tumors. Thus, many of the characteristics acquired by MSU-1.1 cells malignantly transformed by carcinogens resemble those acquired by cells malignantly transformed by a transfected *ras* gene. There were, however, two important differences. First, there was no evidence of overexpression of *ras* oncoprotein in the carcinogen-transformed cells. We have not yet determined whether the *ras* proto-oncogenes have been activated by a mutation. Clearly, however, some genetic change other than higher *ras* expression is at least partly responsible for the transformation. Second, all carcinogen-induced malignant transformants that we have identified so far exhibit chromosomal changes

beyond those found in MSU-1.1 cells (21). Because carcinogens cause random damage throughout the cells' DNA, this result is not surprising. Such chromosomal changes were not seen in malignant transformants created by transfection of oncogenes such as *ras*, but one does not expect chromosome breakage to result from mere random integration of plasmid DNA into the human genome. The fact that the carcinogen-induced malignant cells have such chromosomal changes may indicate that loss of chromosomes plays a role in bringing about the required number of changes essential for tumorigenicity. However, the altered karyotype of the tumor-derived cell strains is not evidence that the malignant cells have acquired genetic instability, because each tumor-derived cell strain maintains its uniquely altered karyotype throughout continuous passage in culture (J. J. McCormick, unpublished studies).

MULTISTEP NATURE OF THE MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS

In the above experiments, we transfected various oncogenes (v-*myc*, H-*ras*, N-*ras*, and v-K-*ras*) into cells to confer on them the various properties of transformed cells. However, we also took advantage of spontaneous changes that occurred within the cells. Figure 16 indicates two places in the MSU 1 lineage, designated Q-1 and Q-2, where such spontaneous changes have occurred. In both cases, the cells that underwent this spontaneous change(s) exhibited a distinctive phenotype that allowed us to detect and isolate the cells in which the change had occurred. For example, the MSU-1.0 cells have an infinite life span, and the MSU-1.1 cells have a shorter doubling time than MSU-1.0 cells in medium supplemented with 10% serum.

As indicated above, there is no simple way to determine what genetic changes have occurred in such a situation. One can, however, determine whether a particular change has occurred in a proto-oncogene or a tumor suppressor gene by carrying out cell fusion experiments between, for example, a spontaneous transformant and its parental cell. If the resulting hybrid cells have the phenotype of the parental cells, one can conclude that the hybrid cells received a gene from the parental cells that had been lost in the generation of the variant (i.e., a suppressor gene). If the hybrid cells exhibit the phenotype of the variant cells, one can conclude that the variant cells gained their distinctive phenotype because of a dominant change (e.g., the activation of a proto-oncogene). We and our colleague, A. Ryan, carried out such fusion studies with the cells of the MSU-1. lineage (22). The results indicate that the MSU-1.1 cells have lost the function of a suppressor gene that is present in the MSU-1.0 cells. They also indicate that the MSU-1.0 cells have lost the function of a suppressor gene present in normal fibroblasts. Because the function of both copies of a suppressor gene must be eliminated, two independent genetic events must

have occurred (Figure 16). We are presently working to determine what suppressor gene function has been lost in these cell strains. Even without this information, we are able to conclude, as shown in Figure 16, that the malignant transformation of human fibroblasts minimally requires six or seven genetic changes. These results are consistent with the prediction of Renan's study and with the studies by Vogelstein and his colleagues with colorectal tumors.

CONCLUSION

In humans, it is clear that carcinogenesis is a multistep process in which cells acquire by sequential clonal selection, the phenotypic changes required for malignancy. We utilized this principle (sequential clonal selection) to develop a lineage (family) of cells (MSU-1) in which clonal variants in a population were selected for a tumor cell-like phenotype. As one moves from parent to daughter to granddaughter, the characteristics of the cells become more and more similar to those derived from malignant tumors. Using these cell strains, we have been able to demonstrate that human fibroblasts must acquire the expression of specific dominant-acting genes (oncogenes) and the loss of various supressor gene activities. From these cells, we have observed tumors composed of spindle-shaped cells (fibromas, spindle cell sarcomas, and fibrosarcomas). We also observed tumors made up of cells with other morphologies (myxoid sarcomas, rhabdomyosarcomas, malignant fribrous histiocytomas, and round cell sarcomas). Our studies indicate that at least six or seven genetic changes are required for normal human fibroblasts to become malignantly transformed. Among the early events must be those that cause immortalization. Our data suggest that for fibroblasts in culture, the other genetic changes can occur in any order. This multiple mutagenesis model fits well with the results of recent studies on the origin of human carcinomas.

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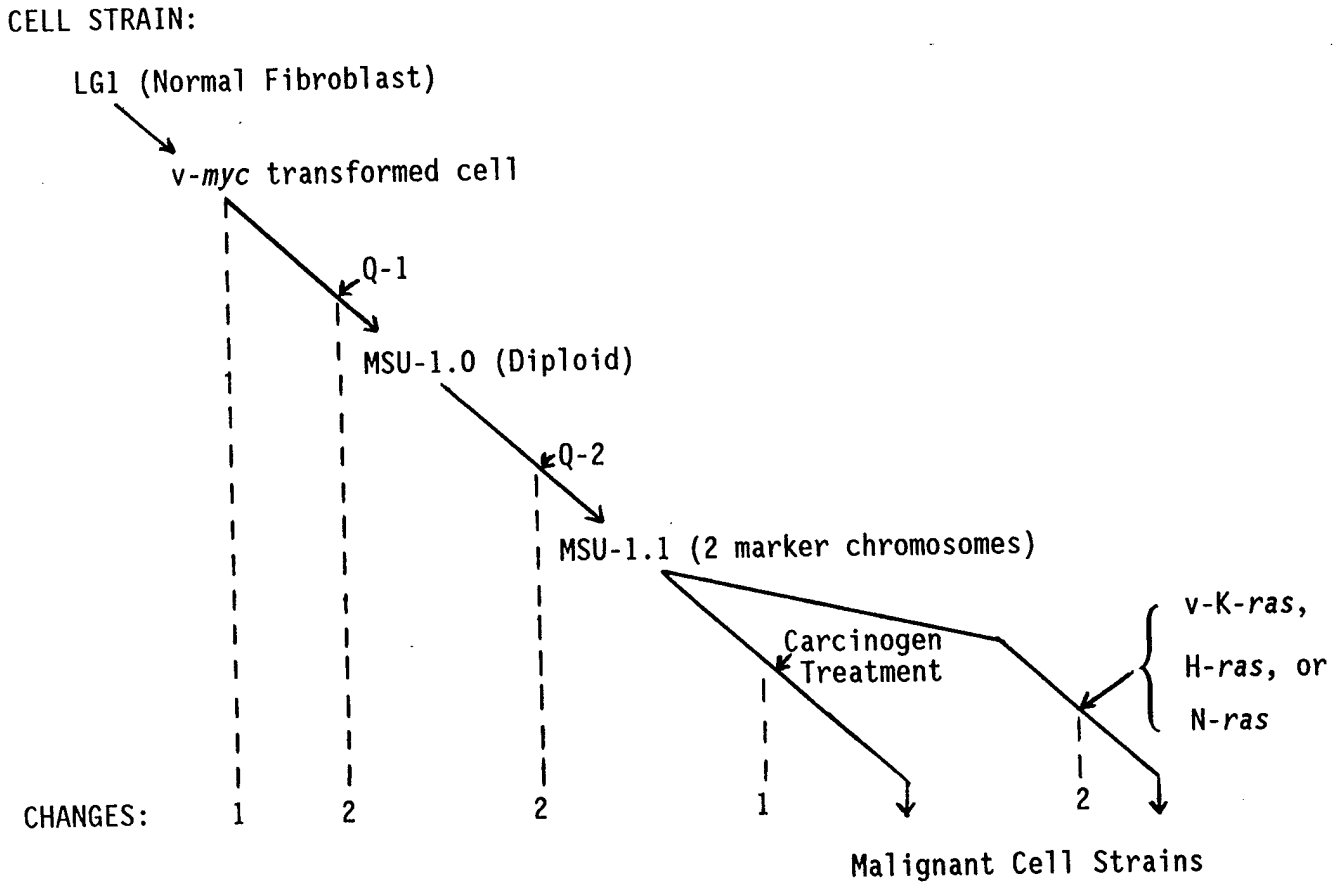


Figure 16. **Clonal Evaluation of the MSU-1 Lineage Showing the Important Properties of the Various Clonally-Derived Cell Populations and the Number of Genetic Changes Required to Cause These Changes.** "Q-1" and "Q-2" represent the loss of suppressor gene activity, although the specific genes involved have not yet been identified. See the text for details.

REFERENCES

1. A.G. Knudson, "Genetics of human cancer," *Annual Rev. Genet.* **20**, 231-251 (1986).

2. C.E. Jackson, "Limiting subsequent mutagenic events in carriers of hereditary tumor genes," *Am. J. Hum. Genet.* **50**, 1350-1351 (1992).

3. B. Vogelstein, B.A. Fearon, S.R. Hamilton, S.E. Kern, A.C. Preisinger, M. Lappert, Y. Nakamura, R. White, A.M.M. Smits, and J.L. Bos, "Genetic alterations during colorectal tumor development," *New Eng. J. of Med.* **319**, 525-532 (1988).

4. E.R. Fearon and B. Vogelstein, "A genetic model for colorectal tumorigenesis," *Cell* **61**, 759-767 (1990).

5. M.J. Renan, "How many mutations are required for tumorigenesis? Implications from human cancer data," *Mol. Carcinogenesis* **7**, 139-146 (1993).

6. P. Armitage and R. Doll, "The age distribution of cancer and a multistage theory of carcinogens," *Br. J. Cancer* **8**, 1-12 (1954).

7. F.A. Ray, J. Meyne, and P.M. Kraemer, "SV40 T antigen induced chromosomal changes reflect a process that is both clastogenic and aneuploidogenic and is ongoing throughout neoplastic progression of human fibroblasts," *Mutat Res.* **284**, 265-273 (1992).

8. Advances in Modern Environmental Toxicology (Volume 1) *Mammalian Cell Transformation by Chemical Carcinogens,* in N. Mishra, V. Dunkel, and M. Mehlman (eds.), (Senate Press, Inc., Princeton Junction, NJ, 1980).

9. V.M. Maher, J. Domoradzki, N.P. Bhattacharyya, T. Tsujimura, R.C. Corner, and J.J. McCormick, "Alkylation damage, DNA repair, and mutagenesis in human cells," *Mutat. Res.* **233**, 235-245 (1990).

10. M. Namba, K. Nishitani, F. Hyodoh, F. Fukushima, and T. Kimoto, "Neoplastic transformation of human diploid fibroblasts (KMST-6) by treatment with ^{60}Co gamma rays," *Int. J. of Cancer* **35**, 275-280 (1985).

11. D.G. Fry, L.D. Milam, V.M. Maher, and J.J. McCormick, "Transformation of diploid human fibroblasts by DNA transfection with the v-*sis* oncogene," *J. Cellular Physiol.* **128**, 313-321 (1986).

12. P.J. Hurlin, D.G. Fry, V.M. Maher, and J.J. McCormick, "Morphological transformation, focus formation, and anchorage independence induced in diploid human fibroblasts by expression of a transfected H-*ras* oncogene," *Cancer Res.* **47**, 5752-5757 (1987).

13. P.J. Hurlin, V.M. Maher, and J.J. McCormick, "Malignant transformation of human fibroblasts caused by expression of transfected T24 H-*ras* oncogene," *Proc. Natl. Acad. Sci.* U.S.A. **86**, 187-191 (1989).

14. D.M. Wilson, D.G. Fry, V.M. Maher, and J.J. McCormick, "Transformation of diploid human fibroblasts by transfection of N-*ras*-oncogenes," *Carcinogenesis* **10**, 635-640 (1989).

15. D.M. Wilson, D. Yang, J.E. Dillberger, S.E. Dietrich, V.M. Maher, and J.J. McCormick, "Malignant transformation of human fibroblasts by a transfected N-*ras* oncogene," *Cancer Res.* **50**, 5587-5593 (1990).

16. D.G. Fry, L.D. Milam, J.E. Dillberger, V.M. Maher, and J.J. McCormick, "Malignant transformation of infinite life span human fibroblast cell strain by transfection with v-Ki-*ras*," *Oncogene* **5**, 1415-1418 (1990).

17. T.L. Morgan, D. Yang, D.G. Fy, P.J. Hurlin, S.K. Kohler, V.M. Maher, and J.J. McCormick, "Characteristics of an infinite life span diploid human fibroblast cell strain and a near-diploid strain arising from a clone of cells expressing a transfected v-*myc* oncogene," *Exp. Cell Res.* **197**, 125-136 (1991).

18. C. Lin, Q. Wang, C. Louden, V.M. Maher, and J.J. McCormick, submitted, "Malignant transformation of a human fibroblast cell strain by a transfected viral *fes* oncogene."

19. D. Yang, S.K. Kohler, V.M. Maher, and J.J. McCormick, submitted, "v-*sis* oncogene-induced transformation of human fibroblasts into cells capable of forming benign tumors," *Cancer Res.*

20. J.J. McCormick and V.M. Maher, "Towards an understanding of the malignant transformation of diploid human fibroblasts," *Mutat. Res.* **199**, 273-291 (1988).

21. D. Yang, C. Louden, D.S. Reinhold, S.K. Kohler, V.M. Maher, and J.J. McCormick, "Malignant transformation of human fibroblast cell strain MSU-1.1 by (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. *Proc. Natl. Acad. Sci.* U.S.A. **89**, 2237-2241 (1992) .

22. P.A. Ryan, V.M. Maher, and J.J. McCormick, submitted, "Failure of infinite life span human cells from different immortality complementation groups to yield finite life span hybrids," *J. Cell. Physiol.*

Incorporation of Pharmacokinetics in Noncancer Risk Assessment: Example with Chloropentafluorobenzene

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ABSTRACT

Noncancer risk assessment traditionally relies on applied dose measures, such as concentration in inhaled air or in drinking water, to characterize no-effect levels or low-effect levels in animal experiments. Safety factors are then incorporated to address the uncertainties associated with extrapolating across species, dose levels, and routes of exposure, as well as to account for the potential impact of variability of human response. A risk assessment for chloropentafluorobenzene (CPFB) was performed in which a physiologically based pharmacokinetic model was employed to calculate an internal measure of effective tissue dose appropriate to each toxic end point. The model accurately describes the kinetics of CPFB in both rodents and primates. The model calculations of internal dose at the no-effect and low-effect levels in animals were compared with those calculated for potential human exposure scenarios. These calculations were then used in place of default interspecies and route-to-route safety factors to determine safe human exposure conditions. Estimates of the impact of model parameter uncertainty, as estimated by a Monte Carlo technique, also were incorporated into the assessment. The approach used for CPFB is recommended as a general methodology for noncancer risk assessment whenever the necessary pharmacokinetic data can be obtained.

INTRODUCTION

For a number of years, the U.S. Air Force has been performing research to develop safe intake simulants for chemical warfare (CW) agents, in order to provide accurate and quantitative real-time assessment of troop proficiency and gear efficacy during CW field exercises. Chloropentafluorobenzene (CPFB) was identified and evaluated as a candidate inhalation simulant and was determined to possess desirable physicochemical and toxicological properties. These include rapid uptake, low metabolism and toxicity, rapid and predictable clearance, real-time detectability by existing portable "breathalyzer"

technology, gas mask breakthrough similar to the actual agents, and commercial availability. Before using CPFB in human trials, it was important to determine safe exposure conditions, taking into consideration the exposure levels at which toxicity was observed in animal studies.

Because of the need to balance protection of personnel during training with the ability to provide effective training for a dangerous wartime scenario, an accurate (as opposed to simply safe-sided) estimate of acceptable human exposure was needed. The usual practice for noncancer risk assessment (1) uses measures of applied dose to relate to toxicity. Safety factors are then applied to account for uncertainty regarding the relationship between applied dose and effective target tissue dose across routes of exposure and species, as well as for variability in the human population. A more scientifically based approach would be to use a measure of tissue dose directly and to use known principles of pharmacokinetics to relate different exposure scenarios. For this purpose a physiologically based pharmacokinetic (PBPK) model was developed that could be used to perform the route-to-route and cross-species extrapolations necessary to develop a human risk estimate. The model also was evaluated by a Monte Carlo analysis to estimate the uncertainty associated with the risk estimate.

PBPK MODEL DEVELOPMENT

Structure

The structure of the model is shown in Figure 17, and the assumptions underlying the mathematical description follow those of Ramsey and Andersen (2) with the following exceptions:

1. The model of Ramsey and Andersen included only saturable metabolism. An additional pathway of metabolism has been added in this model which is linear in concentration. Thus the equation for the rate of change of amount of CPFB in the liver contains an additional term:
   - KF * CL * VL / PL (where the parameters are defined in Table 25)

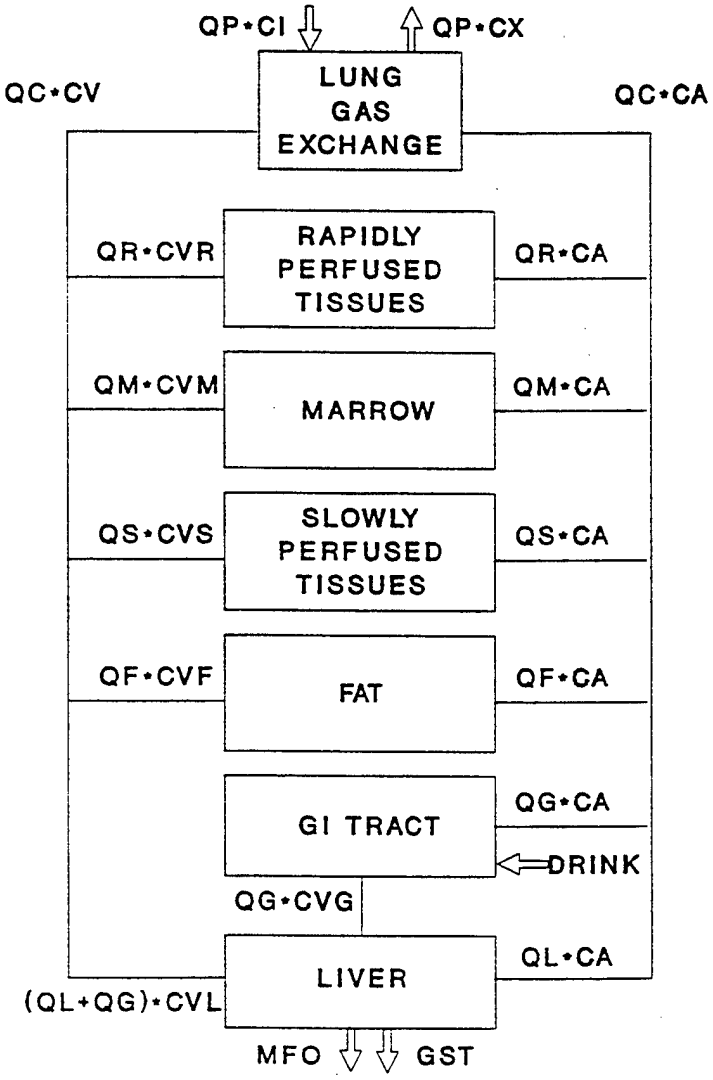


Figure 17. Diagram of the PBPK Model of CPFB.

TABLE 25: PBPK MODEL PARAMETERS UNSCALED PARAMETERS

		Mouse	Rat	Monkey	Human
BW	Body Weight (kg)	0.023	0.22	8.7	70.0
KA	Oral Uptake Rate (/h)	5.0	5.0	5.0	5.0
ALVS	Alveolar Dead Space (Fraction)	0.0	0.0	0.4	0.0
DS	Bronchiolar Dead Space (Fraction)	0.3	0.3	0.45	0.3
QCC	Cardiac Output (L/h, 1 kg animal)	16.5	11.6	12.0	18.0
QPC	Alveolar Ventilation (L/h, 1 kg	29.0	21.2	17.0	35.0
Tissue Blood Flows (Fraction of Cardiac Output):					
QFC	Flow to Fat	0.030	0.058	0.052	0.052
QGC	Flow to GI Tract	0.166	0.183	0.185	0.185
QLC	Flow to Liver	0.036	0.032	0.065	0.065
QMC	Flow to Bone Marrow	0.110	0.110	0.110	0.110
QRC	Flow to Rapidly Perfused Tissues	0.409	0.362	0.348	0.348
QSC	Flow to Slowly Perfused Tissues	0.249	0.255	0.240	0.240
Tissue Volumes (Fraction of Body Weight):					
VBL	Volume of Blood	0.070	0.070	0.070	0.070
VFC	Volume of Fat	0.100	0.070	0.050	0.190
VGC	Volume of GI Tract	0.033	0.033	0.045	0.045
VLC	Volume of Liver	0.050	0.040	0.027	0.027
VMC	Volume of Bone Marrow	0.030	0.030	0.020	0.020
VRC	Volume of Rapidly Perfused Tissues	0.041	0.020	0.026	0.026
VSC	Volume of Slowly Perfused Tissues	0.550	0.600	0.709	0.569

TABLE 25. Continued

PB	Blood/Air	12.3	12.3	7.0	7.0
PF	Fat/Blood	75.0	75.0	93.0	93.0
PG	GI Tract/Blood	2.55	2.55	3.65	3.65
PL	Liver/Blood	2.77	2.77	8.0	8.0
PM	Bone Marrow/Blood	11.6	11.6	16.0	16.0
PR	Richly Perfused Tissue/Blood	2.55	2.55	3.65	3.65
PS	Slowly Perfused Tissue/Blood	1.07	1.07	2.1	2.1
Metabolic Parameters:					
KFC	Rate Constant for 1st Order Pathway (/h – 1 kg animal)	2.0	2.0	2.0	2.0
KM	Affinity of Saturable Pathway (mg/L)	0.4	0.4	0.4	0.4
VMAXC	Maximum Velocity of Saturable Pathway (mg/h, 1 kg animal)	0.	0.	0.	0.

SCALED PARAMETERS

```
QC = QCC*BW**0.75
QP = QPC*BW**0.75

QF = QFC*QC
QG = QGC*QC
QL = QLC*QC
QM = QMC*QC
QR = QRC*QC
QS = QSC*QC

VBL = VBLC*BW
VF = VFC*BW
VG = VGC*BW
VL = VLC*BW
VM = VMC*BW
VR = VRC*BW
VS = VSC*BW

KF = KFC/BW**.25
VMAX = VMAXC*BW**0.75
```

TABLE 25. Continued

DOSE SURROGATES

Amet	Total amount metabolized per unit body weight (mg/kg)
AUCB	Area under the curve of arterial blood concentration of CPFB (mg/L-h)
AUCL	Area under the curve of liver concentration of CPFB (mg/L-h)
AUCM	Area under the curve of CPFB in the bone marrow (mg/L-h)
CA	Concentration of CPFB in the arterial blood (mg/L)
CL	Concentration of CPFB in the liver (mg/L)
CM	Concentration of CPFB in the bone marrow (mg/L)
CV	Mixed venous blood concentration of CPFB (mg/L)
Dose	Total amount inhaled during exposure (mg/kg) = integral of QP * (CALV - CX) / BW

2. A GI tract compartment has been added. Oral absorption takes place in this compartment by a first order process: KA * AST (where AST represents the amount of CPFB remaining in the stomach). The liver receives the blood flow from this compartment (QG) as well as its own arterial supply (QL). Thus, the equations for the rate of change in the amount of CPFB in the stomach (RAST), GI tract (RAG), and the liver (RAL) are:

RAST =- KA * AST
RAG =QG * (CA - CG / PG) + KA * AST
RAL =QG * (CG / PG - CL / PL) + QL * (CA - CL / PL)
- VMAX * CL / PL / (KM + CL / PL) - KF * CL * VL / PL

3. A bone marrow compartment has been added. The form of the equation for the rate of change in the amount of CPFB in the bone marrow (RAM) is identical to that of the other basic tissues in Ramsey and Andersen (2) (e.g., fat, slow, rapid):
RAM = QM * (CA - CM / PM)

4. In order to better simulate the measurements of exhaled breath in anesthetized monkeys, the description of gas exchange between the lung and the blood was modified to explicitly model an alveolar space in which inhaled air at concentration CI and air in equilibrium with the blood were mixed. In place of the steady-state assumption used in Ramsey and Andersen, the following rate equation for the amount of CPFB in the blood (ABL) was integrated along with the equations for the tissue compartments:

RABL = QP * (CALV - CX) + QC * (CV - CA)

where:

CALV = ALVS * ABL / (VBL*PB) + (1. - ALVS) * CI

The measured exhaled air concentration in parts per million (CXPPM) was then described by the equation:

CXPPM = [DS * CI + (1. - DS) * ABL / (VBL * PB)] * 24450. / 202.51

The parameters for the model are shown in Table 25. Physiological parameters for mouse, rat, and human were developed from literature data collected (Stan Lindstedt, Northern Arizona University, personal communication) as part of an ongoing Physiological Parameters Work Group effort sponsored by the International Life Sciences Institute (ILSI), Risk Science Institute. Physiological parameters and partition coefficients for the rhesus monkey were adapted from Crank and Vinegar (3). Partition coefficients for the rat were taken from Jepson *et al.* (4). Partition coefficients for humans were assumed to be the same as for monkeys, while those for mice were assumed to be the same as for rats. Metabolism was modeled as a first-order process, scaled allometrically from the value determined in rats (4). The model was written in the Advanced Continuous Simulation Language (ACSL; Mitchell and Gauthier, Boston, MA) and was compared with experimental data using SimuSolv (Dow Chemical Co., Midland, MI). Monte Carlo analysis was performed on the ACSL model with PBPK_SIM (K.S. Crump Group, ICF Kaiser International, Ruston, LA).

Figure 18 shows the results of gas uptake analysis of CPFB in rats (4). In the gas uptake analysis, several animals are maintained in a closed chamber, and the air is continuously recirculated. Oxygen is replenished and carbon dioxide is scrubbed as necessary to maintain stasis. A known amount of a volatile chemical is then added to the chamber, and the concentration of the chemical in the chamber is monitored over time. The rapid initial decline in the chamber concentration of CPFB seen in Figure 18 is due to uptake by the animals' tissues and demonstrates that CPFB is readily absorbed. Following the tissue uptake phase, any further decline in chamber concentration would indicate loss of chemical due to metabolism. The fact that the concentration curve for CPFB almost levels out after the first few hours reflects the fact that CPFB is not extensively metabolized. By way of comparison, the chamber concentration of a more rapidly metabolized chemical, bromopentafluorobenzene, decreased by more than 20% between hours 3 and 6 under the same conditions. Using a PBPK model for CPFB, the closed chamber data was analyzed to quantify the rate of metabolism. It was determined in that study that metabolism was first-order, with a rate constant of 2/h (scaled to a 1 kg animal by body weight to the -0.25 power).

Model Validation

As a test of the model, a study was simulated in which rats were exposed 6-h per day by inhalation for 21 days to CPFB at 30, 100, and 300 ppm (5). Figure 19 shows the measured and

simulated venous blood concentration of CPFB for the eleventh day of the exposure. As a further evaluation of the model, inhalation exposures to CPFB on eight anesthetized rhesus monkeys (3) were simulated. In these experiments CPFB concentrations in expired breath were measured during and after 15 min exposures at 300 ppm. The PBPK model was evaluated in terms of its ability to relate exposure concentration and exhaled-air concentrations. The results are shown in Figure 20.

Monte Carlo Analysis of PBPK Uncertainty

In the Monte Carlo method, a probability distribution for each of the model input parameters is randomly sampled, and the model is run using the chosen set of parameter values. This process is repeated a large number of times (250 in this study) until the probability distribution for the desired model output has been created. To the extent that the input parameter distributions adequately characterize the uncertainty in the inputs, and assuming that the parameters are reasonably independent, the resulting output distribution will provide a useful estimate of the uncertainty associated with model predictions.

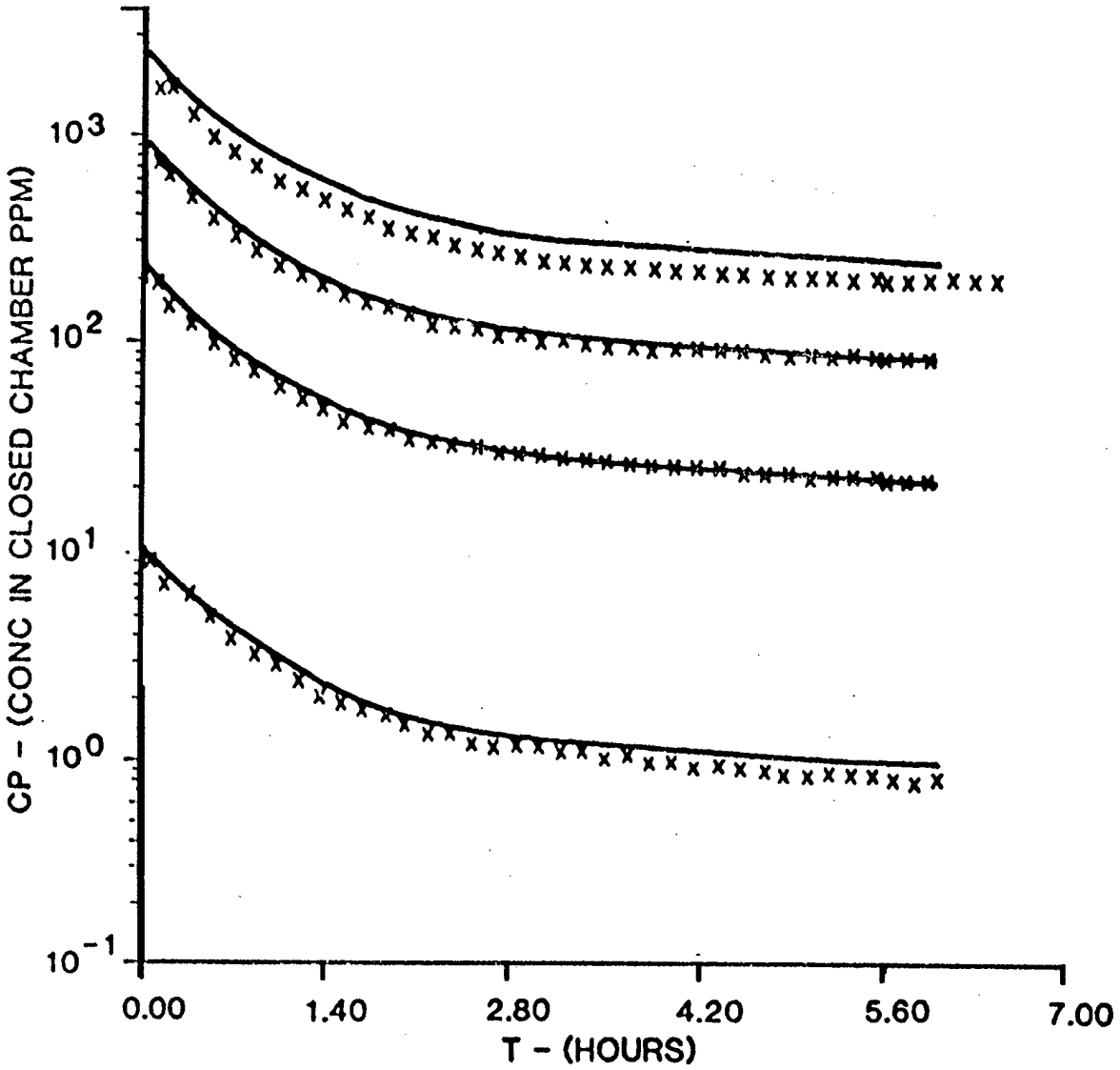


Figure 18. **Computer Simulation (Solid Line) Versus Observed (x) Chamber Concentrations (ppm) Over Time (h) for Rats Exposed to Initial Concentrations of 10, 250, 1000, and 1800 ppm CPFB in a Closed, Recirculating Chamber System (Reproduced from Reference 4).**

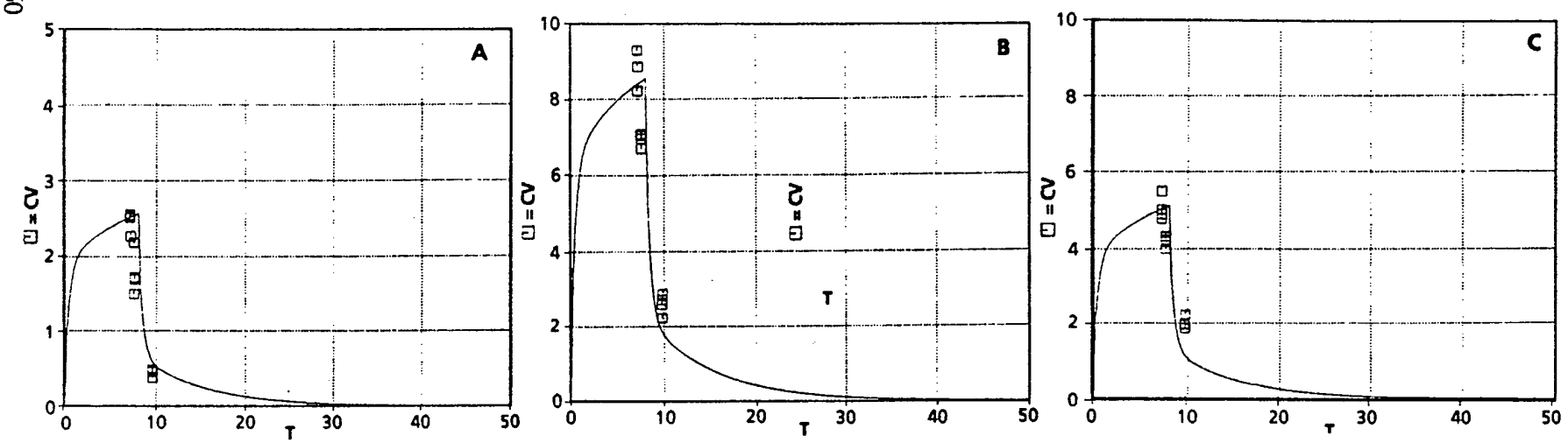


Figure 19. **Model-Predicted (Lines) and Measured (Boxes) Venous Blood Concentrations on 11th Day of Exposure of Rats to CPFB for 6 h Per Day at 30 ppm (a), 100 ppm (b), and 300 ppm (c).**

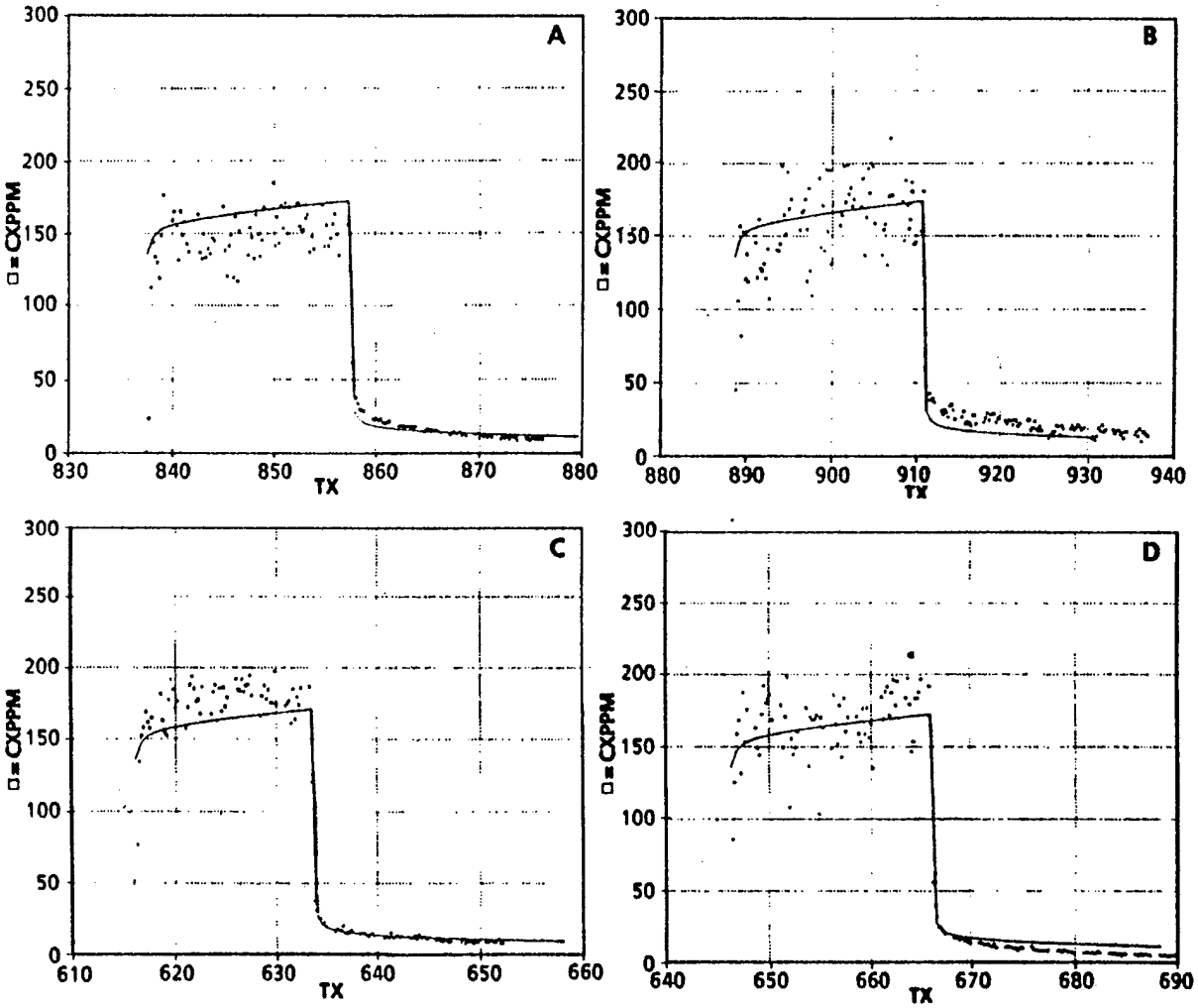


Figure 20. **Model Predicted (Lines) and Measured (Points) Exhaled Air Concentrations for Rhesus Monkeys Exposed to 300 ppm CPFB for 17 to 20 min.**

The Monte Carlo method was used to investigate the impact of parameter uncertainty on the PBPK-based risk estimates for CPFB. Information from repeated assays of the metabolic parameter and partition coefficients (Allen Vinegar, ManTech Environmental Technology, Inc., personal communication) was used together with estimates from the literature of uncertainty in the physiological parameters (Stan Lindstedt, Northern Arizona University, personal communication) to characterize the uncertainty in the input parameters for the CPFB model. The coefficients of variation used for the parameters in the Monte Carlo analysis are shown in Table 26. Truncated normal distributions were used for all parameters except for metabolism and oral bioavailability, for which lognormal and uniform distributions, respectively, were used. The estimated uncertainty distributions for each of the parameters were input into PBPK_SIM, and the ACSL model for CPFB was exercised using the Monte Carlo approach to generate a distribution for the dose surrogate of interest in both the animal and human.

It is important to distinguish uncertainty from variability. As it relates to the issue of using PBPK modeling in risk assessment, uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative ("average") animal. Variability, on the other hand, should only be considered to represent true interindividual differences. Understood in these terms, uncertainty is a defect (lack of certainty) that can typically be reduced by experimentation, and variability is a fact of life that must be considered regardless of the risk assessment methodology used. The parameter distributions used in the Monte Carlo analysis described here were chosen to represent uncertainty, not variability.

TABLE 26. COEFFICIENTS OF VARIATION (PERCENT) FOR CPFB MODEL INPUT PARAMETERS
(All distributions are truncated normal unless specified otherwise)

		Mouse	Rat	Human
BW	Body Weight (kg)	11.	11.	30.
KA	Oral Uptake Rate (/h)	70.	--[a]	--
--	Oral Bioavailability (Fraction)	0.3-1.0[b]	--	--
QCC	Cardiac Output (L/h, 1 kg animal)	8.5	14.	10.
QPC	Alveolar Ventilation (L/h, 1 kg animal)	58.	26.	30.
Tissue Blood Flows (Fraction of Cardiac Output):				
QFC	Flow to Fat	60.	60.	30.
QGC	Flow to GI Tract	25.	25.	10.
QLC	Flow to Liver	96.	96.	35.
QRC	Flow to Rapidly Perfused Tissues	50.	50.	20.
QSC	Flow to Slowly Perfused Tissues	40.	40.	15.
Tissue Volumes (Fraction of Body Weight):				
VFC	Volume of Fat	30.	30.	30.
VGC	Volume of GI Tract	30.	30.	10.
VLC	Volume of Liver	6.	6.	5.
VRC	Volume of Rapidly Perfused Tissues	30.	30.	10.
VSC	Volume of Slowly Perfused Tissues	30.	30.	30.
Partition Coefficients:				
PB	Blood/Air	15.	15.	10.
PF	Fat/Blood	30.	30.	30.
PG	GI Tract/Blood	30.	30.	30.
PL	Liver/Blood	20.	20.	20.
PR	Richly Perfused Tissue/Blood	20.	20.	20.
PS	Slowly Perfused Tissue/Blood	20.	20.	20.
Metabolic Parameters:				
KFC	Rate Constant for 1st Order Pathway	30.[c]	30.[c]	50.[c]

[a] Not applicable
[b] Uniform distribution
[c] Lognormal distribution

The process of determining the PBPK uncertainty factor for risk estimates derived from a selected animal toxicity and dose surrogate can be described as follows:

1. Monte Carlo simulations are performed with the model using parameters appropriate for the animal exposure of interest (e.g., the experimental conditions yielding a no observable adverse effect level [NOAEL] for a particular effect), and the distribution of the predicted values for a dose surrogate appropriate for the toxicity of concern is generated. The uncertainty in the model-predicted dose surrogate for the animal can be characterized by the ratio of the mean of the dose surrogate distribution to the 5th percentile (the value which is lower than 95% of the predicted dose surrogates). The target dose surrogate value is then defined as the 5th percentile of the animal dose surrogate distribution divided by any safety or modifying factors.

2. Monte Carlo simulations are performed with the model using parameters appropriate for the human exposure of interest, and the distribution of the predicted values for the same dose surrogate is generated. The human dose or exposure concentration is then adjusted and the Monte Carlo analysis is repeated until the 95th percentile of the resulting human dose surrogate distribution is equal to the target dose surrogate value. The uncertainty in the model-predicted dose surrogate for the animal can be characterized by the ratio of the 95th percentile (the value which is greater than 95% of the predicted dose surrogates) to the mean.

The PBPK uncertainty factor is defined as the product of the animal and human uncertainty estimates derived in Steps 1 and 2. Equivalently, the PBPK uncertainty factor can be calculated from the ratio of the animal mean from Step 1 to the human mean form Step 2. The final value for the human dose or exposure concentration derived from the iterative process in Step 2 can be used as the human exposure guideline. Alternatively, the PBPK uncertainty factor can be used with model calculations based only on the preferred values of the parameters, as described below in the section on exposure guideline determination.

TOXICOLOGICAL EVALUATION

In order to ensure that CPFB could safely be used as an intake simulant, a number of studies were performed to evaluate its potential toxicity. These studies were designed to elucidate any short-term or long-term effects, and to assess the likelihood that CPFB could be carcinogenic or teratogenic.

Acute Toxicity

The primary irritation hazard, sensitization potential, and acute inhalation toxicity of CPFB were evaluated by Kinkead et al. (6). CPFB demonstrated no potential for skin sensitization in tests on guinea pigs, and was only a mild skin and eye irritant in rabbits. Short-term exposure to CPFB vapor poses no

serious hazard by the inhalation route as all rats survived a 4-hour exposure to an upper limit concentration of 4.84 mg/L (581 ppm), a concentration many orders of magnitude higher than that which is likely to be encountered in the field. Similarly, oral dosing indicates an LD_{50} of greater than 5 g/kg, which would classify CPFB as "practically nontoxic" (7).

Mutagenicity/Genotoxicity

Chloropentafluorobenzene was tested for potential genotoxic activity by three different laboratories (8,9,10) using a battery of *in vitro* assays (Table 27). The first attempt to perform these assays (8) was compromised by experimental difficulties associated with the tendency of CPFB to precipitate out of solution and to dissolve the dishes. In the second study (9), it was again noted that CPFB dissolved the standard plastic dishes, so the study was performed in specially designed glass dishes. A third study (10) was performed by a reference laboratory because the results of the first two studies seemed to be somewhat equivocal.

TABLE 27. SUMMARY OF *IN VITRO* RESULTS FOR CPFB

In Vitro Assay	Tu et al.	Steele	Kutzman et al.
Ames Salmonella mutagenicity:			
- S9 activation	-	-	-
+ S9 activation	-	-	-
CHO/HGPRT gene mutation:			
- S9 activation	-	-	-
+ S9 activation	-	-	-
CHO sister chromatid exchange:			
- S9 activation	-	+/-	-
+ S9 activation	-	+/-	+
CHO chromosome aberration:			
- S9 activation	+/-	+/-	-
+ S9 activation	+/-	+/-	-
Primary rat hepatocyte DNA repair	+/-	-	-
BALB/c-3T3 cell transformation:			
- S9 activation	-	+/-	-
+ S9 activation	[a]	+	-

[a] Not reported.

Chloropentafluorobenzene does not appear to be mutagenic. The Ames Salmonella reverse mutation assay was uniformly negative in all studies, both with and without the addition of a rat liver S9 metabolic activation system. Similarly, all of the laboratories obtained negative results when CPFB was tested in mammalian cell culture for mutagenic activity at the hypoxanthine-guanine-phosphoribosyl locus in Chinese hamster ovary cells.

The results of tests for genotoxicity were less consistent. There was some evidence of CPFB-induced sister chromatid exchange (SCE) and/or chromosomal aberration in the earlier studies, but the final study detected no increases in chromosomal aberrations and only observed SCE with the addition of liver S9 metabolic activation (suggesting that generation of significant levels of metabolite may be required to observe this effect). In the case of the assay for unscheduled DNA repair synthesis in primary rat hepatocytes, the first study suggested that CPFB produced increased repair of DNA damage; however, both the second and third studies failed to confirm this finding. Cell transformation results were also variable, with only the second study showing any indication of an ability of CPFB to induce morphological transformation *in vitro* in BALB/c-3T3 cells.

To resolve the question of whether CPFB could act as a genotoxic or cytotoxic agent under *in vivo* conditions, a 21-day exposure of mice to CPFB at 30, 100, and 300 ppm was performed (11). Under these conditions CPFB did not induce an increase in SCE in the bone marrow of the exposed mice, and the rate of cellular proliferation in the bone marrow was not altered. Similarly, assessment of the micronucleated polychromatic and normochromatic erythrocyte populations during the exposures indicated a general absence of genotoxic activity. A PBPK model for CPFB was used to assess the tissue exposure to CPFB during this study (5). Based on the modeling, bone marrow tissue exposure to CPFB during the *in vivo* study was similar to or greater than the concentrations used in the *in vitro* assays.

The PBPK model described in this paper was used to reconfirm the results of this earlier analysis in the particular case of SCE. A dose-related increase in SCE was observed in 2-h *in vitro* exposures to CPFB ranging from 100 to 250 mg/L (area under the curve ranging from 200 to 500 mg/L-h) in the presence of metabolic activation. For the *in vivo* study, bone marrow exposure to CPFB (as estimated by the model) averaged 288 mg/L during the daily 6-h inhalation exposures to 300 ppm CPFB, with a daily area under the curve in the marrow of 2023 mg/L-h. The lack of *in vivo* response appears therefore to reflect differences between the *in vivo* and *in vitro* situation rather than failure to achieve sufficient

tissue exposure levels. It is possible that the bone marrow does not possess sufficient metabolic activity, in comparison with the *in vitro* situation, to generate the active chemical species.

Full evaluation of the potential for CPFB to be carcinogenic would require a lifetime animal bioassay. However, a reasonable assessment of the likelihood that CPFB could act as a carcinogen can be made on the basis of the above results, taken together with the rather unremarkable results of the subchronic exposures. Chloropentafluorobenzene does not appear to be mutagenic, either in the presence or absence of metabolic activation, and the questionable *in vitro* suggestions of genotoxicity were not born out by the *in vivo* studies. In addition, subchronic exposure (7) did not produce any of the tissue changes, such as peroxisomal proliferation, which typically accompany promotional carcinogenesis in rodents. Therefore, it is not likely that CPFB would be carcinogenic, even under the conditions of a lifetime bioassay.

Toxicity from Repeated Exposure

Repeated exposure of rats to high concentrations of CPFB produced lethargy and incoordination (1000 ppm, 6 h/day, 4 days) or unresponsiveness (500 ppm, 6 h/day, 15 days), but no tissue pathology (12). No behavioral or histological effects were observed for exposure to 250 ppm, 6 h/day, for 15 days (12). (Note: Gage (12) incorrectly shows the concentration of the lowest exposure level as 50 ppm; the original ICI report, TR/449, records the concentration as 250 ppm – J.C. Gage, personal communication.)

In a more recent study (11), ten Fischer-344 rats and six B6C3F1 mice of each sex were exposed to 30, 100, and 300 ppm CPFB for 3 weeks (15 exposures). Exposure to the highest concentration caused a reduction in the growth rate of rats, but did not affect the growth rate of mice. Both rats and mice showed a dose-related increase in liver-to-body-weight ratios. Mice showed clear evidence of liver toxicity (hepatocytomegaly and hypertrophy) at the highest exposure concentration. Another treatment-related change in the livers of male and female mice and female rats was an increase in the incidence of single-cell necrosis in all CPFB-exposed groups. The formation of hyaline droplets in the kidneys of male rats was also noted, but the severity of the lesion was minimal, and no other kidney effects were seen. Consistent with the earlier study, no behavioral effects were noted, even at the highest dose.

In order to better evaluate the impact of prolonged or repeated exposure to CPFB, as well as to determine a NOAEL, a 13-week exposure of rats and mice was carried out at concentrations of 1.2, 6.0, and 30 ppm (7,13). No treatment-related effects were observed at any concentration in either species. In particular, the single cell necrosis seen in the 3-week study at 30 ppm was not observed in the 13-week study at the same concentration. A review of the tissues from the earlier study confirmed the finding of an increase over control, but both the number and severity of the lesions were so slight that it was felt the finding was biologically unimportant. Thus, the only adverse effects seen were those noted for the 300 ppm exposure concentration in the 3-week study. A concentration of 30 ppm was therefore recommended by the investigators as a NOAEL in humans to protect individuals subjected to repeated inhalation of CPFB for extended periods.

Reproductive Toxicity

To evaluate the teratogenic potential of CPFB, time-mated Sprague Dawley rats were dosed orally at 0.3, 1.05, and 3.0 g/kg/day on days 6 through 15 of pregnancy (14). There was a significant reduction in maternal body weight and a significant increase in maternal liver weight at the highest dose. The percentage of post-implantation fetal loss was also greater only at the highest dose. Fetal weight and length differed significantly from the controls at both the high and intermediate doses, indicating a slightly increased fetotoxicity compared to the dam. The number of malformations and variations observed at any of the doses did not differ from controls, suggesting that CPFB is not teratogenic.

Metabolism

Studies of the uptake of CPFB in a closed, recirculated chamber were consistent with a slow rate of first order metabolism (4). In the same studies, the rate of metabolism of the related compound, bromopentafluorobenzene, was unaffected by pretreatment with the potent P450 inhibitor, pyrazole, suggesting that metabolism of these two compounds is not associated with the mixed function oxidase system. This finding contrasts with the metabolism of the related compound, hexachlorobenzene (HCB), which is characterized by both an oxidative (P450) pathway and a glutathione conjugation (GST) pathway (15). This apparent difference between CPFB and HCB is consistent with the results of a comparative study of a series of dihalomethanes (16), which also feature competitive P450 and GST metabolism. This study demonstrated that the fluorine-substituted congeners, CH_2F_2 and CH_2FCl, showed little evidence of P450 activity, whereas compounds containing chlorine and/or bromine, but not fluorine, were readily metabolized by both pathways. Of course, these results were observed in rodents, and the possibility of

species differences in the metabolism of CPFB cannot be ruled out. Evaluation of CPFB metabolism in human tissues would be necessary to confirm the assumption of equivalent metabolism across species.

In the case of HCB, the GST pathway initially produces *N*-acetyl cysteine conjugates which cleave to form chlorothiophenols, which are in turn subject to further metabolism.(15) It can therefore be hypothesized that the liver toxicity associated with repeated exposure to CPFB may result from the generation of the analogous metabolite, pentafluorothiophenol, a toxic compound with an LD_{50} of 56 mg/kg (17).

EXPOSURE GUIDELINE DETERMINATION

The critical effect for evaluation of safe exposure to CPFB is the liver toxicity associated with repeated exposure (5). Specifically, hepatocytomegaly and hypertrophy were observed in mice following exposure to 300 ppm CPFB, 6 h/day, for 3 weeks, and the liver-to-body-weight ratio in rats and female mice were increased in a dose-related fashion. Increased single cell necrosis was also observed at 30 ppm and 100 ppm in the same study, but this effect was not considered toxicologically significant, and neither the necrosis nor the increased liver-to-body-weight ratio were reproduced in a subsequent study at 30 ppm for 13 weeks (13). In the traditional approach, taking 30 ppm as a NOAEL, adjusting for the difference in daily exposure duration (6 h for animal studies, 8 h for humans), and dividing by a factor of 33 to provide a margin of safety, yields a recommended exposure guideline of 0.7 ppm for a daily (8-h) time-weighted average.

The rationale for the factor of 33 used in the traditional guideline calculation is as follows. First, the animal NOAEL must be adjusted for the relationship between the duration of exposure in the animal study and the anticipated duration of exposure in the human scenario. One aspect of this adjustment is described above: adjusting for the difference in daily exposure duration. Assuming a maximum daily exposure duration of 8 h when simulant training is performed, the adjusted NOAEL is 30 * 6/8 = 22.5 ppm. However, the actual anticipated human exposures are brief and infrequent, associated with special training exercises which are not expected to be a common occurrence. Therefore the 3-week and 13-week rodent studies represent much more prolonged exposures than the human exposure scenario, with less opportunity for recovery between exposures. It is common practice to apply factors of up to 10 to extrapolate from short-term to longer-term toxicity (18). In this case, the extrapolation is in the other direction, from relatively long-term to shorter-term, so an inverse factor is justified. To be conservative,

a factor of one-third was selected, yielding an adjusted NOAEL of 22.5 / 0.33 = 67.5. The traditional guideline then applies a safety, or uncertainty, factor of 100, with one factor of 10 to account for uncertainty in the extrapolation from animal to man and a second factor of 10 to account for human variability, resulting in the guideline of 0.7 ppm.

The selection of 100 as the safety factor to be applied in this case follows a convention which, although basically empirical, can be at least partially justified on the basis of quantitative pharmacokinetic principles (18). For example, the factor of 10 usually applied for extrapolation from animals to humans reflects a conventional wisdom based primarily on experience with the more common exposure routes, oral and intravenous, for chemicals that are themselves toxic and are cleared or detoxified by processes that scale roughly with surface area rather than body weight. Under these conditions, pharmacokinetic and empirical allometric considerations justify such a factor for rodent-to-human extrapolation based on the relationship between applied dose and tissue exposure (area under the concentration-time curve) as a function of body weight (19). However, for inhalation exposure to a volatile, poorly soluble chemical such as CPFB, these same principles lead to an expectation of similar area under the curve (AUC) of both parent and metabolites for equivalent external concentration and exposure duration (that is, for equal time-weighted average concentrations). In order to make quantitative use of these pharmacokinetic principles, the model described in this paper was used to calculate the daily AUC in the liver for exposure of rats and humans to 30 ppm CPFB for 6 h. The AUC in the liver predicted for rats was 46 mg/L-h, whereas for humans, even under conditions of moderate exercise, it was 72 mg/L-h, a difference of less than a factor of 2. Thus, the usual animal to human extrapolation factor of 10 is not justified in this instance.

The second factor of 10, which accounts for human heterogeneity, would similarly be susceptible to quantitative evaluation if the distribution of human susceptibilities could be estimated. For a specific chemical toxicity, the variance of the response distribution in the human population will depend on the steepness of the chemical dose-response curve, which can be determined from animal studies, and on the extent of variability in the human population of the pharmacokinetic and pharmacodynamic parameters mediating the response (20). Coupling of Monte Carlo analysis and PBPK modeling provides a method for directly estimating the impact of parameter variation on risk (21).

A pharmacokinetically driven guideline calculation is based on calculation of equivalent effective tissue doses for the animal and human scenarios. In the case of the liver toxicity associated with

prolonged exposure, the AUC for CPFB in the liver was selected as the appropriate tissue dose. The AUC is generally regarded as an appropriate dose surrogate for cumulative, reversible toxicity, such as that seen with CPFB. The daily AUC in the liver calculated by the model at the rodent subchronic NOAEL of 30 ppm was 46 mg/L-h. The selection of an appropriate uncertainty/safety factor for the pharmacokinetic approach was based on three considerations. First, it was determined that there was not yet sufficient information on the variability of human susceptibility to liver toxicity to permit calculation of a more accurate substitute for the default uncertainty factor of 10, so the default value was used. Second, based on the 95th percentile of the Monte Carlo analysis of the impact of parameter uncertainty on the predictions of the CPFB model for this dose surrogate (AUC in the liver), an uncertainty factor of 2.3 was included for animal-to-human extrapolation. This factor represents uncertainty in the accuracy of the PBPK model predictions, as distinguished from the default factor of 10, which represents total uncertainty in the animal to human extrapolation when pharmacokinetics is not considered. The major contributor to the PBPK uncertainty factor is the lack of data on the human metabolic capability for CPFB. Additional data (e.g., from *in vitro* metabolism studies on human liver tissue), could reduce this uncertainty factor.

The final consideration for the overall uncertainty factor was the infrequent nature of the anticipated human exposure, as discussed for the traditional guideline. A factor of one-third was again used. Thus the overall safety factor for the pharmacokinetic guideline is 2.3 * 10 * 1/3 = 7.7. The model was therefore exercised to predict the exposure concentration at which the AUC in the liver for a human would be one-eighth of the value in the animal at the NOAEL. For an 8-h time-weighted average exposure at 1.8 ppm, the calculated AUC in the liver was 5.6 mg/L-h, a factor of 8 below that at the animal subchronic NOAEL. The pharmacokinetically based guideline of 1.8 ppm is more than a factor of 2 higher than the traditionally derived guideline.

In addition to liver toxicity, there is limited evidence (12) of behavioral effects at higher CPFB concentrations (500 to 1000 ppm). Any behavioral deficit induced by CPFB could not only degrade performance during a training exercise, but could also increase the likelihood of subsequent exposure through improper use of protective gear. To avoid any behavioral effects potentially associated with brief exposure to higher concentrations, a short-term guideline was also developed. The traditional guideline is a 3 ppm ceiling limit based on the 300 ppm NOAEL for behavioral effects (13), with a safety factor of 100. The rationale for this guideline is the same as for the liver toxicity except that no exposure

duration adjustment is required since this is a ceiling limit. In the case of acute behavioral effects, toxicity generally appears to be correlated with peak concentration rather than AUC. The model predicts peak blood concentrations of 24.6 mg/L and 26.6 mg/L in rats and mice, respectively, at the acute NOAEL of 300 ppm. The Monte Carlo analysis of the uncertainty in model predictions of peak blood levels indicated that a factor of 1.8 should be used in this case for PBPK uncertainty. Adding a factor of 10 for interindividual variability, the target blood level was
24.6/18.0, or 1.4 mg/L. Iterative simulation with the model determined that an exposure of 31 ppm CPFB would produce a peak blood level of 1.4 mg/L in humans. This pharmacokinetically derived ceiling is roughly a factor of 10 higher than the traditionally derived value.

Finally, fetotoxic effects were observed in rats dosed orally, with a NOAEL of 300 mg/kg/day (14). The traditional guideline calculation requires a dose-route adjustment from the oral route used in the animal study to the inhalation route of concern for human exposure. The default calculation equates routes on a mg/kg basis, assuming an inhalation rate of 10 cu.m per 8 h:

300 mg/kg * 70 kg / 10 cu.m. = 2100 mg/cu.m

2100 mg/cu.m. * 24.45 cu.m./mole / 202.5 g/mole = 254 ppm

Using safety factors of 10 for animal-to-human extrapolation uncertainty, 10 for oral to inhalation extrapolation uncertainty, and 10 for human variability results in a guideline of 0.25 ppm.

In the pharmacokinetic approach, the oral exposure can be used to develop an inhalation guideline by using the PBPK model to estimate the peak blood concentrations and AUC for CPFB in the oral rodent study and comparing them with those achieved during human inhalation exposures. Both peak concentration and AUC are evaluated as dose surrogates, because the mechanism of fetotoxicity in this case is not established, and the choice of preferred dose surrogate is not clear. For an oral dose of 300 mg/kg in the rat, the model estimates a peak blood level of 123 mg/L and an area under the blood curve of 109.2 mg/L-h. In this case, the Monte Carlo analysis indicated that PBPK uncertainty factors of 4.8 and 5.1, respectively, are required, due to additional uncertainty from the oral uptake parameters. Taken together with a factor of 10 for human variability, the target dose surrogates in the human are a peak blood level of 2.56 mg/L and an AUC of 2.14 mg/L-h. For human inhalation, the peak blood level is predicted to be 2.5 mg/L at 56 ppm, whereas the area under the blood curve for an 8-h exposure is 2.04 mg/L-h at 5.2 ppm. Thus the pharmacokinetically derived inhalation guideline for the prevention of

fetotoxic effects is 5 ppm, based on AUC in the blood. This guideline is a factor of 20 above the traditionally derived guideline.

CONCLUSION

Chloropentafluorobenzene possesses a remarkable combination of properties, making it an attractive candidate for use as an intake simulant in chemical defense field training exercises. It is volatile and unreactive, simplifying dissemination, and mimics the performance of typical vapor threats in terms of persistence and canister penetration. It does not appear that CPFB would present any significant health hazards to personnel under the envisioned use. A thorough toxicological evaluation indicates that CPFB is not acutely toxic or teratogenic and is not likely to be carcinogenic. Chronic liver toxicity was observed only after prolonged exposure to high concentrations. Based on a pharmacokinetic analysis, it is recommended that field exercises be designed to avoid short-term exposures to concentrations greater than 30 ppm, with the daily (8-h) time-weighted average not to exceed 2 ppm. Because field analytical methods can measure CPFB at part per billion levels, this should not be an impediment to its use in training exercises. By comparison, a traditional approach to guideline generation would suggest a short-term limit of 3 ppm with an 8-h time-weighted average of 0.25 ppm.

The pharmacokinetic approach described in this paper for CPFB provides a general alternative to the traditional applied-dose methodology when pharmacokinetic data is available. The presumed advantage of the pharmacokinetic approach is that using an internal measure of effective tissue exposure should provide a more meaningful basis for estimating risk than using applied dose, and that the incorporation of pharmacokinetic information should increase the accuracy of the dose, route, and species extrapolations required in the risk assessment process. The level of pharmacokinetic information required to support a given risk assessment depends on the chemical and the nature of its effects on the animal system. For simple chemical toxicities, such as those seen with CPFB, a fairly uncomplicated pharmacokinetic description can suffice. The key information required includes the partitioning and metabolism of the chemical, together with a sufficient understanding of the nature of the toxicity to select the appropriate dose surrogate (22). For other chemicals and toxicities, a more significant effort may be required to develop an adequate description of the pharmacokinetics and pharmacodynamics involved. Nevertheless, there are a number of examples in the literature of successful pharmacokinetic/pharmacodynamic models for relatively complex chemical effects, including cofactor depletion and enzyme inhibition (23,24).

Countering the desire to incorporate as much science as possible into the risk assessment process is the concern for residual uncertainty in the qualitative and quantitative factors underlying the risk calculation. For example, in the application of pharmacokinetics for risk assessment there are at least two levels of uncertainty at issue: (1) the correctness of the structure of the pharmacokinetic model and the values of its parameters, and (2) the relationship of the pharmacokinetic information to the semiempirical heuristics of the traditional approach. The first level of uncertainty can be dealt with by Monte Carlo analysis as described here and elsewhere (21). To the extent that the uncertainty in the model structure and parameters can be characterized, the risk calculations can be performed using a conservative measure (the 5th or 95th percentile of the dose surrogate distribution in this case) which takes these uncertainties into account.

The second level of uncertainty is more problematic. The traditional approach has withstood the test of time, and has been invested with a level of confidence based more on its success than on its scientific basis. For example, the safety factors typically applied in noncancer risk assessment have been derived heuristically, over the course of many years and chemicals, and only after the fact have their theoretical or experimental basis been postulated (18). Therefore, when new scientific information is introduced into the risk assessment process, the inevitable question results: Does this new information replace, supplement, or confound the use of the traditional methods and factors? In the methodology described in this paper, the calculation of an internal dose surrogate representing tissue exposure was used to replace the cross-species and route-to-route safety factors traditionally applied for risk extrapolations based on an external dose measure. The assumptions underlying this use were that the model correctly predicts the internal dose measure, and that the expected tissue response (toxicity) would be the same, regardless of exposure route and species, for the same tissue exposure. As mentioned above, the first assumption was dealt with quantitatively using Monte Carlo analysis. The second assumption equates to a judgement that for the particular chemical and toxicities dealt with in this paper, there is no expectation of species-specific or route-dependent sensitivities, and the purpose for which the safety factors would be applied in the traditional process is completely served by the pharmacokinetic calculations. For other chemicals and toxicities, it could well be the case that this second assumption could not be supported, and that the incorporation in some quantitative fashion of pharmacodynamic considerations into the risk assessment would be necessary.

REFERENCES

1. D.G. Barnes and M.L. Dourson, "Reference Dose (RfD): Description and Use in Health Risk Assessments," *Reg. Toxicol. Pharmacol.* **8**, 471-486 (1988).

2. J.C. Ramsey and M.E. Andersen, "A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Humans and Rats," *Toxicol. Appl. Pharmacol.* **73**, 159-175 (1984).

3. W.D. Crank and A. Vinegar, "A Physiologically Based Pharmacokinetic Model for Chloropentafluorobenzene in Primates to be Used in the Evaluation of Protective Equipment Against Toxic Gases," *Toxicol. Ind. Health*, **8**, 21-35 (1992).

4. G.W. Jepson, H.J. Clewell, and M.E. Andersen, "A Rapid, Physiologically Based Method for Evaluating Candidate Chemical Warfare Agent Uptake Simulants," (AAMRL-TR-85-045, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1985).

5. E.R. Kinkead, H.G. Wall, C.J. Hixson, R.R. Tice, R.S. Kutzman, and A. Vinegar, "Chloropentafluorobenzene: Short-term Inhalation Toxicity, Genotoxicity and Physiologically-based Pharmacokinetic Model Development," *Toxicol. Ind. Health* **6**, 533-550 (1990a).

6. E.R. Kinkead, W.J. Bashe, D.M. Brown, and S.S. Henry, "Evaluation of the Inhalation Toxicity and Sensitization Potential of Chloropentafluorobenzene," *1986 Toxic Hazards Research Unit Annual Report* (AAMRL-TR-87-020, NMRI-87-2, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH), pp. 131-135 (1987).

7. E.R. Kinkead, S.K. Bunger, E.C. Kimmel, C.D. Flemming, H.G. Wall, and J.H. Grabau, "Effects of a 13-week Chloropentafluorobenzene Inhalation Exposure of Fischer 344 Rats and B6C3F1 Mice" (AAMRL-TR-90-064, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1990b).

8. A. Tu, M.G. Broome, and A. Sivak, "Evaluation of Chloropentafluorobenzene in a Battery of *In Vitro* Short Term Assays" (AAMRL-TR-86-003, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1986) .

9. V. Steele, "Biological Activity of Chloropentafluorobenzene" (AAMRL-TR-87-039, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1987) .

10. R.S. Kutzman, B.C. Myhr, T.E. Lawlor, D.C. Valentine, R.R. Young, H. Murli, M.A. Cifone, and B.M. Jarnot, "Genetic Toxicity Assessment of Chloropentafluorobenzene" (AAMRL-TR-90-048, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1990).

11. E.R. Kinkead, B.T. Culpepper, H.G. Wall, R.S. Kutzman, C.D. Flemming, C.J. Hixon, and R.R. Tice, "Evaluation of the Potential of Inhaled Chloropentafluorobenzene to Induce Toxicity in F-344 Rats and B6C3F1 Mice and Sister Chromatid Exchanges and Micronuclei Formation in B6C3F1 Mice" (AAMRL-TR-89-037, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH, 1989).

12. J.C. Gage, "The Subacute Inhalation Toxicity of 109 Industrial Chemicals," *British J. Ind. Med.*, **27**, 1-18 (1970).

13. E.R. Kinkead, S.K. Bunger, E.C. Kimmel, C.D. Flemming, H.G. Wall, and J.H. Grabau, "Effects of a 13-week Chloropentafluorobenzene Inhalation Exposure of Fischer 344 Rats and B6C3F1 Mice," *Toxicol. Ind. Health* **7**, 309-318 (1991).

14. J.R. Cooper and B.M. Jarnot, "An Evaluation of the Teratogenicity of Chloropentafluorobenzene (CPFB)," *The Toxicologist* **12**, 105 (1992).

15. G. Renner, "Hexachlorobenzene and Its Metabolism," *Toxicol. Environ. Chem.* **18**, 51-78 (1988).

16. M.L. Gargas, H.J. Clewell, and M.E. Andersen "Metabolism of Dihalomethanes *In Vivo*: Differentiation of Kinetic Constants for Two Independent Pathways," *Toxicol. Appl. Pharmacol.* **82**, 211-223 (1986).

17. National Institute for Occupational Safety and Health, *Registry of Toxic Effects of Chemical Substances* (U.S. Department of Health and Human Services, Washington, DC, 1992).

18. M.L. Dourson and J.F. Stara, "Regulatory History and Experimental Support of Uncertainty (Safety) Factors," *Reg. Toxicol. Pharmacol.*, **3**, 234-228 (1983).

19. National Research Council, *Drinking Water and Health*, Vol. 6 (National Academy Press, Washington, DC, pp. 193-200, 1986).

20. D. Hattis, L. Erdreich, and M. Ballew, "Human Variability in Susceptibility to Toxic Chemicals – A Preliminary Analysis of Pharmacokinetic Data From Normal Volunteers," *Risk Anal.* **7**, 415-426 (1987).

21. H.J. Clewell, "The Use of Physiologically Based Pharmacokinetic Modeling in Risk Assessment: A Case Study with Methylene Chloride," *Cancer Dose-Response* (ILSI Press, Washington, DC) in press (1993).

22. M.E. Andersen, "Tissue Dosimetry in Risk Assessment, or What's the Problem Here Anyway?" National Research Council. *Pharmacokinetics in risk assessment. Drinking water and health. Volume 8.* (National Academy Press, Washington, DC) 8-23 (1987).

23. H.J. Clewell and M.E. Andersen, "Dose, Species, and Route Extrapolation using Physiologically Based Pharmacokinetic Models." National Research Council. *Pharmacokinetics in risk assessment. Drinking Water and Health. Volume 8.* (National Academy Press, Washington, DC) 159-182 (1987).

24. H.J. Clewell, "Coupling of computer modeling with *in vitro* methodologies to reduce animal usage in toxicity testing," *Toxicol. Lett.* **68**, 101-117 (1993).

Significance of the Dermal Route of Exposure to Risk Assessment

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ABSTRACT

The skin is a route of exposure that needs to be considered when conducting a risk assessment. It is necessary to identify the potential for dermal penetration by a chemical as well as to determine the overall importance of the dermal route of exposure as compared with inhalation or oral routes of exposure. The physical state of the chemical, vapor or liquid, the concentration, neat or dilute, and the vehicle, lipid or aqueous, are also important. Dermal risk is related to the product of the amounts of penetration and toxicity. Toxicity involves local effects on the skin itself and the potential for systemic effects. Dermal penetration is described in large part by the permeability constant. When permeability constants are not known, partition coefficients can be used to estimate a chemical's potential to permeate the skin. With these concepts in mind, a tiered approach is proposed for dermal risk assessment. A key first step is the determination of a skin-to-air or skin-to-medium partition coefficient to estimate a potential for dermal absorption. Building a physiologically based pharmacokinetic (PBPK) model is another step in the tiered approach and is useful prior to classical *in vivo* toxicity tests. A PBPK model can be used to determine a permeability constant for a chemical as well as to show the distribution of the chemical systemically. A detailed understanding of species differences in the structure and function of the skin and how they relate to differences in penetration rates is necessary in order to extrapolate animal data from PBPK models to the human. A study is in progress to examine anatomical differences for four species.

INTRODUCTION

BACKGROUND

The skin, constituting approximately 10% of total human body weight, acts as the major interface between the homeostatic internal environment of the body and the comparatively unregulated and potentially hostile external environment. The skin primarily functions as a protective barrier that restrains entry of chemical substances into the body. The potential for occupational or accidental skin exposure to nonvolatile and volatile chemicals (both of which may penetrate the barriers of the skin) requires an

experiment-based understanding of chemical absorption through the skin to adequately determine risks of such exposures.

Personnel working in an occupational environment are often exposed to a variety of chemicals. Maintenance, repair, and fueling operations expose workers to engine oils, lubricants, fuels, hydraulic fluids, paints, and solvents. All of these types of compounds present a potential for dermal exposure. Up to 40% of all occupational illness may involve the skin (1). For some substances, cutaneous absorption is a major contributor to overall exposure (2). The absorbed total uptake of xylene from hand skin contact with solvent mixtures for 15 min was greater than that from inhalation over a full 8-h shift in auto body repair shops (3). The dermal route was found to be the major contributor to total polychlorinated biphenyls body burden of transformer maintenance and repair personnel (4). Gloves are of limited protection, as permeation of chemicals through glove material is known to occur (5). Absorption of chemicals through the skin now appears to be of greater significance than previously suggested by industry or epidemiological experience (6). The dermal route of exposure may not always be the most important route, but it may often contribute significantly to total exposure. For a highly fat soluble chemical such as dibromomethane, the body burden from dermal penetration compared with inhalation was approximately 6% in one rat study (7). If respiratory protection were worn but the skin was exposed, absorption of this chemical vapor would still occur. A method to compare dermal vapor exposure to inhalation exposure at the same concentration has been described as a ratio of input functions for the contribution of each route of exposure, provided that the permeability constant, surface area of skin exposed, and aveolar ventilation rate are known or can be determined (8).

Chemicals in the liquid state must also be considered because many exposure chemicals exist as a neat liquid or dissolved in a liquid medium such as water. Concentrations of pure liquid are much greater than in their vapor form. This results in greater total penetration through the skin. Even though the concentration on the skin is different between a vapor and the liquid form of a chemical, the solubility of a chemical in the skin should not be affected once the chemical enters the skin unless the liquid form of the chemical alters the skin barrier. Tsuruta (9) and others have reported on the percutaneous absorption of organic solvents. Morgan *et al.* (10) demonstrated that significant amounts of volatile organic chemicals (VOCs) can be absorbed through the skin during dermal exposure of rats to low levels of this class of chemical in aqueous solutions. Absorption to neat chemical did not appear to result in the same absorption rate as chemical in aqueous solution. Permeability constants were not determined

by Morgan *et al.* (10) but peak blood levels after neat chemical exposure were approximately an order of magnitude greater than after chemical in aqueous solution. Estimation of the significance of dermal absorption of VOCs from aqueous solutions based on data for pure liquids may not provide an accurate assessment of actual exposure levels.

Additional studies in this area have looked at chloroform, a VOC that contaminates chlorine-treated municipal tap water (11). Therefore, individuals are exposed to chloroform while showering with chlorine-treated tap water. In situations where water should not be consumed due to contamination with VOCs, individuals should also consider avoiding bathing with the water.

Dermal risk is a function of exposure penetration and toxicity. A toxic chemical that cannot penetrate the skin may be limited to local toxic effects on the skin. A chemical with a relatively low toxicity potential that readily penetrates the skin and enters circulation may have systemic effects or produce target organ toxicity. Therefore, it is necessary to know the capacity of a chemical for percutaneous absorption in order to assess its overall potential risk.

ESTIMATES OF DERMAL PENETRATION

Various methods have been used to measure the potential of a chemical to penetrate the skin. The permeability constant (K_p) of a chemical is a quantitative expression of the capacity of a chemical to enter and diffuse through the skin. Permeability constants are used to predict the absorption rate or flux, which is the mass of chemical absorbed per unit area of skin per unit time. The solubility of a chemical in skin (partition coefficient [PC]) is an important parameter for determining the permeability constant when the diffusion coefficient for skin is known.

Flux, or rate of penetration of a chemical across the skin, is determined by concentration at the skin surface, the surface area exposed, and solubility of chemical in the skin (12,13). Skin-to-air PC values for a chemical are a measure of the solubility of the chemical in skin and should correlate with the permeability constant as shown in the following equation for flux:

$$\text{Flux} = \frac{Dk_mC}{} = k_pC$$

where Flux is $mg/cm^2/hr$, D is the diffusion constant of the chemical in the skin (cm^2/h), k_m is the solubility or PC of the chemical in skin (unitless), C is the concentration of chemical on the surface of the skin (mg/cm^3), l is the skin thickness (cm), and k_p is the permeability constant (cm/h).

Physical and chemical properties of chemicals such as solubility are important descriptors of skin penetration (6,14,15). The PC for skin, a measure of the affinity of a chemical for skin tissue, is the ratio of concentrations at equilibrium between the tissue and an adjacent medium, such as air, water, or other environmental vehicle. Various experimental methods have been reported in the literature for determining PC values for skin. One method uses the octanol/water PC as a surrogate for partitioning between the skin (octanol phase) and the environment or vehicle (water phase) (16,17). Octanol/water PC values are typically determined by shaking the test compound in a mixture containing equal parts of water and octanol. After sufficient time for equilibration to occur, the ratio of the amount of test compound in each solvent is determined (16). Hawkins and Reifenrath (18) compared octanol/water PC values to the percent of applied dose of pesticides and steroid hormones after exposure *in vitro* through pig and human skin. Kasting, Smith, and Cooper (17) used octanol/water PC values in a mathematical model to estimate the flux of chemicals across the skin. Berner *et al.* (19) used octanol/water PC values to confirm skin permeation rates for a series of chemicals prior to examining the relationship between the pKa of these chemicals and acute skin irritation. Octanol/water PC values have been used to estimate dermal flux for setting a skin notation guideline for a threshold limit value-time weighted average (20). Although the octanol/water PC has been used extensively in estimating dermal penetration, it is an oversimplification of the process of chemical interaction with the skin. The octanol/water PC assumes that skin is homogenous with respect to octanol and that water is the environmental medium.

Surber *et al.* (15) measured stratum corneum (SC)/water and SC/isopropyl myristate PC values. In their study, PCs were determined as a function of equilibration time, initial concentration of drug in the vehicle, delipidization of stratum corneum, and source and preparation of stratum corneum. The PCs were considered as predictors of percutaneous penetration for the purpose of conducting dermal risk assessments (15).

TIERED APPROACH

A tiered approach is proposed for determining the potential hazard of a chemical for dermal risk assessment as shown in Figure 21. This approach employs toxicity tests in an orderly sequence that can be stopped at various levels depending on the application and potential for exposure of the chemical, potential for full scale development of the system of intended use, initial toxicity results, and such.

The first phase is conducted completely with *in vitro* tests and structure–activity comparisons. Dermal PCs are proposed as an important first step at this level. A procedure for determining skin:air PC values was developed in this laboratory and will be summarized in this paper. Exposure assessment is also an important early component of the tiered approach. Knowing the physical form of the chemical, the expected concentration, and possible environmental medium are essential in planning the appropriate tests to conduct initially as well as throughout the tiered approach.

The second phase involves acute *in vivo* toxicity studies such as a dermal limit test. The Phase I screen is used to eliminate as many chemicals as possible in order to decrease the number of animal studies.

Another relatively early step in the tiered approach is the development of a physiologically based pharmacokinetic (PBPK) model with a skin compartment. The use of PBPK models will also be discussed and an example of their use will be presented in this paper. Physiologically based pharmacokinetic model development spans two levels of the tiered approach because development of a model involves *in vivo* procedures. A PBPK model could still be developed without completing all of the end points for a Phase II screen. Completion of a PBPK model and short-term dermal exposure studies represent the Phase III screen.

Phase IV is the screening phase for genotoxicity and carcinogenicity. Completion of this phase would provide a comprehensive hazard assessment of potential dermal risk. It is possible that *in vitro* genotoxicity testing will need to be conducted prior to the completion of earlier phases.

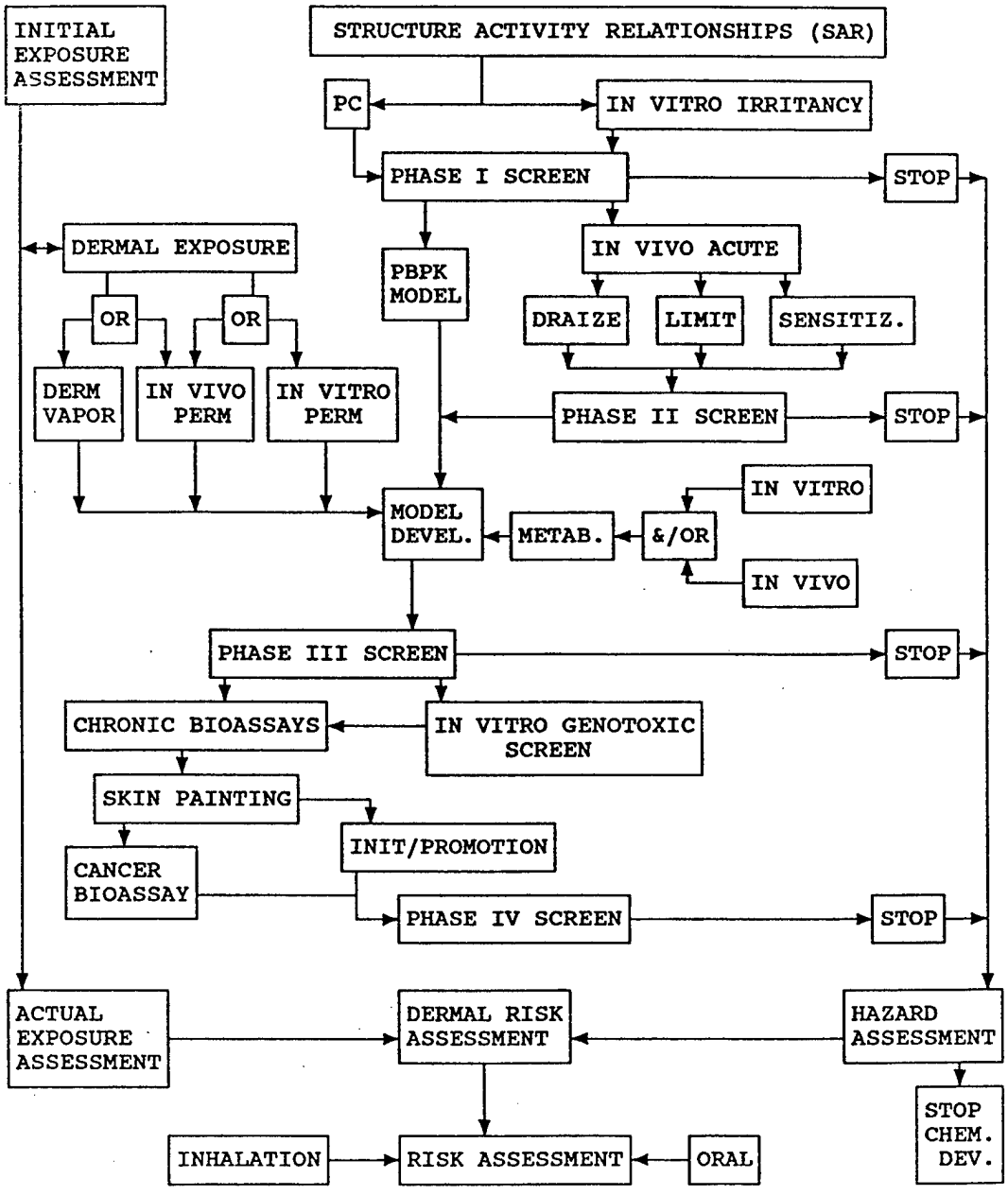


Figure 21. Tiered Approach to Dermal Risk Assessment.

SKIN:AIR PARTITION COEFFICIENTS

The headspace method for PC determination, developed by Sato and Nakajima (21) and modified by Gargas *et al.* (22), has been used extensively in this laboratory for determining PCs of a variety of biological tissues. However, the methodology was not adequate for measuring the skin:air PC. A modification in the preparation of skin for the headspace method was developed in order to measure skin:air PC values.

Dibromomethane was used as a prototype for skin:air PCs. Male Fischer 344 (F-344) rats (Charles River Laboratories) were between 8 and 16 weeks old at the time the skin was collected for PC determination. Clipped dorsal skin was collected and cut into 1 by 0.5 cm strips. The pieces of skin were placed on the walls of scintillation vials (24.65 mL volume) without saline. Sample vials containing skin and the corresponding empty reference vials were injected with an equal concentration of chemical vapor. At equilibrium, vapor from the headspace of the sample and reference vials were measured on a gas chromatograph with a flame ionization detector. After measuring the amount of chemical as area counts from all of the reference and sample vials in a set, sample vials were compared with corresponding reference vials using the following equation modified from Gargas *et al.* (22):

$$PC = \frac{(\text{reference vial area cts})(\text{vial volume}) - (\text{skin sample area cts})(\text{vial volume} - \text{sample volume})}{(\text{skin sample area cts})(\text{sample volume})}$$

After developing the technique for determining a skin:air PC value using dibromomethane, the procedure was used for other VOCs of interest (Table 28).

TABLE 28. RAT SKIN:AIR PARTITION COEFFICIENTS FOR SELECTED VOLATILE ORGANIC CHEMICALS

Chemical	Skin:Air PC (± S.E.)	n	Equilibration Time (hours)
Dibromomethane	68.3±3.1	10	4
Perchloroethylene	41.5±1.2	16	4
Trichloroethylene	31.8±1.5	19	4
Benzene	34.5±1.9	19	4

(continued)

TABLE 28. Continued

Chemical	Skin:Air PC (± S.E.)	n	Equilibration Time (hours)
Hexane	1.9±0.1	18	4
Toluene	43.0±1.8	16	4
Xylene	50.4±1.7	24	2
Styrene	91.9±6.8	20	3
Methylene chloride	13.6±0.5	17	2
Carbon tetrachloride	12.4±0.6	24	4
Methyl chloroform	10.8±0.6	18	4
Halothane	10.6±0.7	17	3
Isoflurane	4.5±0.3	16	6

The skin:air partition coefficient for dibromomethane and selected VOCs is shown in Table 28. Approximately 19 samples were analyzed for each chemical. The most common equilibration time for this group of volatile chemicals was 4 h. The skin:air PC values ranged from 1.9 for hexane to 91.9 for styrene. The octanol/water PC values (23) were compared with the corresponding skin:air PC values for 11 of the above chemicals. There was no correlation ($r^2=0.09$) between the octanol/water PC values and skin:air PC values (Figure 22). Permeability constants were available for nine of the chemicals for which skin:air PC values were measured in this study (7,13,24). There was good correlation ($r^2=0.93$) between permeability constants and skin:air PC values (Figure 23). When octanol/water PC values were compared directly to eight of the permeability constants (minus the octanol/water PC for Isoflurane), the correlation was also poor ($r^2=0.04$). If a saline:air or water:air PC value is determined for a chemical, a skin:saline or skin:water PC value can be calculated for the chemical by dividing the skin:air PC value by the saline:air or water:air PC value. Comparison of octanol/water PC values with skin:saline PC values still resulted in a poor correlation ($r^2=0.20$).

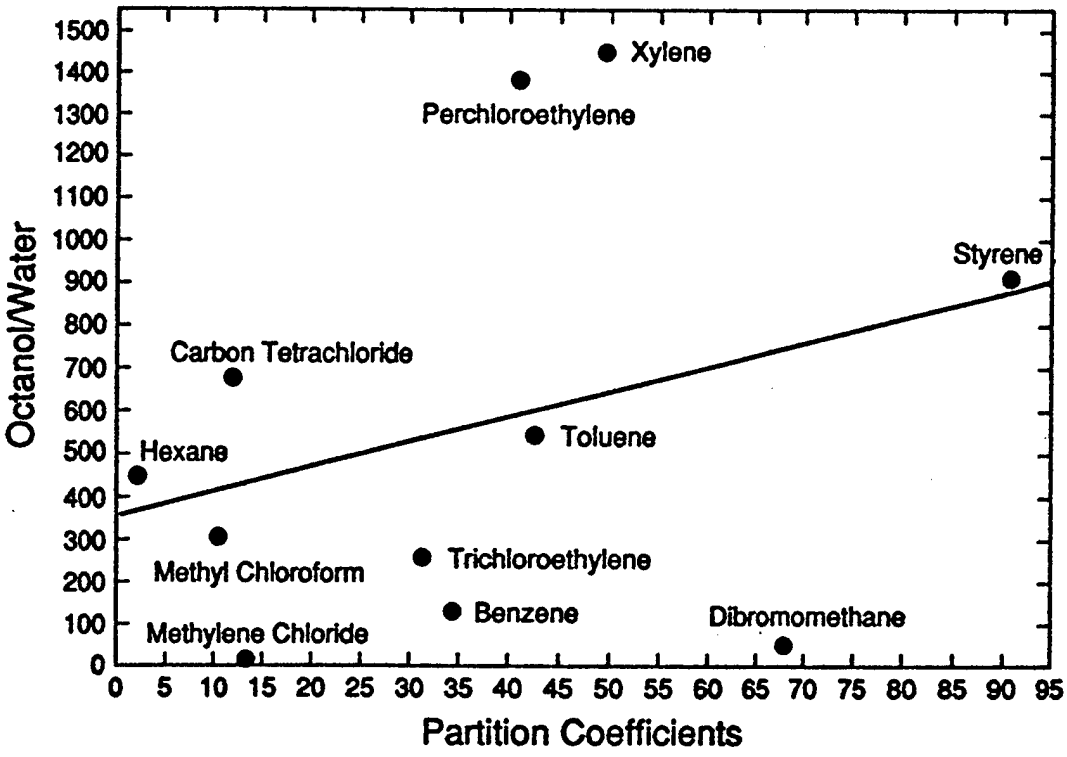


Figure 22. Comparison of Skin:Air Partition Coefficients with Octanol/Water PC Values.

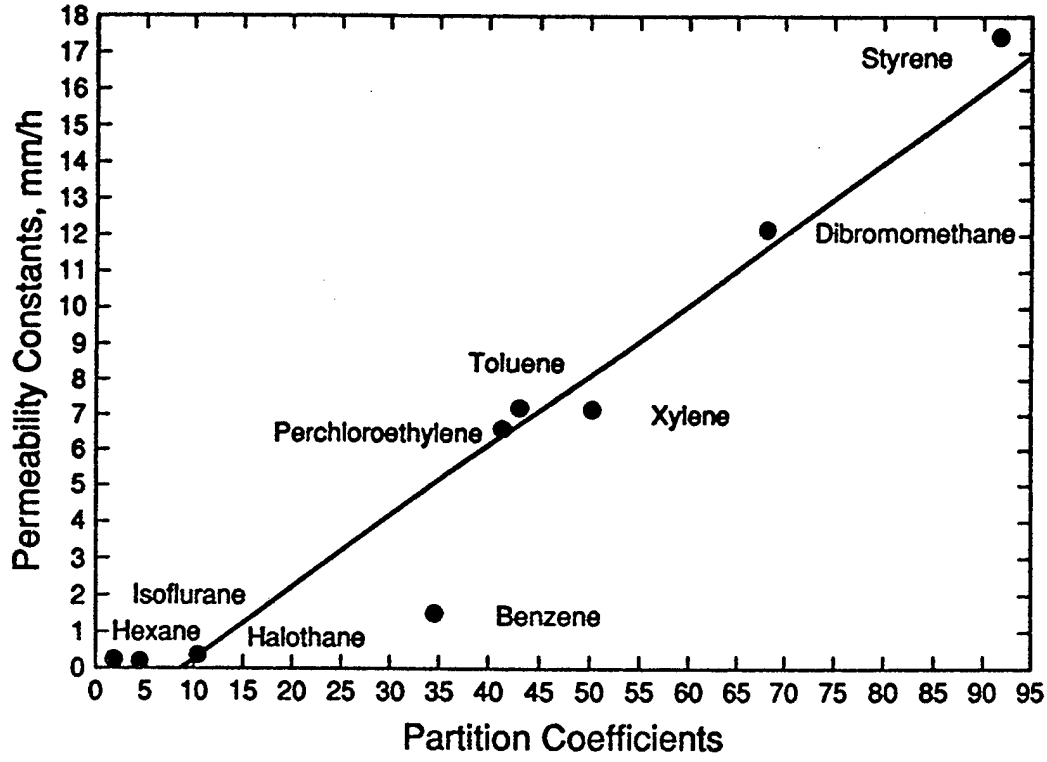


Figure 23. Comparison of Skin:Air Partition Coefficients with Permeability Constants.

The skin:air PC values were compared to both octanol/water PC values and permeability constants. Octanol/water PC values have been used as a qualitative measure of skin permeability (16-18,25,26). Skin:air PC values for the chemicals that were tested showed a correlation with permeability constants, but did not show as good a correlation with octanol/water PC values. Octanol/water PC values for the VOCs examined in this study appeared to be poor indicators of the solubility of these chemicals in skin. No single bulk solvent, such as octanol, precisely mimics the solvent properties of the stratum corneum transport barrier (25). In addition, the skin:air PC values were determined for chemicals with poor water solubility. The predictive ability of octanol/water PC values is most likely lower for these volatile chemicals because octanol/water PC values are based on water representing the vehicle or environmental medium. The data in this study suggest that skin:air PC values are a better indicator of the relative skin permeability for the volatile chemicals examined in this study. Skin:saline PC values would be representative of permeability into skin from an aqueous environmental medium. Determining a skin:air PC or skin:saline PC is proposed as an initial screen to identify the potential for skin absorption of volatile chemicals with unknown permeability constants.

PBPK MODELING

In addition to indicating potential permeability, skin PCs are necessary for developing the dermal compartment in a PBPK model. Physiologically based pharmacokinetic models mathematically describe the dynamics of chemicals in the body including permeability of membranes and partitioning of chemicals into tissues. A PBPK model is developed by grouping various tissue types together based on similar blood flows and PCs. Each compartment has a measured physiological blood flow. Model parameters are determined from laboratory studies or literature values, and can be changed to extrapolate across species. Absorption, distribution, metabolism, and elimination of a chemical are then mathematically described for each compartment which has such a process. The skin:air PC is essential for the rate equation in the dermal compartment describing the uptake of chemical from air into the skin. A skin:saline PC value is calculated, as described above, for the rate equation for uptake into skin from an aqueous medium. A PBPK model with a dermal compartment can then be utilized to determine the permeability constant for a chemical. The difference in concentration, surface area, and exposure duration between the laboratory and an actual occupational situation can also be described using a PBPK model. In addition, metabolism of the chemical, which may be quantitatively or qualitatively different between experimental species and humans, can be estimated with existing methods and their impact on penetration described using a PBPK model. Physiologically based pharamacokinetc modeling provides

the means to relate laboratory animal exposures to the human situation by extrapolating across exposure concentrations, routes of exposure, and species (8). Accurate extrapolation from animal exposures to personnel in the workplace will provide the means to more quickly and efficiently set safe, but not overly restrictive, dermal exposure standards.

For a PBPK model to accurately estimate a permeability constant for a chemical in skin, a number of conditions are important. A PBPK model with a dermal compartment must be validated based on exposure for a second route of exposure, such as the inhalation route. Skin PC data should be experimentally determined for the dermal compartment. Actual dermal exposures should be conducted in order to measure the uptake of chemical into the blood. The concentration of chemical in blood after dermal exposure is then used in model simulations to estimate the permeability constant for that chemical.

Previous work with PBPK models in this laboratory has demonstrated their usefulness in extrapolation and the risk assessment process (27-33). The PBPK models were developed, based on the work of McDougal *et al.* (7,13,24), which described the following three different *in vivo* dermal exposures in rats: whole body dermal exposure to benzene vapor (13), exposure to neat benzene from a closed cell on the dorsal skin (unpublished data and 10), and exposure to saturated solutions of benzene in water also from a closed cell (10). The models were used to estimate the permeability constants of benzene from blood concentrations achieved during exposure to each form of the chemical. The estimated permeability constant for dermal vapor was 0.152 cm/h, for neat benzene 0.0025 cm/h, and for aqueous solutions 0.05 cm/h. The physical form of the chemical and the presence of water resulted in different rates of absorption. The permeability constant for rat skin from aqueous solutions was one-half the human permeability constant value used for dermal risk assessment, 0.111 cm/h (34). Rat skin has been reported to be more permeable than human skin by a factor of two to four (8), so the rat permeability constant was expected to be at least twice as high as the human value for benzene.

SPECIES DIFFERENCES IN SKIN PENETRATION

In an attempt to better understand factors affecting dermal penetration and to be able to better extrapolate between animal species and humans, a study was initiated to quantitate selected anatomical differences in skin from a number of animal species. Anatomical differences that may affect permeability include density and size of hair follicles, density of sebaceous and apocrine glands, capillary density and distance from the surface, as well as thickness of the various layers of the epidermis and dermis.

Anatomical differences in skin between species will be compared to permeability constants for three model chemicals to determine possible correlations between structure and permeability. Permeability constants will be determined using PBPK models for chloropentafluorobenzene, perfluoroheptane, and dichlorobenzene.

Sections of dorsal skin were collected from IAF/HA Hairless and Hartley Guinea Pigs, Fuzzy and F-344 Rats, B6C3F1 and Crl:SKH1 Hairless mice, and farm pigs. Pieces of skin were processed at the same time and under identical conditions for standard histopathology sections in paraffin. One set of sections was stained with hemotoxylin and eosin and another set with Massons trichrome. Image analysis was conducted on sections from each strain using an image analysis system. Parameters measured were thickness (stratum corneum, stratum granulosum, viable epidermis, and total epidermis); average depth and distribution of capillaries, venules and arterioles; surface area of each type of blood vessel relative to basement membrane of the epidermis; and depth and surface area of hair follicles and sebaceous glands relative to the basement membrane of the epidermis.

Exploratory data (Table 29) showed that the hairless guinea pig and the farm pig have the thickest epidermal layers and F-344 rat and the mice have the thinnest epidermal layers. There was a wide range for average depth of capillaries, venules, and arterioles with the hairless guinea pig and mice having capillaries and venules closer to the epidermis and F-344 rats having all vessels farther away from the epidermis. The farm pig had the greatest volume of hair follicles and sebaceous glands; the F-344 rat had the least follicular volume and the Hartley guinea pig had the least gland volume.

Additional skin samples will be analyzed to confirm these preliminary data. A PBPK model will be built for each of the three strains showing the widest variation in anatomical parameters. Exploratory information suggests the use of F-344 rats and Hartley and Hairless guinea pigs. A better understanding of the anatomical differences of skin in animal species may lead to a better extrapolation of animal skin data to human skin.

TABLE 29. ANATOMICAL PARAMETERS IN SKIN FROM FOUR ANIMAL SPECIES (N=3)

	Mean Microns ± S.E.						
	Mouse		Guinea Pig		Rat		Swine
	SKH1	B7C3F1	Hartley	Hairless	Fuzzy	F-344	Farm
Total Epidermis	26.4±1.7	13.2±1.5	26.8±0.7	59.6±3.9	47.0±3.0	20.9±2.2	52.2±7.2
Capillary Depth	299±39	421±55	446±149	333±48	519±138	803±83	511±13
Veinule Depth	389±19	536±20	903±218	610±59	723±188	970±57	623±87
Arteriole Depth	479±20	410±109	602±275	743±298	715±89	1340±63	792±123
Follicle Volume[a] ($X10^{-3}$)	1.7±0.4	1.2±0.6	1.5±0.1	2.5±0.1	2.0±0.5	0.5±0.1	6.4±2.0
Gland Volume[a] ($X10^{-3}$)	1.4±0.2	1.0±0.3	0.3±0.1	1.1±0.2	2.4±0.3	1.0±0.2	5.1±2.5

[a]ratio of follicle or sebaceous gland area to total area.

SUMMARY

The dermal route is an important potential route of exposure. There is still much research to be conducted to understand the skin and its significance in risk assessment.

ACKNOWLEDGEMENTS

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The animals used in this study were handled in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

REFERENCES

1. K.F. Wheeler, "Barrier Lotions, Along With Gloves, Can Help Deter Occupational Dermatitis," *Occupational Health and Safety* Jan., 60-61 (1992).

2. G. Scansetti, G. Piolatto, and G.F. Rubino, "Skin Notation in the Context of Workplace Exposure Standards," *Am. J. Ind. Med.* **14**, 725-732 (1988).

3. W. Daniell, A. Stebbins, D. Kalman, J.F. O'Donnell, and S.W. Horstman, "The Contributions to Solvent Uptake by Skin and Inhalation Exposure," *Am. Ind. Hyg. Assoc. J.* **53**, 124-129 (1992).

4. P.S.J. Lees, M. Corn, and P. Breysse, "Evidence for Dermal Absorption as the Major Route of Body Entry During Exposure of Transformer Maintenance and Repairmen to PCBs," *Am. Ind. Hyg. Assoc. J.* **48**, 257-264 (1987).

5. J.L. Perkins and V.B. Knight, "Risk Assessment of Dermal Exposure to Polychlorinated Biphenyls Permeating a Polyvinyl Chloride Glove," *Am. Ind. Hyg. Assoc. J.* **50**, A-171-172 (1989).

6. P. Grandjean, A. Berlin, M. Gilbert, and W. Penning, "Preventing Percutaneous Absorption of Industrial Chemicals: The "Skin" Denotation," *Am. J. Ind. Med.* **14**, 97-107 (1988).

7. J.N. McDougal, G.W. Jepson, H.J. Clewell III, and M.E. Andersen, "Dermal Absorption of Dihalomethane Vapors," *Toxicol. Appl. Pharmacol.* **79**, 150-158 (1985).

8. J.N. McDougal and H.J. Clewell III, "Dermal to Inhalation Extrapolation for Organic Chemicals," in T.R. Gerrity and C.J. Henry (eds.), *Principles of Route-to-Route Extrapolation for Risk Assessment* (Elsevier Science Publishing Co., Inc., New York, 1990), pp. 313-317.

9. H. Tsuruta, "Percutaneous Absorption of Organic Solvents. 1. Comparative Study of the *In Vivo* Percutaneous Absorption of Chlorinated Solvents in Mice," *Ind. Health* **13**, 227-236 (1975).

10. D.L. Morgan, S.W. Cooper, D.L. Carlock, J.J. Sykora, B. Sutton, D.R. Mattie, and J.N. McDougal, "Dermal Absorption of Neat and Aqueous Volatile Organic Chemicals in the Fischer 344 Rat," *Environ. Res.* **55**, 51-63 (1991).

11. W.K. Jo, C.P. Weisel, and P.J. Lioy, "Chloroform Exposure and the Health Risk Associated with Multiple Uses of Chlorinated Tap Water," *Risk Anal.* **10(4)**, 581-585 (1990).

12. G.L. Flynn, S.H. Yalkowsky, and T.J. Roseman, "Mass Transport Phenomena and Models: Theoretical Concepts," *J. Pharm. Sci.* **63**, 479-510 (1974).

13. J.N. McDougal, G.W. Jepson, H.J. Clewell III, M.L. Gargas, and M.E. Andersen, "Dermal Absortion of Organic Chemical Vapors in Rats and Humans," *Fundam. Appl. Toxicol.* **14**, 299-308 (1990).

14. L.K. Pershing, L.D. Lambert, and K. Knutson, "Partition Coefficient and Solubilities of Estradiol in a Variety of Vehicles Predict the *In Vivo* Flux Across the Human Skin Sandwich Flap," *Clin. Res.* **37**, 727A (1989).

15. C. Surber, K.-P. Wilhelm, H.I. Maibach, L.L. Hall, and R.H. Guy, "Partitioning of Chemicals into Human Stratum Corneum: Implications for Risk Assessment Following Dermal Exposure," *Fundam. Appl. Toxicol.* **15**, 99-107 (1990).

16. R.L. Bronaugh and E.R. Congdon, "Percutaneous Absorption of Hair Dyes: Correlation with Partition Coefficients," *J. Invest. Dermatol.* **83**, 124-127 (1984).

17. G.B. Kasting, R.L. Smith, and E.R. Cooper, "Effect of Lipid Solubility and Molecular Size on Percutaeous Absorption," *Pharmacol. Skin* **1**, 138-153 (1987).

18. G.S. Hawkins and W.G. Reifenrath, "Influence of Skin Source, Penetration Cell Fluid, and Partition Coefficient on *In Vitro* Skin Penetration," *J. Pharm. Sci.* **75**, 378-381 (1986).

19. B. Berner, D.R. Wilson, R.H. Guy, G.C. Mazzenga, F.H. Clark, and H.I. Maibach, "The Relationship of pK_a and Acute Skin Irritation in Man," *Pharm. Res.* **5**, 660-663 (1988).

20. V. Fiserova-Bergerova, J.T. Pierce, and P.O. Droz, "Dermal Absorption Potential of Industrial Chemicals: Criteria for Skin Notation," *Am. J. Ind. Med.* **17**, 617-635 (1990).

21. A. Sato and T. Nakajima, "Partition Coefficients of Some Aromatic Hydrocarbons and Ketones in Water, Blood, and Oil," *Brit. J. Ind. Med.* **36**, 231-234 (1979).

22. M.L. Gargas, R.J. Burgess, D.E. Voisard, G.H. Cason, and M.E. Andersen, "Partition Coefficients of Low-Molecular-Weight Volatile Chemicals in Various Liquids and Tissues," *Toxicol. Appl. Pharmacol.* **98**, 87-99 (1989).

23. A. Leo, C. Hansch, and D. Elkins, "Partition Coefficients and Their Uses," *Chem. Rev.* **71**, 525-616 (1971).

24. J.N. McDougal, G.W. Jepson, H.J. Clewell III, M.G. MacNaughton and M.E. Andersen, "A Physiological P0harmacokinetic Model for Dermal Absorption of Vapors in the Rat," *Toxicol. Appl. Pharmacol.* **85**, 286-294 (1986).

25. B.D. Anderson and P.V. Raykar, "Solute Structure-Permeability Relationships in Human Stratum Corneum," *J. Invest. Dermatol.* **93**, 280-286 (1989).

26. N.E. Tayar, R.-S. Tsai, B. Testa, P.-A. Carrupt, C. Hansch, and A. Leo, "Percutaneous Penetration of Drugs: A Quantitative Structure-Permeability Relationship Study," *J. Pharm. Sci.* **80**, 744-749 (1991).

27. M.E. Andersen, H.J. Clewell III, M.L. Gargas, F.A. Smith, and R.H. Reitz, "Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride," *Toxicol. Appl. Pharmacol.* **87**, 185-205 (1987).

28. H.J. Clewell III and M.E. Andersen, "Risk Assessment Extrapolations and Physiological Modeling," *Toxicol. Ind. Health* **1**, 111-131 (1985).

29. H.J. Clewell III and M.E. Andersen, "Improving Toxicology Testing Protocols using Computer Simulations," *Toxicol. Lett.* **49**, 139-158 (1989).

30. R.A. Corley, A.L. Mendrala, F.A. Smith, D.A. Staats, M.L. Gargas, R.B. Conolly, M.E. Andersen, and R.H. Rietz, "Development of a Physiologically Based Pharmacokinetic Model for Chloroform," *Toxicol. and Appl. Pharmacol.* **103**, 512-527 (1990).

31. J.W. Fisher, T.A. Whittaker, D.H. Taylor, H.J. Clewell III, and M.E. Andersen, "Physiologically Based Pharmacokinetic Modeling of the Pregnant Rat: A Multiroute Exposure Model for Trichloroethylene and its Metabolite, Trichloroacetic Acid," *Toxicol. Appl. Pharmacol.* **99**, 395-414 (1989).

32. M.L. Gargas, M.E. Andersen, and H.J. Clewell III, "A Physiologically Based Simulation Approach for Determining Metabolic Constants from Gas Uptake Data," *Toxicol. Appl. Pharmacol.* **86**, 341-352 (1986).

33. H.R. Reitz, J.N. McDougal, M.W. Himmelstein, R.J. Nolan, and A.M. Schumann, "Physiologically-Based Pharmacokinetic Modeling with Methylchloroform: Implications for Interspecies, High Dose/Low Dose, and Dose Route Extrapolations," *Toxicol. Appl. Pharmacol.* **95**, 185-199 (1988).

34. I.H. Blank and D.J. McAuliffe, "Penetration of Benzene Through Human Skin," *J. Invest. Dermatol.* **85**, 522-526 (1985).

Risk Above the Reference Dose (RfD)/Benchmark Dose (BMD)

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ABSTRACT

Current methods to estimate noncancer health risk are limited. Situations exist where subthreshold doses such as reference doses (RfDs) or reference concentrations (RfCs) are exceeded and little is known about the possible health risk. A recent model indicates that toxicity data viewed as categories of pathology has potential for exploring such risk. What appear to be reasonable estimates of risk above the RfC are found with toxicity data for manganese.

This model is also compared to another new approach — the benchmark dose (BMD) for several chemicals. Differences between these two approaches are briefly discussed.

INTRODUCTION

The RfD and RfC have been the mainstay of noncancer risk assessment in the U.S. Environmental Protection Agency (EPA) for several years. The RfD and RfC are defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily (for RfD) or continuous (for RfC) exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

As displayed in Figure 24, much interest exists in the estimation of health risk above some level, such as an RfD or RfC. Little progress has been made, however, primarily due to the multiplicity of effects, the changing severity and intensity of individual effects as dose increases, and the lack of mathematical tools.

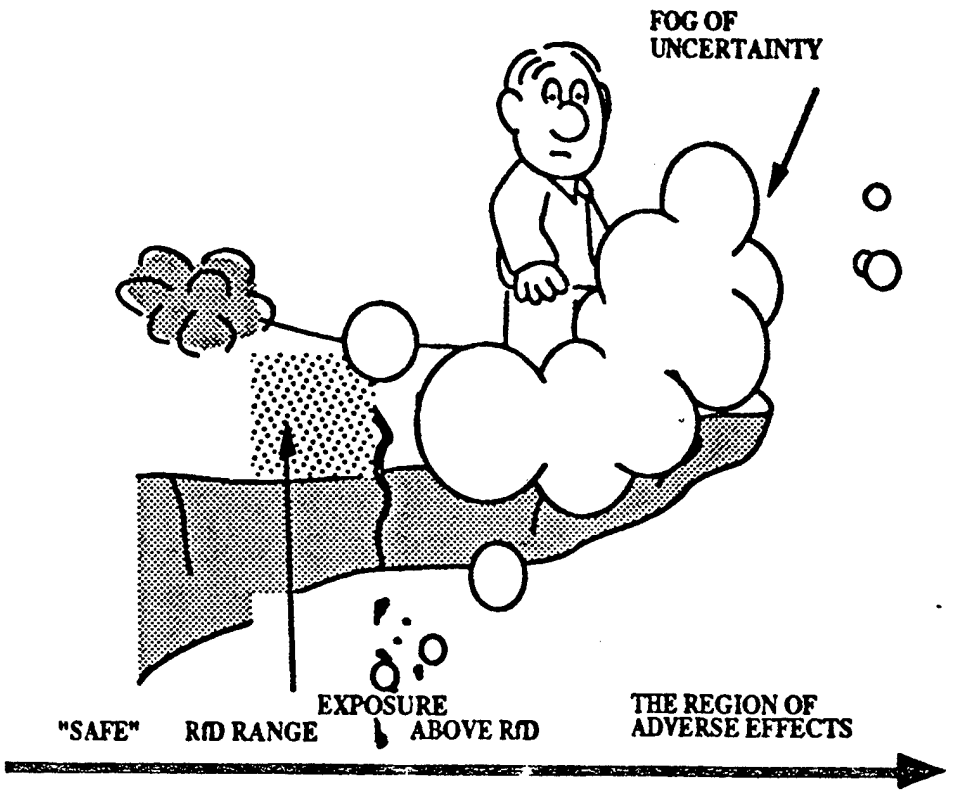


Figure 24. **Much Interest Exists in the Risk Between the RfD or RfC and the Toxicity Data.** To date, few methods have been able to address this problem due to a number of uncertainties.

The purpose of this manuscript is to present and analyze toxicity data for manganese with a method of Hertzberg (1) that addresses some of these issues. Risks above the RfD or RfC are estimated with this model.

In addition, recent interest has been expressed in replacing the no-observable-adverse-effect level (NOAEL) with a benchmark dose (BMD): a statistically derived lower confidence limit on a dose associated with a specified level of excess risk such as 1, 5, or 10%. The arguments favoring the BMD are that statistical models can be used, and that a consistent interpretation can be made of the BMD across studies and across chemicals. We show briefly in this paper, and more extensively elsewhere (2), that the BMD, although an improvement in some respects to the NOAEL, still leaves several issues unresolved.

METHODS

We reviewed inhalation toxicity data for manganese and judged exposure or dose groups as one of four very broad categories of toxicity; either no-observed-effect level (NOEL), NOAEL, adverse-effect level (AEL), or frank-effect level (FEL). We regressed these ordered categories against both concentration (or dose) and exposure duration using a logit model. Ordered regression obviates pathologic "distances" among categories.

Based on an analysis of all data as shown schematically in Figure 25 for one study, it is possible to determine the probability of NOEL, NOAEL, lowest-observed-adverse-effect level, and FEL for a given chemical. This is shown hypothetically in Figure 26. In mathematical terms, categorical regression can be seen as follows:

RfD Definition	Regression Model
"is likely to be"	$P(*) > 0.95$
"without appreciable risk"	$r < 10^{-2}$
"deleterious effect"	toxicity category = moderate or lethal adverse effect

This leads to a new RfD definition: $P(r < 10^{-2} | dose < RfD > 0.95$, where r = P (severity > 1). We selected 10^{-2} risk and the 95% confidence level only to illustrate the method. Standard values for these decision criteria have not been adopted by EPA. The value 10^{-2} is a more realistic goal than the 10^{-6} risk often used for carcinogenic risk because most of the noncancer effects we consider are sublethal, and many are reversible.

RESULTS

Toxicity data for manganese were excerpted from available literature. These were available incidence data from human studies as shown in Table 30. The resulting probability statements for manganese are interpretable as human incidence for either an adverse effect (e.g., finger tremor) or frank effect (e.g., disturbed gait) as shown in Figure 27. For most published toxicity studies, effects are noted only for the dose group, so the probability statements are likewise interpretable only at the dose group level (i.e., the probability that a dose group will have the effect).

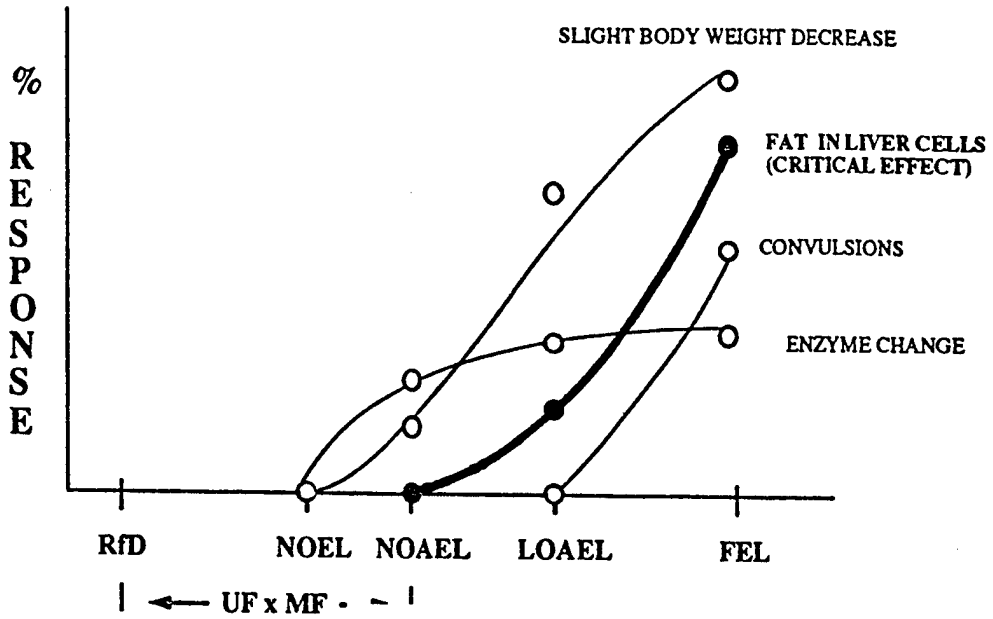


Figure 25. Typical Judgments on Doses Used to Determine RfDs in a Hypothetical Study.

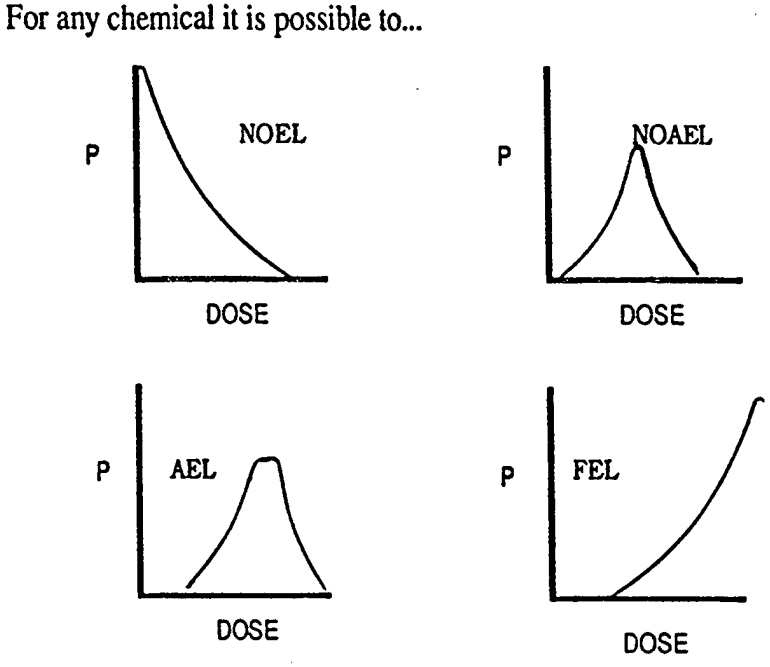


Figure 26. Probabilities of Various Effect or No-Effect Levels with Dose Based on a Review of all Data.

TABLE 30. TOXICITY DATA ON MANGANESE IN HUMANS USED IN THE CATEGORICAL REGRESSION

Geometric Mean Exposure (mg/m^3)	Arithmetic Mean Duration (days)	Effect	Number of People	Incidence[a]			Study
				NOEL	AEL	FEL	
0.00006	3464	None	204	1.00	0.00	0.00	Saric et al., 1977
0.11	7370	CNS, pulmonary	20	0.75	0.25	0.00	Chandra et al., 1981
0.20	7670	CNS, pulmonary	20	0.50[b]	0.50[b]	0.00	Chandra et al., 1981
0.25	4015	None	66	1.00	0.00	0.00	Saric et al., 1977
0.35	2592	CNS, pulmonary	141	0.85	0.15	0.00	Roels et al., 1987
0.63	5150	CNS, pulmonary	20	0.60	0.40	0.00	Chandra et al., 1981
1.8	2980	CNS	83	0.44[b]	0.45[b]	0.11	Schuler et al., 1957
3.2	7300	CNS	1	0.00	0.00	1.00	Saric et al., 1977
3.7	4015	CNS	17	0.82	0.18	0.00	Saric et al., 1977
4.8	6205	CNS	71	0.46[b]	0.47[b]	0.07	Smyth et al., 1973
6.0	3100	CNS	36	0.41[b]	0.42[b]	0.17	Emara et al., 1971
6.6	4015	CNS	18	0.72	0.28	0.00	Saric et al., 1977
19.0	730	CNS	34	0.38[b]	0.38[b]	0.24	Flinn et al., 1941

[a] See Table 30 and text for explanation of severity codes. Incidences are based on judgment of effects reported and should be considered approximate.

[b] This study did not clearly distinguish the incidence of NOEL for AEL. As a conservative assumption, these incidences were considered equivalent.

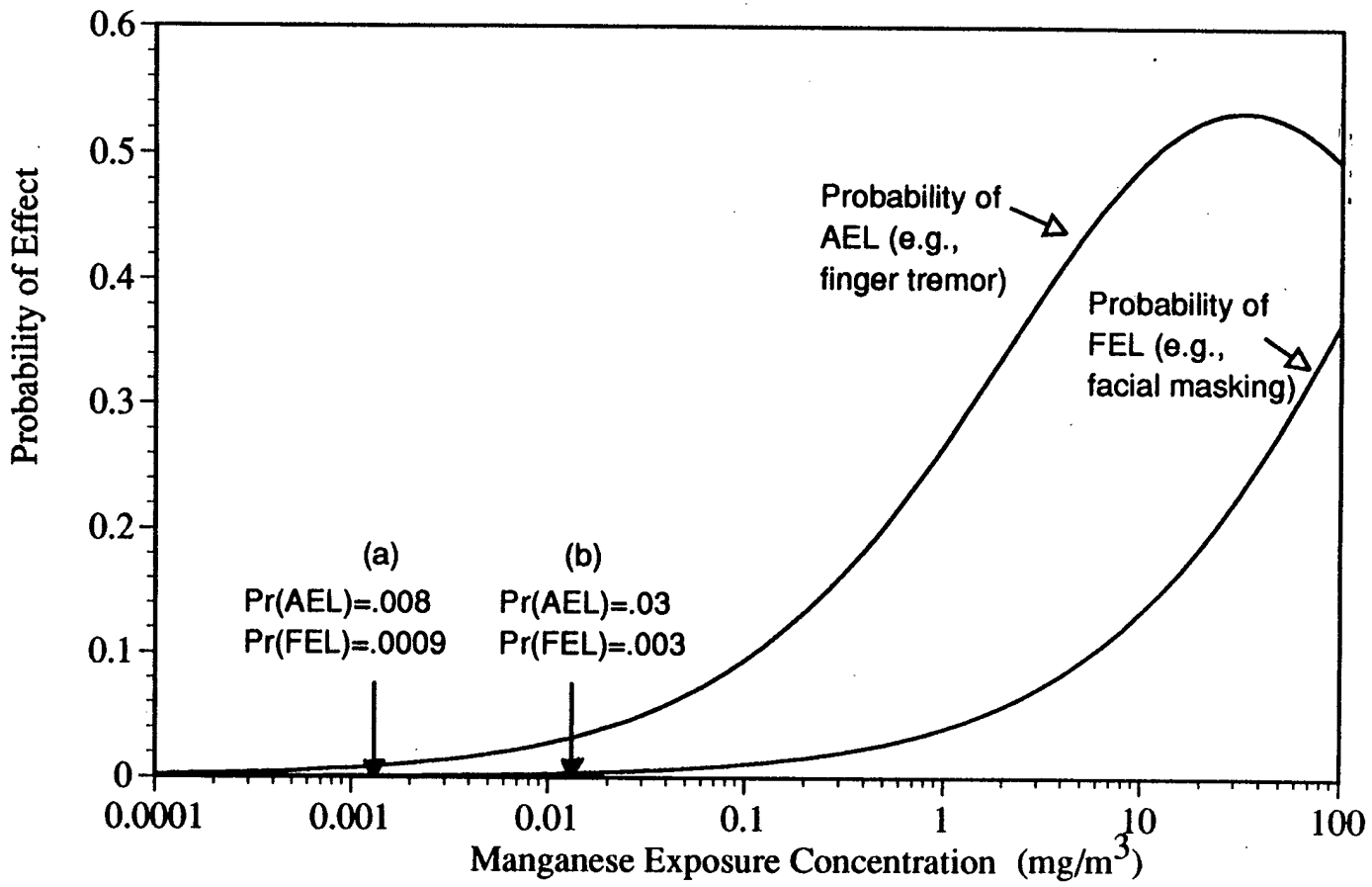


Figure 27. **Probability of Either an AEL or FEL at Increasing Manganese Concentrations.** Data are from Table 30. Designated doses "a" and "b" and associated probabilities are either at 3-fold or 30-fold of the RfC of $4x10^{-4}$ mg/m^3. See text for further discussion.

DISCUSSION

The categorical regression model described by Hertzberg and Miller (3) and later papers (Hertzberg,[4,5] Hertzberg and Wymer,[6]; Guth et al.,[7]; Farland and Dourson,[8]) is one approach that incorporates judgments of toxicity along with response rate into a statistical characterization of the overall exposure-response relationship. The model can be used to estimate a BMD, or can be used to estimate toxicity risk at any exposure level. The "risk" is no longer a single number, but a vector of numbers, one for each category.

The categorical regression approach has two distinct advantages over the NOAEL-RfD and BMD-RfD procedures described above. First, the approach is easily adapted to most types of toxicity data, from judgments of overall severity of toxic effect for the dose group to measured responses on each individual. Second, all relevant toxicity data are included in the regression. Third, a consequence of the second advantage, the goal of this approach is highly consistent with that of the NOAEL-RfD method: to produce regulatory information that incorporates all toxic effects. In particular, an exposure level can be estimated by regression that is quite similar in interpretation to the NOAEL-based RfD. Fourth, the judgmental step involves evaluation of overall toxic impact on the exposed individual, allowing comparison across target organs, and across chemicals when several organs are affected.

However, the probabilities generated by categorical regression are usually limited to whether or not a dose or exposure group, and not an individual, is at risk. When incidence data are used in the analysis (such as for manganese shown here), actual population risk estimates are possible.

Perhaps the greatest advantage of categorical regression is that this method can compare the likely health risk above the RfD or RfC for several chemicals. In risk management decisions, such comparison is often necessary. Figure 28 demonstrates this concept hypothetically.

DISCLAIMER

Although the research (or other work) described in this article has been funded wholly or in part by the U.S. EPA, it has not been subjected to the Agency's required peer and administrative review and, therefore, does not necessarily reflect the view of the Agency. No official endorsement should be inferred.

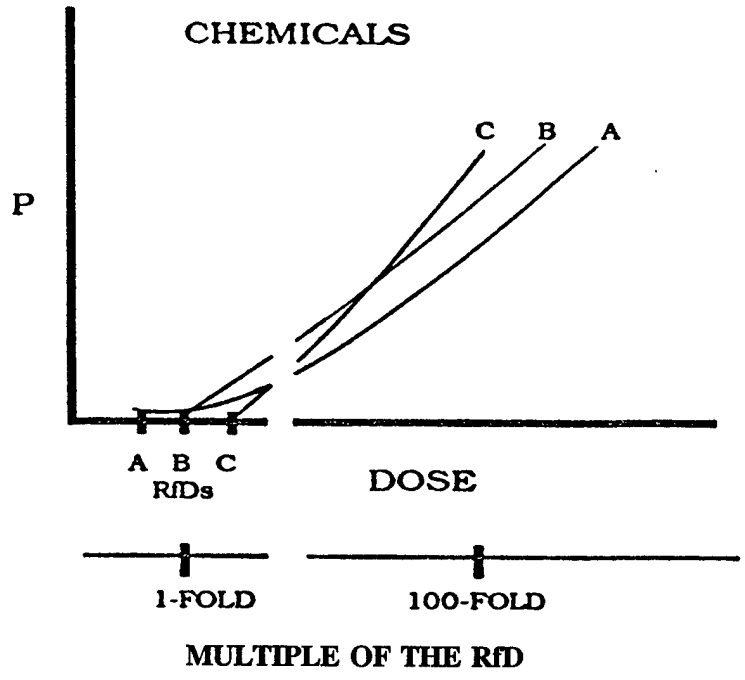


Figure 28. For Multiple Chemicals, It Is Thus Possible To Compare the Risk of Unacceptable Effect at Existing Exposures Above the RfD or RfC.

REFERENCES

1. R.C. Hertzberg, "Fitting a Model to Categorical Response Data with Application to Special Extrapolation to Toxicity," *Health Phys.* **57** (Suppl. 1), 404-409 (1989).

2. R.C. Hertzberg and M.L. Dourson, "Using Categorical Regression Instead of a NOAEL to Characterize a Toxicologist's Judgment in Noncancer Risk Assessment," (submitted).

3. R.C. Hertzberg and M. Miller, "A Statistical Model for Species Extrapolation Using Categorical Response Data," *Toxicol. Ind. Health* **1**, 43-57 (1985).

4. R.C. Hertzberg, "Studies on Toxicity Applicable to Risk Assessment (STARA) User's Guide," Quantitative Toxicity Data and Graphics on Environmental Chemicals, (Environmental Criteria and Assessment Office, U.S. EPA, Cincinnati, OH, February 1988).

5. R.C. Hertzberg, "Studies on Toxicity of Mixtures and Interacting Chemicals User's Guide (MIXTOX) (Available on diskette from U.S. EPA Environmental Criteria and Assessment Office, Cincinnati, OH 45268, 1992).

6. R.C. Hertzberg and L. Wymer, "Modeling the Severity of Toxic Effects," (Proceedings papers from the 84th Annual Meeting and Exhibition of the Air and Waste Management Association, Vancouver, British Columbia, June 16-21, 1991).

7. D.J. Guth, A.M. Jarabek, L. Wymer, and R.C. Hetzberg, "Evaluation of Risk Assessment Methods For Short-Term Inhalation Exposure," Presentation at the 84th Annual Meeting of the Air and Waste Management Association, (Vancouver, British Columbia, June 16-21, 1991).

8. W. Farland and M.L. Dourson. "Noncancer Health Endpoints: Approaches to Quantitative Risk Assessment," in C. R. Cothern (ed.), *Comparative Environmental Risk Assessment,* (Lewis Publishers, Boca Raton, FL, 1992), pp. 87-106.

Defining the "Reference" in the Reference Dose

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ABSTRACT

The U.S. Environmental Protection Agency has established the reference dose (RfD) procedure as a primary method for human health risk assessment for noncancer end points. United States Environmental Protection Agency guidelines for the development of RfDs dictate the use of uncertainty factors (UFs) when the experimental database is less than optimal. Five areas of uncertainty have been defined, with a possible combination of four UFs for any given RfD. Each of these UFs can be thought of as a probability density function (PDF) that must be combined with one another to yield an overall PDF for each RfD. Previous work by the U.S. EPA has focused on establishing expected values and limits on these PDFs. The approach has been to determine the distribution of ratios of fixed experimental dose levels. Consequently, the ratios have exhibited pronounced "spikes," precluding traditional statistical treatment for the most part. Additionally, data supporting several of the UFs are sparse or absent, entailing the *a priori* assignment of distributions. The assignment and combination of these distributions in an overall PDF for the RfD will be illustrated. Biological and statistical issues pertaining to the definition, use and interpretation of such distributions will be discussed.

Combination of Cancer Data in Quantitative Risk Assessments: Case Study Using Bromodichloromethane

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ABSTRACT

There are often several datasets that may be used in developing a quantitative risk estimate for a carcinogen. These estimates are usually based, however, on the dose–response data for tumor incidences from a single sex/strain/species of animal. When appropriate, the use of more data should result in a higher level of confidence in the risk estimate. The decision to use more than one dataset (representing, for example, different animal sexes, strains, species, or tumor sites) can be made following biological and statistical analyses of the compatibility of these datasets. Biological analysis involves consideration of factors such as the relevance of the animal models; study design and execution; dose selection and route of administration, the mechanism of action of the agent, its pharmacokinetics, any species- and/or sex-specific effects, and tumor site specificity. If the biological analysis does not prohibit combining datasets, statistical compatibility of the datasets is then investigated. A generalized likelihood ratio test is proposed for determining the compatibility of different datasets with respect to a common dose–response model, such as the linearized multistage model. The biological and statistical factors influencing the decision to combine datasets are described, followed by a case-study of bromodichloromethane.

INTRODUCTION

The estimation of the carcinogenic hazard posed to humans by a chemical involves a great deal of scientific judgement. Uncertainty is inherent in cancer risk assessments, particularly those developed from animal data, because assumptions must be made in areas for which data are scarce. These include, for example, appropriate transformations for extrapolation of dose from test animals to humans and for high to low doses, and the assumption that the same biological processes leading to cancer in laboratory

animals are operative in humans as well. Statistical uncertainty is also inherent in the use of sample data to derive inferences about a large population.

A cancer bioassay often generates more than one dataset, demonstrating a statistically significant positive tumorigenic response (i.e., by pairwise comparisons with controls or by a trend analysis). The term "dataset" is defined here as the tumor incidence data for a single anatomical site or combination of sites within a single sex/strain/species of animal. It generally is not known whether one particular animal model is more appropriate for extrapolation to humans than another. When the differences in animal sensitivities are small (as measured by the quantitative estimates derived from the data), or when the estimates incorporate large uncertainties, it could be argued that a more reasonable approach would be to derive a risk estimate using more of the available data, rather than just one dataset. A combination of data may result in a higher level of associated confidence or in a risk assessment with improved statistical properties.

The use of more of the available information is complicated by both biological and statistical issues. The available carcinogenicity data for a specific chemical rarely originate from replicate studies; rather, they come from studies using different sexes, strains, or species of animals in which the responses depend on a variety of biological factors. Differences in study design and execution may further complicate the issue of combining data from different sources.

APPROACHES TO COMBINING CARCINOGENICITY INFORMATION

Several methods could be applied to utilize more of the available carcinogenicity information: the choice of a risk estimate derived from a single dataset, with additional risk estimates from other datasets used as corroboration for the chosen value; the use of some average value (e.g., a geometric mean) of risk estimates derived from different datasets; or the combination of individual datasets prior to the calculation of a risk estimate.

Assuming that the datasets represent samples from the same population, then the statistically preferred approach is to combine the datasets prior to calculating the quantitative estimate of cancer potency (e.g., the 95% upper confidence limit on the slope of the animal dose–response curve at low doses). In general, a large dataset will yield tighter confidence limits than a small dataset. Thus, averaging two or more quantitative estimates, each based on a small dataset, will incorporate small

sample uncertainty. In contrast, the combination of datasets prior to deriving a quantitative estimate decreases this uncertainty and better defines the confidence limits.

The combining approach described here requires that the responses being considered for combination be carefully evaluated with respect to their biological similarities and differences to judge whether the datasets can be assumed to represent samples from the same population. Examination of the biological basis for combination is used in conjunction with a statistical analysis based on a likelihood ratio test to evaluate the compatibility of two or more datasets with the same model. If two datasets are determined not to be statistically compatible, this may suggest underlying biological differences.

BIOLOGICAL CRITERIA FOR COMBINING CARCINOGENICITY DATA

The criteria for determining the biologic compatibility of multiple datasets are discussed here as issues relating to two broad categories: (1) study quality; or (2) the mechanism of action or pharmacokinetic disposition of the carcinogen. Study quality factors include elements determined by the design of the study that may affect the biological processes involved in carcinogenesis (e.g., dosing regimen, duration of exposure) as well as factors contributing to the overall adequacy of the study (e.g., number of animals and adequate survival). Other questions pertain directly to the assessment of how the chemical induces cancer, such as genotoxicity, or to species, strain, or sex differences in pharmacokinetics. Several qualitative indicators of differential sensitivity to a carcinogen (e.g., differences in latency, degree of malignancy) also may be important. These considerations have been presented elsewhere in the form of decision trees that can be used in the determination of whether to combine data (1).

STATISTICAL CRITERIA FOR COMBINING CARCINOGENICITY DATA

The traditional interpretation of a cancer bioassay, in which tumor development may be considered a dichotomous response, is to express the number of exposed animals developing cancer using the binomial probability distribution:

$$f(x) = \binom{N}{x} P^x (1-P)^{N-x} \quad for\ x = 0,1,2,\cdots,N \qquad (1)$$

where N is the number of animals tested, X is the number of animals with tumors, and P is the probability of an animal developing cancer. By substituting the sample values of X and N from a

bioassay into Eq.(1), the unknown value of P can be estimated. This value of P maximizes Eq.(1) and corresponds to the highest probability of observing that particular value of X. As a result, the probability of observing any number of animals with cancer can be computed, so that Eq.(1) also represents a likelihood function for the parameter P. For an experiment having k groups (including a control group and k-1 dose groups), the overall likelihood function can be expressed as:

$$L = \prod_{i=1}^{k} \binom{N_i}{X_i} P_i^{X_i} (1-P_i)^{N_i-X_i} \quad . \tag{2}$$

where the data points X_i and N_i represent the total number of animals with tumors, and the number tested in the ith group, respectively.

An alternate interpretation of a cancer bioassay, however, is to consider the individual animal data, indicating a positive or negative carcinogenic response for each animal, rather than for each dose group. Given the dichotomous nature of the response, and assuming that the animals respond independently, the likelihood function is then expressed as a product of Bernoulli distributions, which, except for the exclusion of the combinatoric term, is equivalent to the binomial-based likelihood:

$$L = \prod_{i=1}^{k} P_i^{X_i} (1-P_i)^{N_i-X_i} \quad . \tag{3}$$

The likelihood function expressed in Eq.(3) is simply a product of terms – one for each animal in the experiment. Each tumor-bearing animal contributes a quantity P_i to the likelihood and each nonresponder contributes a term $(1-P_i)$, where P_i is the probability (unknown) of developing cancer for the ith group to which the animal belongs. Using this interpretation, the likelihood function can be specified for combined datasets from two separate studies by combining the individual likelihood functions. The numerical solution to the value of the P_i terms is equivalent for both the binomial and Bernoulli problems.

The problem remains, then, of relating the dose of the carcinogen to the likelihood function. This may be expressed by assuming that the data follow a particular dose–response model that restricts the P_i terms to functions that include a measure of dose. For this application, the linearized multistage (LMS) model is used, but it is noted that other models also may be used. The general form of the LMS model is:

$$P(d) = 1 - \exp[-(\theta_0 + \theta_1 d + \cdots + \theta_s d^s)] . \quad (4)$$

where s is the number of stages, d is dose, and $\Theta' = (\Theta_0, \Theta_1, \ldots, \Theta_s)$ is an unknown model parameter to be estimated from the data.

The compatibility of two or more datasets with a common dose–response model could be determined by testing the following null hypothesis against its alternative:

H_0: The datasets are compatible with a common model,

H_1: The datasets are not compatible with a common model.

To test the null hypothesis, the method of maximum likelihood can be used to estimate the model parameters. If two or more datasets are combined, the resulting likelihood function would be equal to the product of the likelihood functions for the individual datasets:

$$L_T = L_1 \cdot L_2 \quad (5)$$

If each of the individual terms is maximized, then it follows that the product (i.e., the overall likelihood function) is maximized. The joint likelihood L_T can be maximized under either of two assumptions: it can be assumed that the two datasets can be fit to a common dose–response model (H_0); or it can be assumed that the two datasets fit different dose–response models (H_0 U H_1). In order to test H_0, a generalized likelihood ratio test can be conducted by comparing the ratio of the two maxima under the two assumptions:

$$\Lambda = \frac{\max L(H_0)}{\max L(H_0 \cup H_1)} , \quad (6)$$

Under H_0, -2ln Λ has an asymptotic chi-square distribution with one degree of freedom (2). The single degree of freedom for the test results from the fact that H_0 imposes one constraint on the parameter space (2,3). The test, then, is performed by comparing -2ln Λ = 2[max ln L(H_0 U H_1) - max ln L(H_0)] with the tabulated chi-square at a chosen level of significance.

H_0 is rejected when there is a significant difference (p-value $\leq$ chosen significance level) between the values of the joint likelihood functions under the two assumptions defined by H_0 and H_1. In contrast, H_0 is accepted when there is little difference (p-value $>$ chosen significance level) between the maxima

of the joint likelihood functions under these two assumptions. The statistical theory supporting this research is described in more detail elsewhere (4).

CASE STUDY: BROMODICHLOROMETHANE

Bromodichloromethane (BDCM) is a volatile trihalomethane formed when chlorine interacts with organic constituents in water. Several studies have suggested a possible association between water chlorination and cancer incidence, particularly bladder, colon, and rectal cancers (reviewed in 5,6). There are no epidemiological studies of BDCM intake alone, and the intake of chlorinated water involves exposure to a mixture of compounds, including the trihalomethanes.

Several animal studies have been performed to evaluate whether chronic oral exposure to BDCM can induce tumors. Those that did not induce a statistically elevated tumor incidence are not discussed here (see 5 for review).

In a bioassay by the National Toxicology Program (NTP), Fischer 344 (F-344)/N rats (50/sex/dose) were administered 0, 50, or 100 mg/kg/day BDCM (99% pure) in corn oil by gavage for 102 weeks (7). Groups of 50 B6C3F1 mice received 0, 25, or 50 mg/kg/day (males) or 0, 75, or 150 mg/kg/day (females) BDCM by gavage in corn oil for 102 weeks. The study in male rats was restarted because of decreased survival of the vehicle controls after 10.5 months due to excessive room temperature. Several sites showing a statistically significant increased tumor incidence in treated groups relative to controls or a statistically significant dose-related increase in incidence (positive linear trend) were reported: large intestine adenomatous polyp/adenocarcinoma and kidney tubular cell adenoma/adenocarcinoma in both sexes of rats; hepatocellular adenomas/adenocarcinomas in female mice; and kidney tubular cell adenoma/adenocarcinomas in male mice. The tumor incidences for these sites are shown in Tables 31, 32, and 33. The low historical control incidence for the tumor types seen at statistically significant increased incidence in this study indicate that these tumors are uncommon and biologically important, thereby constituting datasets suitable for calculation of a risk estimate (8).

TABLE 31. BROMODICHLOROMETHANE LARGE INTESTINE AND RENAL TUMOR INCIDENCE IN F-344/N RATS[1]

Human Equivalent Dose[2] (mg/kg)/day	Combined Adenomatous Polyp/ Adenocarcinoma		Combined Tubular Cell Adenoma/ Carcinoma	
	Male	Female	Male	Female
0.0	0/50	0/46	0/50	0/50
5.8	-	0/50	-	1/50
6.8	13/49	-	1/49	-
10.9	-	12/47	-	15/50
13.0	45/50	-	13/50	-

[1] Adapted from NTP (4). Animals that did not have the tissue microscopically examined or that died before the appearance of the first tumor were excluded from the denominator.

[2] Based on surface area adjustment (i.e., body weight[2/3]).

TABLE 32. BROMODICHLOROMETHANE LARGE INTESTINE/RENAL TUMOR INCIDENCE (COMBINED) IN F-344/N RATS[1]

Human Equivalent Dose[2] (mg/kg)/day	Male	Female
0.0	0/50	0/46
5.8	-	1/50
6.8	13/49	-
10.9	-	24/48
13.0	46/50	-

[1] Adapted from NTP (4). One high-dose female that was not examined for intestinal tumors had kidney tumors.

[2] Based on surface area adjustment (i.e., body weight[2/3]).

TABLE 33. BROMODICHLOROMETHANE TUMOR INCIDENCE IN B6C3F1 MICE[1]

Human Equivalent Dose[2] (mg/kg)/day	Combined Hepatocellular Adenoma/Carcinoma in Females	Combined Renal Tubular Adenoma/Carcinoma in Males
0.0	3/50	1/46
1.5	-	2/49
3.0	-	9/50
4.2	18/48	-
8.1	29/50	-

[1] Adapted from NTP (4). Animals that escaped or died during Weeks 1 and 9 were excluded.
[2] Based on surface area adjustment (i.e., body weight[2/3]).

Because all the adequate datasets are from the same bioassay, issues related to study quality are minimal. The route of exposure for both sexes of both species was gavage in corn oil at similar dose rates and dose volumes for the lifetime of the animals. Appropriate controls were employed; complete histology was performed; and, with the exception of the female mice, survival was comparable among treated and control groups. Although statistically significant, decreased survival in the female mice was primarily attributable to ovarian abscesses, and does not compromise the hepatocellular tumor dataset for use in quantitative risk estimation. The maximum tolerated dose (MTD) was reached in rats at the high dose as evidenced by decreased body weight and liver and kidney lesions. Similarly, histological findings in the kidney, liver, thyroid, and testis of the low-dose male mice and decreased body weight and thyroid hyperplasia in high-dose female mice suggest the MTD was reached.

Concerns about the use of corn oil as a vehicle in studies of trihalomethanes have been raised (9,10). For this reason, the Science Advisory Board of the United States Environmental Protection Agency (EPA) recommended that the mouse hepatocellular carcinomas not be used in quantitative risk assessment (11).

Limited data are available on the metabolism and pharmacokinetics of BDCM, limiting comparisons between sexes or species of animals. Much of the information for BDCM has been inferred from data on a more studied trihalomethane, chloroform. *In vivo* and *in vitro* studies with the trihalomethanes demonstrate that there are two primary routes of metabolism, oxidative and reductive (reviewed in 5). Thorton-Manning *et al.* (12) recently demonstrated that cytochrome P450 enzymes are

involved in the metabolism of BDCM. The species differences in P450-mediated metabolism may, in part, be responsible for the apparent greater sensitivity of mice to the carcinogenic action of BDCM. Mice have a greater capacity than rats to metabolize trihalomethanes, by both the oxidative pathway (13) and the reductive pathway (14,15). Mink *et al.* (13) administered single oral doses of ^{14}C-labeled BDCM to rats (100 mg/kg) and mice (150 mg/kg). In rats, 14% of the radiolabel was expired as CO_2, and 42% as the parent compound. In mice, 81% was expired as CO_2, and only 7% as the parent compound. Qualitative and quantitative differences in metabolism also have been reported for other halogenated hydrocarbons, all of which are expected to undergo similar metabolic processes. For chloroform-induced renal toxicity, mice have been shown to be more sensitive than rats (16,17); and male mice have been shown to be more sensitive than female mice (18). It has been suggested that human metabolism of halogenated hydrocarbons is more similar to rats than mice (19).

Although the greater metabolism of halogenated hydrocarbons by mice may partially explain the greater sensitivity of mice in the NTP bioassay, strain-specific factors also may be involved. In a two-year drinking water bioassay of BDCM in CBA x C57Bl/6 mice, doses up to 76 mg/kg/day did not result in any increased tumor incidences (20). This difference between the tumor response in mice and rats in the NTP bioassay may be a result of differences in metabolism, strain-specific (e.g., genetic) susceptibility, or a vehicle-related effect (i.e., water versus corn oil).

Bromodichloromethane is considered by the EPA to have genotoxic potential (5). Conflicting results in *in vivo* and *in vitro* test systems have been attributed to inadequacies or variation in experimental protocols, such as difficulty in achieving sufficient exposure to volatile BDCM and different metabolic capability of various cell types (7; reviewed in 5). Based on the premise that BDCM is most likely to act by a genotoxic mechanism, and that similar genetic processes form the basis for carcinogenicity, all datasets, with the exception of female mouse liver tumors, thus far qualify as the basis for a quantitative estimate of risk.

Renal tubular hyperplasia was reported in both sexes of rats. In addition, renal cytomegaly was noted in male rats and male mice at the high-dose levels (7). These findings suggest that an epigenetic mechanism, such as regenerative hyperplasia, also may play a role in carcinogenic activity of BDCM at this site, although the data are insufficient to establish such a role. Reitz *et al.* (21) suggested this mechanism for chloroform carcinogenesis. The NTP reported that hyaline droplet formation in the

kidney was not seen in the male rats; therefore, it appears unlikely that α_{2u}-globulin nephropathy (involving a mechanism not relevant to human cancer) contributes to the formation of these tumors. Hyperplasia was not reported in the large intestine of either sex or species (7).

It is difficult to quantitatively assess the role differential pharmacokinetics among the sexes or species may have on the dose term for BDCM in these datasets. The interaction of the vehicle (corn oil) with BDCM also may be a determinant in site-specificity or sensitivity, although the Science Advisory Board of the EPA did not consider a vehicle effect likely in the development of the renal or large intestine tumors (11). In the absence of additional data, there do not appear to be radically different processes occurring at the different sites or between the different sexes or species that would discourage combining across the sites/sexes/species. Therefore, datasets that can be considered for the calculation of a quantitative risk estimate BDCM include the following tumor types: male mouse kidney, male and female rat kidney, male and female rat large intestine, and combinations thereof (see Table 34).

TABLE 34. BROMODICHLOROMETHANE LIKELIHOOD RATIO TESTS

Datasets	Potency Estimate[1]	p value	Compatible?
♂ Mouse renal	6.2E-2	----	----
♀ Rat renal	9.5E-3	----	----
♂ Rat renal	8.5E-3	----	----
♀ Rat GI/renal	1.0E-2	----	----
♂ Rat GI/renal	2.5E-2	----	----
♂ + ♀ Rat GI/renal	----	<0.001	No
♂ + ♀ Rat renal	6.3E-3	0.067	Yes
♂ + ♀ Rat renal and ♂ Mouse renal	----	<0.001	No

[1]The 95% upper confidence limit on the slope of the dose–response curve at low doses; also referred to as a q_1^* or a slope factor.

The linearized multistage procedure was used to model these datasets, and the likelihood ratio test was applied to evaluate the compatibility of the combined dataset of both sexes as described above. Table 34 shows that the datasets for the combined kidney and large intestine tumors in male and female rats are not statistically compatible with the same multistage model. These results suggest that some of the factors influencing the dose–response relationship are not evident from the available information. Reexamination of the large intestine and renal tumor incidence shows that large intestine tumors occur

at the low-dose in the male rats but not in the female rats. Thus, the incidence of large intestine and kidney tumors in the males is comprised primarily of large intestine tumors, whereas kidney and large intestine tumors contribute equally to the combined incidence in the females. The disparate responses observed across sexes could occur for several reasons, including differential pharmacokinetics, tissue sensitivities, or metabolic pathways (e.g., oxidative in the kidney and reductive in the intestine), although further research is needed to clarify the underlying biological basis.

The observation of renal tubular cell adenomas and adenocarcinomas in both sexes of rats and the male mice may suggest that a similar mechanism of action may be operating in the two species at this site. The renal tumors in mice occurred at lower administered dose levels than the tumors in rats, suggesting the mice may be "more sensitive" than the rats, perhaps a reflection of the increased or differential metabolism in mice. Moore *et al.* (22) showed that nephrotoxicity also occurs to a greater extent in mice than in rats when BDCM was administered in a subchronic drinking water study. Biological similarity of the tumor type may favor combining datasets across both sexes of rats and possibly across both rats and mice. The latter options have the advantage of increasing the number of dose groups to five and seven, respectively.

The results of the statistical analysis show that the male and female rat kidney tumor data are compatible with the same multistage model (Table 34). The potency estimate of the combined dataset of male and female kidney tumors is slightly lower (6.5E-3 per [mg/kg]/day) than either estimate derived from the two individual datasets (9.5E-3 for females and 8.5E-3 per [mg/kg]/day for males) because the small sample size variability affecting the confidence limits has been decreased. When these combined data are tested with the male mice kidney data, however, the likelihood ratio test indicates that these datasets are not compatible with the same multistage model, suggesting the possibility of underlying biological differences.

The use of a pharmacokinetic model to account for the greater metabolic capacity of mice for halogenated hydrocarbons would allow for a reevaluation of the likelihood ratio test for the rat and mouse kidney tumor datasets using the target organ doses as the common denominator. The results of such an analysis may indicate whether differences in metabolism are responsible for the greater tumorigenic response in mice, or whether other factors also play a significant role. If available, metabolic information

in humans can then be factored into this assessment. Ultimately, the result will be a risk characterization with more emphasis on biological mechanisms and relevance to the carcinogenic process in humans.

DISCUSSION

Our confidence in quantitative cancer risk assessments may be increased by the use of as many of the available data as possible. A case study on BDCM illustrated many of the biological and statistical issues that must be considered in combining multiple datasets used to calculate a cancer risk estimate. A more comprehensive discussion of these issues has been presented elsewhere (1,4). In determining the most suitable basis for estimating a cancer potency estimate for BDCM, the NTP bioassay provides several datasets for consideration. In this experiment, BDCM was administered by gavage to rats and mice, with a positive tumor response observed for the large intestine and kidney of male and female rats, liver tumors in female mice, and kidney tumors in male mice. A study design issue (i.e., use of corn oil gavage) precluded use of the liver tumors. The remaining datasets seemed plausible candidates for combination, and statistical compatibility was subsequently analyzed. Individual risk estimates derived from five different datasets (involving mouse and rat kidney and rat gastrointestinal tract) are shown in Table 4 to be within one order of magnitude. Application of the likelihood ratio test demonstrates that the male and female rat kidney data are statistically compatible with the same model. A further attempt to combine these with the male mouse kidney data, which provide the highest risk estimate, reveals that the mouse data are not statistically compatible with the rat data from the same tumor site.

The results of this research provide the risk manager with two reasonable options for estimating a cancer potency factor for BDCM: (1) using the male mouse kidney tumor data, which results in the highest risk estimate; or (2) using the combined male and female rat kidney tumor data, which is supported by data from more dose groups and therefore incorporates less of the uncertainty inherently associated with small sample size. As a regulatory Agency charged with the protection of human health, the EPA has generally opted to use the dataset that results in the highest risk estimate, unless relevance to humans can be disproved. Although a quantitative assessment of species-specific differences in the metabolism of BDCM is not yet possible, there are data to suggest that humans may be more similar to rats than mice. Together with the larger database provided by the rat kidney tumor incidence, this provides a basis for the risk manager to decide to use the rat data in calculating a cancer potency factor.

This research demonstrates a systematic approach for considering the biological and statistical issues involved in combining datasets in quantitative cancer risk assessments. The limited amount of data precludes a definitive answer for this case study; however, when additional pharmacokinetic data become available, the compatibility of the mouse and rat tumor incidences can be determined based on target organ doses (e.g., kidney) of the active metabolites. Such an analysis would indicate whether the difference in tumor responses is a result of differential metabolism, or whether other factors also play significant roles. As more of this type of information becomes available, and as physiologically based pharmacokinetic models are generated for cross-species comparisons, the use of the likelihood ratio test for determining the compatibility of tumor incidence datasets will gain greater utility.

REFERENCES

1. S.T. Vater, P.M. McGinnis, R.S. Schoeny, and S.F. Velazquez, "Biological Considerations for Combining Carcinogenicity Data for Quantitative Risk Assessment," *Reg. Toxicol. Pharmacol.*, In Press (1993).

2. B.W. Lindgren, *Statistical Theory*, 3rd ed. (MacMillan Publishing Co., Inc. New York, NY, 1976).

3. C.R. Rao, *Linear Statistical Inference and Its Applications* (John Wiley & Sons, Inc., New York, NY, 1965).

4. W.M. Stiteler, L.A. Knauf, R.C. Hertzberg, and R.S. Schoeny, "A Statistical Test of Compatibility of Datasets to a Common Dose Response Model," *Reg. Toxicol. Pharmacol.*, In Press (1993).

5. U.S. Environmental Protection Agency, "Drinking Water Criteria Document for Trihalomethanes. Revised External Review Draft," (Office of Science and Technology, Washington, DC, 1992).

6. U.S. Environmental Protection Agency, "Integrated Risk Information System, Online," (Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, 1993).

7. National Toxicology Program, "Toxicology and Carcinogenesis Studies of Tribromomethane (Bromoform) in F344/N Rats and B6C3F1 Mice (Gavage Studies)," (U.S. Department of Health and Human Services, Research Triangle Park, NC, Technical Report Series No. 321, 1987).

8. U.S. Environmental Protection Agency, "Guidelines for Carcinogen Risk Assessment," *Federal Register* **51(185)**, 33992-34003 (1986).

9. J.R. Withey, B.T. Collins, and P.G. Collins, "Effect of Vehicles on the Pharmacokinetics and Uptake of Four Halogenated Hydrocarbons From the Gastrointestinal Tract of the Rat," *J. Appl. Toxicol.* **3,** 249-253 (1983).

10. T.A. Jorgenson, E.F. Meierhenry, C.J. Rushbrook, R.J. Bull, and M. Robinson, "Carcinogenicity of Chloroform in Drinking Water to Male Osborne-Mendel Rats and Female B6C3F1 Mice," *Fundam. Appl. Toxicol.*, **5,** 760-769 (1985).

11. SAB. (Science Advisory Board), Drinking Water Committee, "Review of Health Criteria Document for Trihalomethanes by the Drinking Water Committee of the Science Advisory Board," (U.S. Environmental Protection Agency Science Advisory Board, Washington, DC, 1992).

12. J.R. Thorton-Manning, P. Gao, and P.D. Lilly, "Acute Bromodichloromethane Toxicity in Rats Pretreated with Cytochrome P450 Inducers and Inhibitors," *The Toxicologist* **13(1),** 361 (Abstract 1412) (1993).

13. F.L. Mink, J. Brown, and J. Rickabaugh, "Absorption, Distribution and Excretion of ^{14}C-trihalomethanes in Mice and Rats," *Bull. Environ. Contam. Toxicol.* **37**, 752-758 (1986).

14. E. Testai and L. Vittozzi, "Biochemical Alterations Elicited in Rat Liver Microsomes by Oxidation and Reduction Products of Chloroform Metabolism," *Chemico-Biol. Inter.* **59**, 157-171 (1986).

15. E. Testai, F. Gramenzi, S. DiMarzio, and L. Vittozzi, "Oxidative and Reductive Biotransformation of Chloroform in Mouse Liver Microsomes," *Arch. Toxicol.*, Supplement **II,** 42-44 (1987).

16. J.E. Klaunig, R.J. Ruch, and M.A. Pereira, "Carcinogenicity of Chlorinated Methane and Ethane Compounds Administered in Drinking Water to Mice," *Environ. Health Persp.* **69,** 89-96, (1986).

17. A.E. Munson, L.E. Sain, V.M. Sanders, B.M. Kauffmann, K.L. White Jr., D.G. Page, D.W. Barnes, and F. Borzelleca, "Toxicology of Organic Drinking Water Contaminants: Trichloromethane, Bromodichloromethane, Dibromochloromethane and Tribromomethane," *Environ. Health Persp.* **46**, 117-126, (1982).

18. A.B. Eschenbrenner and E. Miller, "Induction of Hepatomas in Mice by Repeated Oral Administration of Chloroform, with Observations on Sex Differences," *J. Nat. Cancer Inst.* **5**, 251-255, (1945).

19. H.M. Bolt, "Pharmacokinetic Factors and their Implication in the Induction of Mouse Liver Tumors by Halogenated Hydrocarbons," *Arch. Toxicol.* Supplement **10**, 190-203, (1987).

20. V.M. Voronin, A.I. Donchenko, and A.A. Korolev, "An Experimental Study of the Carcinogenicity of Dichlorobromomethane and Dibromochloromethane Released During the Water Chlorination Process," *Gigiena I Sanitariia (Moskva)* **O (1),** 19-21, (1987) (English translation).

21. R.H. Reitz, T.R. Fox, and J.F. Quast, "Mechanistic Considerations for Carcinogenic Risk Estimation," *Chloroform Environ. Health Persp.* **46**, 163-168, (1982).

22. T.C. Moore, A.B. DeAngelo, and R.A. Pegram, "Subchronic Toxicity of Bromodichloromethane and Bromoform Administered to Mice and Rats in Drinking Water," *The Toxicologist* **13(1),** 359, (Abstract 1405) (1993).

SESSION V

ADVANCING THE SCIENCE OF RISK ASSESSMENT – PART 2

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Morphometry Approaches for Evaluating Pulmonary Toxicity in Mammals: Implications for Risk Assessment

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ABSTRACT

Recent advances in quantitative morphology provide all the tools necessary to obtain structural information in the lung that can be quantified and interpreted in the three-dimensional world of toxicology. Structural hierarchies of conducting airways and parenchyma of the lung provide (1) numbers of cells per airway, lobe, or lung; (2) surface areas of cells, airways, and alveoli; (3) length of airways and vessels; and (4) volumes of cells, alveoli, airways, vessels, and individual lobes or the entire lung. Unbiased sampling of these subcompartments of the lung requires fractionation of lobes or individual airways. Individual airways of proximal and distal generations are obtained by airway microdissection along one axial pathway and comparisons are made between airway generations. Vertical sections of selected airways are used to sample epithelium and interstitium. Using this unbiased approach of quantitative morphology, we have shown that inhalation of low ambient concentrations of ozone ([O_3] 0.15 ppm) near or at the U.S. National Ambient Air Quality Standard (NAAQS) (0.12 ppm O_3) induces significant alterations in bronchiolar epithelium and interstitium in nonhuman primates but not rats. The alterations do not appear to be concentration or time-dependent, thereby bringing into question the current NAAQS that may be at or above the threshold for distal airway injury in primates. Unbiased morphometric methods are critical in a quantitative evaluation of toxicological injury of mammalian tracheobronchial airways.

INTRODUCTION

An unbiased morphometric assessment of pulmonary toxicity in animal lungs must consider the complexity and diversity of the entire branching airways and parenchyma. The extrapolation to humans of studies of toxic agents injurious to the respiratory system using animal models assumes comparability in the structure and function of the respiratory system of these model species and humans. The underlying assumption is that data, especially morphometric data of lung structure, obtained in model species can be extrapolated to humans.

Ozone (O_3) is the most reactive and toxic oxidant gas in photochemical air pollution. Ozone is also the principal air pollutant in many urban areas during the summer months. In recent years, maximal monthly concentrations of O_3 ranging from 0.1 to near 0.3 parts per million (ppm) have been reported in Mexico City (1,2). Ozone inhalation produces injury in at least three target areas of the respiratory system of animals: nasal cavity, trachea, and the centriacinar region (3-13). Recent reports that some pulmonary function impairment was induced in exercising children and adult humans exposed to O_3 concentrations at or near the ambient concentration in the current U. S. National Ambient Air Quality Standard (NAAQS) for O_3 (0.12 ppm) have opened to question the health safety of the NAAQS (14-21).

The majority of studies defining the pathogenesis of O_3-induced injury have been conducted using the laboratory rodents. Small laboratory mammals show an initial response of cellular necrosis, exfoliation, degranulation of secretory cells, followed by epithelial hyperplasia and hypertrophy (6,8,9,11,22,23). Experimental studies with rats at concentrations near the NAAQS, suggest that the lungs of rats are relatively insensitive to these environmentally relevant concentrations of O_3 (4,24). Direct assessment of the human susceptibility to injury by O_3 inhalation is limited to physiologic assessment or measurement of bronchoalveolar lavage cells and fluid following short-term, low level exposures; thus, accurate assessment of the risk of ambient O_3 to humans is difficult (25,26).

Extrapolation from data collected using the laboratory rat suggests that there is minimal risk for humans at ambient concentrations of O_3. In contrast, monkeys exposed to 0.15 ppm O_3 for 6 or 90 days, 8 h per day, had significant nasal epithelial lesions of ciliated cell necrosis, attenuated cilia, secretory cell hyperplasia, bronchiolar epithelial lesions of hyperplasia, hypertrophy of nonciliated bronchiolar epithelial cells, and intraluminal accumulations of macrophages (10,27). The bronchiolar lesion was also characterized by significant thickening of the interstitium (27). These comparative quantitative results of

differential sensitivity to O_3 inhalation of the nonhuman primate versus the rodent were only possible because of the use of carefully applied morphometric methods. This paper reviews the state-of-the-art application of morphometric methods to pulmonary tissue evaluated in the assessment of O_3-induced lung injury. It should be noted that the application of morphometric methods to the assessment of O_3-induced lung injury is only one example of the beneficial sensitivity of morphometry in the assessment of organ toxicity.

METHODS

We will use three guiding principles in morphometry: (1) design-based methods to quantify structural changes, (2) structural hierarchies to link and interpret experimental data, and (3) collected critical data to detect structural changes (28).

Design-Based Sampling

A sample is considered unbiased when all the compartments of the structure have an equal chance of being sampled. The most reliable method of satisfying this need of unbiased sampling is to introduce randomness in the sampling process. This is a true departure from the traditional approach of assuming randomness in the lung. For example, if a structure such as the lung does not have a uniform distribution of components, then the best way to avoid sampling bias is to collect samples systematically with a design-based approach (29,30). A general approach is to take a lung lobe, measure its longest dimension, and divide the length by 10 to obtain 10 equally spaced slabs; use a random start within the width of the first slab and space the remaining slab cuts by the equal slab width. All components in the lung lobe have an equal chance of being sampled because the cuts can fall anywhere within the intervals, and thus the sampling is unbiased. Usually, we are interested in quantitating structures at various magnifications, but all compartments must have an equal chance of being sampled. Designed-based sampling ensures unbiased sampling at all magnifications and even when the components exhibit striking anisotropy.

Hierarchical Data

The lung is a complex organ composed of numerous compartments ranging in size from molecules to tissues. Hierarchies allow us to organize data according to the size of the structures. Hierarchy equations define the relationships among and link data within and across hierarchical levels (28). For example, if we desire the number of Type I epithelial cells in the lung, we only need to multiply the number of Type I epithelial cells per volume of interalveolar septal tissue by the volume

density of the interalveolar septal tissue per parenchymal tissue, the volume density of parenchymal tissue per lung, and the volume of the lung. In this example, the object at one magnification becomes the reference at the next higher level of magnification. Also, information is provided at each level or magnification and not just at the organ level. Two general guidelines emerge from hierarchy organization: (1) use the lowest reasonable magnification (acceptable resolution) to increase sample size for measurements, and (2) if major compartments and their subcompartments cannot be measured at the same magnification, then the magnification should be increased to optimize resolution in the subcompartment.

Critical Data

A critical dataset is required to detect and interpret quantitative data in any organ (28). These data include the volume of the structure, the number of cells in the structure, and the structural components or densities. The critical dataset allows one to move data about a structural hierarchy, detect and interpret structural changes, and create links to other data types.

Abbreviations, Symbols, and Terminology

Pulmonary structures can be described as having volumes (V), surfaces (S), lengths (L), and numbers (N). Structural densities relate these parameters to a unit of reference volume: V/V, S/V, L/V, N/V (represented as V_V, S_V, L_V, N_V). These four defining parameters are further defined by accompanying symbols. The symbol (i/ref) defines the ratio of two compartments as given for the densities: V_V(i/ref), S_V(i/ref), L_V(i/ref), N_V(i/ref). The compartment of interest, the small "i", is related to the reference compartment, "ref". For example, the volume density of capillaries within the lung is represented as V_V(ca, lu). We use the first two letters of the compartment to abbreviate the names. For example, lung becomes lu, trachea tr, capillaries ca and collagen co. Extra letters can be added to avoid duplicates.

Test Grid Systems

Point and intersection counting, using coherent test systems are more efficient for collecting raw data than digitizers requiring hand tracing of objects (31). Hence, for all stereologic measurements other than those that can be done by image analysis (32), point and intersection counting using test grid systems prevail. It should be noted that even when image analysis can be applied to lung tissue, such as quantitating the volume of stored mucosubstance per surface area of epithelial basal lamina, it is only

12-fold more efficient than manual methods (33). We prefer using the efficient point and intersection counting methods by employing coherent test systems designed to give reference points (P_r), reference line lengths (L_r) and reference areas (A_r) after Weibel (31), Cruz-Orive (34), and Baddeley *et al.*, (35). The counting rule of Gundersen (36) should be followed when making profile counts on any of the grids. The rule is to count all profiles totally within the counting frame that do not intersect the "forbidden lines" at any point. The forbidden lines include two adjacent borders of the frame (left and bottom as marked by solid lines on the grids) and extended lines from left top and lower right corners.

Volume of the Structure

One of the most common starting points and our first critical data is the volume of the lung or its individual lobes. One of the most direct methods is to systematically cut fixed lung lobes into slabs of equal thickness, dehydrate, embed, and section sampled slabs and determine all data within a volume that is common to all levels of observation (e.g., a volume that is fixed, dehydrated, embedded, and sectioned). By incorporating a common reference into the experimental study, it is then possible to move data freely across the various levels of the hierarchy without fear of experimental bias. To estimate the volume of a lung lobe, take approximately 10 samples from the slabs (selected systematically), determine their cumulative area by point counting, and multiply by the average slab thickness (Cavalieri method[a]). The volume of the individual slabs can be estimated more precisely by defining their shape as a prismatoid using computer digitization and analysis, but this level of precision is unwarranted and not a marked improvement on the Cavalieri method of volume estimation (37).

Another approach, the optical volume fractionator (OVF), provides estimates for the volume of the structure, the total number of cells in the structure, and the numerical density of cells (38). It combines two of the primary tools of stereology, the fractionator (39) and the optical "disector" (40). The fractionator systematically subdivides a structure into smaller and smaller fractions until a final fraction is obtained. Volume is estimated in the final fraction by the Cavalieri method and related by fractions to estimate the volume of the entire structure. If we count the number of cells in the final fraction using the OVF method, then we can estimate the number of cells per unit volume of compartment and within the entire structure. The OVF method allows us to build structural hierarchies for the lung by establishing links between light and electron microscopy. As long as specimens are treated the same (similarly fixed, dehydrated, and embedded) the links between light and electron microscopy are valid.

Number of Cells in a Structure

The second critical data required are the number of cells in a structure (lung lobe, airway, vessel, alveolus, etc.). The most direct and unbiased method is to count cells in three-dimensional (3-D) space (41,42). This is the basis of the "disector" principle. Counting methods based on the disector include the fractionator (40), optical fractionator (43), OVF (38), and selector (44).

Using direct counting of cells in 3-D space, we are given three options for estimating the number of cells in the structure. If we want to estimate only total cell number, the fractionator or optical fractionator will be the easiest. Both methods are efficient and independent of shrinkage and swelling artifacts. For hierarchical studies, wherein data are collected from several levels within the structure, we will want numerical density estimates for cells (number in the volume of the various compartments). These estimates become critical for detecting changes in cell compartments, such as organelles, because they allow us to calculate average cell data from stereological densities. For example, assume we estimate cell numerical density of Type II cells within the volume of interalveolar septa (N_V(#2,is)) using the optical disector and the volume density of Type II cells within the volume of interalveolar septa (V_V(ii,is), then we can calculate the average volume of Type II cells (V(ii)) by dividing the volume density by the numerical density as follows:

$$V(ii,\#2) = V_V(ii,is) / N_V(\#2,is),$$

where #2 represents the number of Type II cells which in the denominator of V(ii,#2) becomes 1. The real units for volume are in all the same cm^3 units and the reference volumes for V_V and N_V are the same and thereby divide to 1.

Optical Disector

The optical disector method counts cells directly in a measurable volume (40,45). Whether light or laser confocal microscopes are used to optically focus through a thick section (usually about 20 μm thick), a short depth of focus (1 to 3 μm) is essential to optically section the tissue and a length gauge is required to precisely move in the Z direction. Usually a lens with a high numerical aperture satisfies the short depth of focus problem. This unbiased counting method is direct provided we use a two-dimensional (2-D) unbiased counting frame (36) and extend the counting frame concept by excluding structures counted on either the top or bottom of the cube. We estimate the reference volume by point counting an optical section in the middle of the cube that provides us with a reference area that is

multiplied by the distance traveled in the Z direction for counting structures. Note that we can use the area of the 2-D counting frame if it is totally filled with the reference area.

Densities

A density is the ratio of two compartments, a compartment i in the numerator and a reference compartment, which is usually a volume in cm^3, in the denominator. The four standard densities include volume, surface, length, and number. Because the numerator and denominator in densities are both variables, they cannot reliably detect changes unless they are related to the volume of the structure.

Two types of sections can be generated when structures are sampled systematically: (1) vertical sections (blocks rotated randomly about a vertical axis and sectioned), or (2) isotropic uniform random (IUR) sections (blocks oriented randomly in all directions and sectioned). Because the lung contains many anisotropic (oriented) structures, only systematic sampling and vertical sectioning guarantee unbiased estimates for all four densities. Estimates of surface and length densities in the lung require a cycloid test grid oriented with respect to the vertical axis. Volume and numerical densities can be made with vertical or IUR sections, and thus the grid type is not specific.

Volume Density:

Volume density, V_V, is independent of the sectioning angle and the orientation of anisotropic structures because it affects both the object and reference phase equally. Volume density should be estimated by point counting techniques. Point counting has been shown to be the most efficient method of estimation (31), and it uses the formula

$$V_V(i,r) = P_i / P_r$$

where P_i is the number of point "hits" on the compartment of interest and P_r is the number of point "hits" on the reference compartment (46).

Surface Density

Surface density, S_V, is influenced by both the sectioning angle and the shape of anisotropic structures (47). For isotropic structures, surface density can be defined

$$S_{Vi,r} = 2I_i / L_r$$

where I_i is the number of intersections of the object surface and L_t is the test system length of the reference component (48,49). This equation is valid for test lines that are isotropic uniform random in 3-D space. To meet this requirement using a lattice grid, the microstructures must be distributed uniformly and randomly and their orientation must be isotropic. The use of vertical sections, defined along the plane of preferred orientation for anisotropic microstructures, and a cycloid test grid system (35), gives surface density estimates that correct for anisotropic orientation directly using the equation for S_V. Vertical sections alone, however, do not guarantee isotropic random encounters with the orthogonal test lines used to estimate surface and length densities in IUR sections. Sin weighted test lines along the vertical axis, arranged continuously as cycloids correct this bias of vertical sections (35). The requirements of vertical sections according to Baddeley *et al.* (35) are as follows: (1) identify a vertical axis (along a preferred or arbitrary axis); (2) all vertical sections must be cut parallel to the vertical axis; the test grid must be oriented with respect to the vertical axis; (3) all vertical sections must have random positions (systematic sampling of slices) and isotropic random orientation (spin about vertical axis); and (4) a test line on vertical sections must be weighted proportional to sin θ, where θ is the angle between the test line and the vertical direction. Some examples of vertical sections are (1) longitudinal sections of skeletal muscle, (2) sections of skin and other flat epithelial (e.g., tracheobronchial epithelium in microscopic windows) normal to the exterior macroscopic surface, and (3) sections of an arbitrary organ, obtained by cutting the organ into parallel slabs (with an arbitrary common direction) and then placing some of the slabs on a flat surface (horizontal plane) and sectioning normal to the flat surface. To obtain vertical sections of tubular organs (e.g., tracheobronchial airways), the organ or airway must be opened along its axis, flattened along its abluminal surface that becomes the horizontal plane. Relative to this defined horizontal plane, the vertical sections must be selected in a random orientation. For test grid systems, superimposed on vertical sections, a test line is given a weight proportional to sin θ, where θ is the angle between the test line and the vertical direction. Either a numerical weight for each intersection count obtained with test lines at a given angle or a test system in which test lines at an angle θ to the vertical have length proportional to sin θ is required for a correct weighting factor. The cycloid grid has a unique property in which the tangent part of the curve is at an angle θ to the vertical axis that has length proportional to sin θ (35). Thus, the sin θ weighting factor is incorporated into the grid and with the intersection count per unit length of cycloid test curve gives an unbiased estimate of surface density.

Length Density

Estimating lengths with IUR sections can become unusually problematic when linear structures have anisotropic orientations in tissues (50). A new design-based method avoids this problem of anisotropy by using vertical sections and projected images (50,51). With vertical sections, all linear structures contained within the volume of a thick section or slice are projected onto a plane. Counting the intersections between a cycloid test line and the linear structure and measuring the section thickness with a length gauge provides an unbiased estimate of L_V. To collect data with this method, a cycloid test grid must be oriented with respect to the vertical axis of the section, not perpendicular to it as with S_V estimates. To estimate the length of capillaries per volume of interalveolar septa (L_V[ca,is]), we need to count the total points hitting on the reference component (is) and the number of intersections I(ca) between test lines and the capillaries. We then evaluate the Gokhale equation (50):

$$L_V(ca,is) = 2/ (t * \Sigma I(ca) / \Sigma L(is)),$$

where t is the mean section thickness and Σ L(is) the total test line length in the reference component (is).

Numerical Density

Numerical density, N_v , was introduced previously in the number of cells in a structure. When N_V is desired, one simply uses the optical disector and measures the volume in which the cells are contained. If the optical disector is combined with the fractionator method, it provides the most powerful method, the optical volume fractionator (38).

Statistical Considerations

Statistical considerations relative to stereological measurements have been presented in detail elsewhere (52-54). However, a compelling argument on the contribution to the total variance of a group of animals in a stereological study of the harmonic mean thickness of the glomerular basement membrane was provided by Gundersen and Osterby (54). They showed that animals contributed 70%, blocks 19%, fields 8% and intercepts and measuring 3% of the total variance. In our laboratory and those of others (55), the results of stereological measurements of lung tissue agree with those in kidney and identify animal and block variance as the greatest source of variation in a study. A logical approach to this problem means that we always use a sufficient number of animals and blocks per animal in our studies. Then, in turn, we use the minimal number of fields per block and points per field to estimate the block values. Using ratio estimators, we sum the values over blocks and then use the means of the block values to estimate the organ or animal value (52).

Fixation

Fixation and sampling are critical aspects of any morphometric study of the lung. If the pulmonary epithelium is to be examined only by light microscopy, a 10% buffered formalin solution is adequate. However, the use of electron microscopy usually requires a glutaraldehyde-paraformaldehyde fixation (440 mOsm, pH 7.4) (56). Because no true dimensions of cells and their organelles are known, some investigators have employed quick-freezing and cryosubstitution to compare morphological features with those in fixed tissues (57), or to examine unfixed antigenic determinants. The relative osmotic effects of glutaraldehyde and buffer solutions were evaluated on the harmonic mean thickness of the blood/air barrier and the shape of erythrocytes in pulmonary capillaries by instilling fixative in lungs of rats (58). Mathieu *et al.* (58) showed that 300 mOsm was required to maintain erythrocyte shape and that glutaraldehyde concentration exerted an osmotic effect, even though it was less important than the buffer. In essence, the harmonic mean thickness of the blood/air barrier varies inversely proportionally to the total osmolarity. Our most commonly used fixative is 1% glutaraldehyde, 1% paraformaldehyde at pH 7.4 and adjusted to 360 mOsm with cacodylate buffer. However, the goal of a study also dictates the fixative to be used. If preservation of antigenic determinants is critical, then mild fixation may be required. This had to be determined empirically in most cases. One percent paraformaldehyde adjusted to 360 mOsm with cacodylate buffer and applied for about 30 min maintains the majority of antigens we are interested in seeing in lung tissue. Perhaps the most critical element of a morphometric study is that the composition of the fixative, dehydrating, and embedding solutions be constant. This consideration should extend to embedding and sectioning tissue from all experimental groups at the same time.

Airway Microdissection

The approach we have used for precise sample selection is to employ airway microdissection with a specific binary numbering system (59). This can be applied to lungs preserved by a variety of methods. Airway microdissection has been used in lungs inflated at a standard air pressure and fixed by perfusion of the pulmonary vascular bed with aldehyde fixatives (60). We have also employed specimens that have been frozen after expansion with air to total lung capacity (TLC) and lyophilized in a freeze-drying chamber. Beginning at the lobar bronchus, the intrapulmonary airways and accompanying parenchyma are split down the long axis of the largest daughter branch or down the axial pathway of the primary airway. An attempt is made to expose as many minor daughter side branches as possible. Each airway is numbered by a binary system originally described by Phalen *et al.* (61). The binary system is a simple

numbering system that provides a branching history for each segment of the conducting airways. Each time an airway branches, the larger of the two daughter branches, or the one with the smallest angle of deviation from the long axis, is designated with the number one. The smaller branch, or the one with the larger angle of deviation from the previous pathway, is designated with a 0. This number provides a branching history, the number of generations of branching, and a general size relationship of specific levels. From these dissected specimens, samples can be taken from specific locations with known generation numbers.

Tracheobronchial and Centriacinar Airways

The tracheobronchial airways are uniquely characterized by ciliated pseudostratified columnar epithelium, a submucosa with glands and cartilage. Because epithelial and interstitial cell populations vary by airway and show different injury and repair patterns by airway generation (62), it seems only appropriate to focus our attention on airways to individual generations. Airway microdissection is the only practical way to sample airways and still retain knowledge of the generation number (59).

We will use an example (Figure 29) of airway microdissection, fractionator, optical disector and local vertical section method to illustrate how one can estimate the number of epithelial, interstitial or inflammatory cells for one airway generation. Airway microdissection uses airway casts in the appropriate species to select the best plane to select desired airways. Airways are exposed along the longest axial pathway with as many small branching airways as possible in one plane. A binary system is used to uniquely record airway branching from the trachea. Once an airway is selected, both halves are cut out of the lung for trimming and subsampling. The airway is cut transversely into 2 mm rings. Every third ring is selected using a random start, laid flat with the luminal surface up, rotated randomly and local vertical sections cut perpendicular to the epithelial basal lamina (Figure 29). Every fifth block is selected from the series of rings, and tissue blocks are embedded and cut in alternating step serial sections (1 and 20 μm). We use the OVF to count cells in 20 μm sections and 1 μm sections to estimate Vv and Sv with local vertical sections. With those unbiased measurements, we can calculate the volume of the airway wall and its components, epithelium and interstitium; the number of epithelial and interstitial cells in the airway; the total surface area of the airway and number of epithelial or interstitial cells per unit of surface. Data and calculations related to Figure 29 are given below.

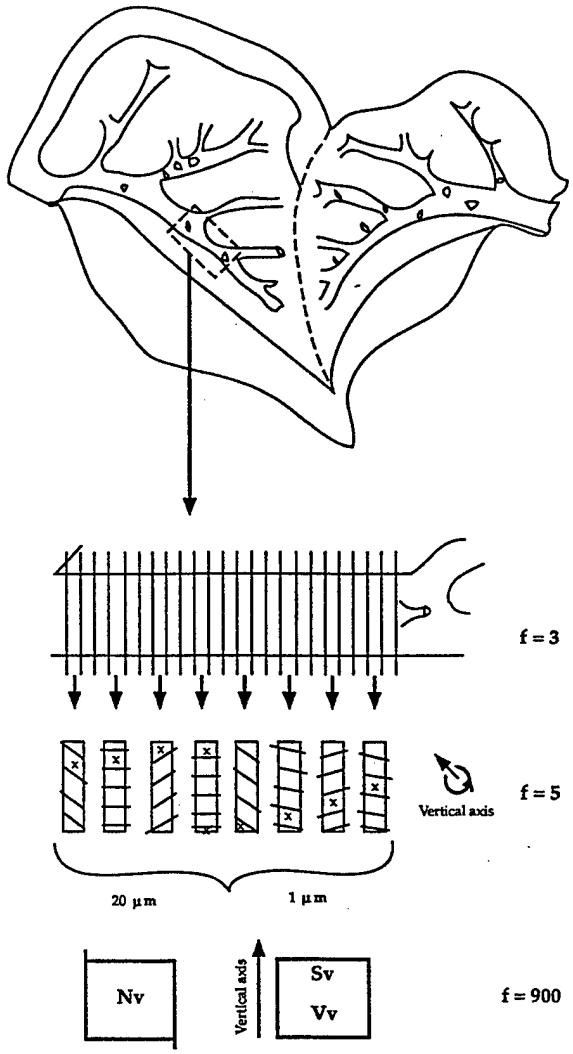


Figure 29. An Illustration of Airway Selection, Sampling, and Sectioning for Estimating the Total Number of Cells in the Airway, the Mean Volume of the Cells, and the Number Per Surface of the Airway. Airway microdissection is used to expose airways along the longest axial pathway with as many small branching airways as possible in one plane. An airway is selected using both sides of the dissected lobe, and cutting the lobes into 2 mm transverse rings. Every third ring (f = 3) is selected by stratified sampling with a random start, laid flat with the luminal surface up and rotated randomly, and local vertical sections are cut perpendicular to the epithelial basal lamina. Every fifth block (f = 5) is selected from the series of rings, it is embedded and cut in alternating step serial sections (20 μm and 1 μm) and with a random start every ninehundreth section/field is selected (f = 900) to estimate N_V using the OVF method to count cells in a known volume, and S_V and V_V using point and intersection counting. The "f" is for fraction which represent the fraction used for sampling and to estimate total values for the sampled airway. Note that S_V requires a cycloid grid and a local vertical section.

The volume of the fixed, dehydrated, embedded airway wall (AW).

$$V_{(AW)} = f(1) \times f(2) \times f(3) \times \Sigma V(fi) = 3 \times 5 \times 900 \times (2.5 \times 10^{-7} cm^3) = 0.003375\ cm^3$$

where f(1)....f(3) are the fractions sampled at each level from airway rings (f(1)) to fields (f(3)). The sums of the individual section/field volumes (V(fi)) are used to estimate the volume of the airway wall. If we counted 100 epithelial cells in the sections with the optical disector, then we can estimate the total number of epithelial cells (epce) in the wall of the airway as:

$$N_{(epce,\ AW)} = f(1) \times f(2) \times f(3) \times \Sigma N_{ce} = 3 \times 5 \times 900 \times 100 = 1{,}350{,}000$$

In turn, we can calculate the surface area of the epithelial basal lamina (epbl), a good reference surface, of the airway indirectly from the Sv of epithelial basal lamina to the volume of the airway wall as:

$$S_V(epbl,AW) = 2\Sigma I_{(epbl)} / L_{(AW)} = 650\ cm^2 / cm^3$$

where $L_{(AW)}$ is the total length of the grid line in the reference space (airway wall). The total surface of the epithelial basal lamina of the airway is simply

$$S_{(AW)} = Sv_{(epbl,\ AW)} \times V_{(AW)} = 650\ cm^2/cm^3 * 0.003375\ cm^3 = 2.1938\ cm^2$$

In turn, the number of cells per surface of the airway is calculated as:

$$N_S(ep,AW) = N_{(epce,AW)} / S_{(AW)} = 1350000 / 2.1938\ cm^2 = 6.1538 \times 10^5 / cm^2$$

One can also calculate the average volume of an airway epithelial cell as:

$$V_{(ep,ce)} = V_V(epce,AW) / N_V(\#/AW) = 0.2498 / (4 \times 10^8 / cm^3) = 6.24 \times 10^{-10}\ cm^3 = 624\ \mu m^3$$

where

$$N_V(\#/AW) = N_{(epce,AW)} / V_{(AW)} = 1350000 / 0.003375\ cm^3 = 4 \times 10^8 / cm^3$$

With these calculations, one can detect hyperplasia, hypertropy, and differential growth of airways under numerous experimental or disease conditions.

DISCUSSION

Ozone is well recognized as an oxidant injurant to the mammalian respiratory system. A pertinent question, particularly considering the recent efforts to assess the costs of bringing major metropolitan areas in the United States into compliance with the current NAAQS for O_3 at 0.12 ppm, is the level of risk posed to human beings by exposure to current ambient levels of O_3. Most of the assessment of health risks and health effects of O_3 have been based on extrapolation of findings reported using laboratory rats. Comparisons of quantitative measures of cellular changes occurring in two of the three target zones within the respiratory system demonstrate a substantial difference in the sensitivity of rats and nonhuman primates. The nonhuman primate appears to be at least one order of magnitude more sensitive at low-level concentrations of O_3 than is the laboratory rat.

The assumption that all species have the same susceptibility to injury from toxic substances such as O_3 is one of the primary difficulties complicating attempts to extrapolate toxicological data from damage in rats to assessment of risks for human health. Clearly, the respiratory system in various orders of mammals is substantially different in architectural and cellular composition including the nasal cavity, the tracheobronchial airways, and the centriacinar region of the lung. All three of these regions are targets for oxidant injury from O_3. Recently, quantitative information became available that allowed direct comparisons, using the same types of morphometric measurements, of effects of inhalation of O_3 on the respiratory system of species of different orders (Rodentia and Primates). These studies indicate that there are both topographic and cellular differences between the rat and macaque monkey.

This review suggests that nonhuman primates have less ability to protect their respiratory system, at least from a cellular perspective, than do rats (i.e., their cells are inherently more susceptible to O_3-induced injury than are those of the rat). The nasal cavity that could be expected to remove less O_3 in the primate than in the rat is more affected at lower concentrations of O_3 in the primate. Similarly, the centriacinar region of primates is more susceptible to lower concentrations of O_3 when compared with rats by an order of magnitude. What these comparative findings imply for an assessment of risks to humans from inhalation of ambient concentrations of O_3 is still questionable. It is reasonable to conclude that basing the assessment of human health risk on studies without nonhuman primates and unbiased morphometric methods would at best grossly underestimate the potential susceptibility of humans to chronic lung injury from ambient concentrations of O_3.

Lesions in the bronchiolar epithelium of the central acinus are also observed from a variety of organic compounds found in the environment. The primary centriacinar response to a single injection of these compounds, and their subsequent delivery to the bronchiolar epithelium by the vascular system, is bronchiolitis (63). The early phase within the first two days shows Clara cell swelling and necrosis. This Clara cell response is followed by about a two-fold increase in ciliated cells. Some organic compounds also appear to injure ciliated cells as well as Clara cells. In either case, assessment of alterations in epithelial cell populations is greatly enhanced by quantitation. It should be noted that the new generation of designed-based morphometric methods will allow more precise comparisons to be made in studies of O_3-induced and organic compound-induced injury in experimental animals and can now provide the essential data required for a more accurate assessment of the risk from these agents on human health.

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FOOTNOTES

[a] Cavalieri method is named for the Italian mathematician Bonaventura Cavalieri (1598-1647), who first proposed the method for estimating volume.

REFERENCES

1. H. Bravo-Alvarez, G. Roy-Occtia, R. Sosa, and R. Torres, "Tendencia del Problema del la Contaminacion Atmosferica por Ozono en la Zona Suroeste de la Ciudad de Mexico (Tendency of the Air Pollution Problem in South-West Mexico City)", Memoirs of the Seventh National Congress of Sanitary and Environmental Engineering, Oaxaca, Mexico, September, 1990.

2. L. Calderon-Garciduenas, A. Osorno-Velazquez, H. Bravo-Alvarez, Delgado-Chavez, and R. Barrios-Marquez, "Histopathologic Changes of the Nasal Mucosa in Southwest Metropolitan Mexico City Inhabitants," *Am. J. Pathol.* **140**, 225-232 (1992).

3. L.W. Schwartz, D.L. Dungworth, M.G. Mustafa, B.K. Tarkington, and W.S. Tyler, "Pulmonary Responses of Rats to Ambient Levels of Ozone: Effects of 7-Day Intermittent or Continuous Exposure," *Lab. Invest.* **34**, 565-578 (1972).

4. C.G. Plopper, D.L. Dungworth, W.S. Tyler, and C.K. Chow, "Pulmonary Alterations in Rats Exposed to 0.2 and 0.1 PPM Ozone: A Correlated Morphological and Biochemical Study," *Arch. Environ. Health* **34**, 390-395, (1979).

5. G.A. Boorman, L.W. Schwartz, and D.L. Dungworth, "Pulmonary Effects of Prolonged Ozone Insult in Rats: Morphometric Evaluation of the Central Acinus," *Lab. Invest.* **43**, 108-115 (1980).

6. W.L. Castleman, D.L. Dungworth, L.W. Schwartz, and W.S. Tyler, "Acute Respiratory Bronchiolitis: An Ultrastructural and Autoradiographic Study of Epithelial Cell Injury and Renewal in Rhesus Monkeys Exposed to Ozone," *Am. J. Pathol.* **98**, 811-840 (1980).

7. B.E. Barry, F.J. Miller, and J.D. Crapo, "Effects of Inhalation of 0.12 and 0.25 Parts Per Million Ozone on the Proximal Alveolar Region of Juvenile and Adult Rats," *Lab. Invest.* **53**, 692-704 (1985).

8. L.E. Fujinaka, D.M. Hyde, C.G. Plopper, W.S. Tyler, D.L. Dungworth, and L.O. Lollini, "Respiratory Bronchiolitis Following Long-Term Ozone Exposure in Bonnet Monkeys: A Morphometric Study," *Exp. Lung Res.* **8**, 167-190 (1985).

9. R.K. Moffatt, D.M. Hyde, C.G. Plopper, W.S. Tyler, and L.F. Putney, "Ozone-Induced Adaptive and Reactive Cellular Changes in Respiratory Bronchioles of Bonnet Monkeys," *Exp. Lung Res.* **12**, 57-74 (1987).

10. J.R. Harkema, C.G. Plopper, D.M. Hyde, J.A. St. George, D.W. Wilson, and D.L. Dungworth, "Response of the Macaque Nasal Epithelium to Ambient Levels of Ozone: A Morphologic and Morphometric Study of the Transitional and Respiratory Epithelium," *Am. J. Pathol.* **128**, 29-44 (1987).

11. B.C. Barr, D.M. Hyde, C.G. Plopper, and D.L. Dungworth, "Distal Airway Remodeling in Rats Chronically Exposed to Ozone," *Am. Rev. Respir. Dis.* **137**, 924-938 (1988).

12. W.S. Tyler, N.K. Tyler, J.A. Last, M.J. Gillespie, and T.J. Barstow, "Comparison of Daily and Seasonal Exposures of Young Monkeys to Ozone," *Toxicology* **50**, 131-144 (1988).

13. J.R. Harkema, J.A. Hotchkiss, and R.F. Henderson, "Effects of 0.12 and 0.80 PPM Ozone on Rat Nasal and Nasopharyngeal Epithelial Mucosubstances: Quantitative Histochemistry," *Toxicol. Pathol.* **17**, 525-535 (1989).

14. M. Lippmann, P. Lioy, and G. Leikauf, "Effects of Ozone on the Pulmonary Function of Children," *Adv. Mod. Environ. Toxicol.* **5**, 423-446 (1983).

15. P.J. Lioy, T.A. Vollmuth, and M. Lippmann, "Persistence of Peak Flow Decrement in Children Following Ozone Exposures Exceeding the National Ambient Air Quality Standard," *J. Air Pollut. Control Assoc.* **35**, 1068-1071 (1985).

16. W.F. McDonnell, R.S. Chapman, M.W. Leigh, G.L. Strope, and A.M. Collier, "Respiratory Responses of Vigorously Exercising Children to 0.12 Ozone Exposure," *Am. Rev. Respir. Dis.* **132**, 875-879 (1985).

17. S.I. Gibbons and W.C. Adams, "Combined Effects of Ozone Exposure and Ambient Heat on Exercising Females," *J. Appl. Physiol.* **57**, 450-456 (1984).

18. E.L. Avol, W.S. Linn, T.G. Venet, D.A. Shamoo, and J.D. Hackney, "Comparative Respiratory Effects of Ozone and Ambient Oxidant Pollution Exposure During Heavy Exercise," *J. Air Pollut. Control Assoc.* **74**, 804-809 (1984).

19. F. J. Kulle, L.R. Sauder, J.R. Hebel, and M.D. Chatham, "Ozone Response Relationships in Healthy Nonsmokers," *Am. Rev. Respir. Dis.* **132**, 36-41 (1985).

20. H. Gong Jr., P.W. Bradley, M.S. Simmons, and D.P. Tashkin, "Impaired Exercise Performance and Pulmonary Function in Elite Cyclists During Low-Level Ozone Exposure in a Hot Environment," *Am. Rev. Respir. Dis.* **134**, 726-733 (1986).

21. D.M. Spektor, M. Lippmann, G.D. Thurston, P.J. Lioy, J. Stecko, G. O'Connor, E. Garshick, F.E. Spelzer, and C. Hayes, "Effects of Ambient Ozone on Respiratory Function in Healthy Adults Exercising Outdoors," *Am. Rev. Respir. Dis.* **138**, 821-828 (1988).

22. F.J. Stephens, M.F. Sloan, M.J. Evans, and G. Freeman, "Early Response of Lung to Low Levels of Ozone," *Am. J. Pathol.* **74**, 31-58 (1974).

23. C.G. Plopper, C.K. Chow, D.L. Dungworth, M. Brummer, and T.J. Nemeth, "Effect of Low Level of Ozone on Rat Lungs. II. Morphological Responses During Recovery and Re-exposure," *Exp. Mol. Pathol.* **29**, 400-411 (1979).

24. C.K. Chow, C.G. Plopper, M. Chiu, and D.L. Dungworth, "Dietary Vitamin E and Pulmonary Biochemical and Morphological Alterations of Rats Exposed to 0.1 PPM Ozone," *Envir. Res.* **24**, 315-324 (1981).

25. U.S. Environmental Protection Agency, "*Air Quality Criteria for Ozone and Other Photochemical Oxidants*," Volume I, (U.S. Environmental Protection Agency, Research Triangle Park, NC, 1986).

26. U.S. Environmental Protection Agency, "*Summary of Selected New Information on Effects of Ozone on Health and Vegetation: Draft Supplement to Air Quality Criteria for Ozone and Other Photochemical Oxidants*," (U.S. Environmental Protection Agency, Research Triangle Park, NC, 1988).

27. J.R. Harkema, C.G. Plopper, D.M. Hyde, J.A. St. George, D.W. Wilson, and D.L. Dungworth, "Response of Macaque Bronchiolar Epithelium to 0.15 and 0.30 PPM Ozone," *Am. J. Pathol.* (in press).

28. R.P. Bolender, D.M. Hyde, and R.T. DeHoff, "Quantitative Morphology of the Lung: A New Generation of Tools and Experiments for Organ, Tissue, Cell, and Molecular Biology," *J. Appl. Physiol.: Cell and Mol. Biol.* (in press).

29. H.J.G. Gundersen and E.B. Jensen, "The Efficiency of Systematic Sampling in Stereology and Its Prediction," *J. Microsc.* **147**, 229-263 (1987).

30. S. Ogbuihi and L.M. Cruz-Orive, "Estimating the Total Number of Lymphatic Valves in the Infant Lung with the Fractionator," *J. Microsc.* **158**, 19-30 (1990).

31. E.R. Weibel, "*Stereological Methods*, Vol. I, Practical Methods for Biological Morphometry," (Academic Press, New York, NY, 1979), pp. 3-7, 106-108, 139-150, 218-223, 322-331, 354-379.

32. D.M. Hyde, D. Orthoefer, D. Dungworth, W. Tyler, R. Carter, and H. Lum, "Morphometric and Morphologic Evaluation of Pulmonary Lesions in Beagle Dogs Chronically Exposed to High Ambient Levels of Air Pollutants," *Lab. Invest.* **38**, 455-469 (1978).

33. J.G. Heidsiek, D.M. Hyde, C.G. Plopper, and J.A. St. George, "Quantitative Histochemistry of Mucosubstance in Tracheal Epithelium of the Macaque Monkey," *J. Histochem. Cytochem.* **35**, 435-442, (1987).

34. L.M. Cruz-Orive, "The Use of Quadrates and Test Systems in Stereology, Including Magnification Corrections," *J. Microsc.* **125**, 89-102 (1982).

35. A. J. Baddeley, H.J.G. Gundersen, and L.M. Cruz-Orive, "Estimation of Surface Area from Vertical Sections," *J. Microsc.* **142**, 259-276 (1986).

36. H.J.G. Gundersen, "Notes on the Estimation of the Numerical Density of Arbitrary Profiles: The Edge Effect," *J. Microsc.* **111**, 219-223 (1977).

37. D.M. Hyde, D.J. Magliano, E. Reus, N.K. Tyler, S. Nichols, and W.S. Tyler, "Computer-Assisted Morphometry: Point, Intersection, and Profile Counting and Three-Dimensional Reconstruction," *Micros. Res. Tech.* **21**, 262-270 (1992).

38. R.P. Bolender, and J. Charleston, "Software for Counting Cells and Estimating Structural Volumes with the Optical Volume Fractionator," *Micros. Res. Tech.* (in press)

39. H.J.G. Gundersen, "Stereology of Arbitrary Particles. A Review of Unbiased Number and Size Estimators and the Presentation of Some New Ones, in Memory of William R. Thompson," *J. Microsc.* **143**, 3-45 (1986).

40. H.J.G. Gundersen, P. Bagger, T.F. Bendtsen, S.M. Evans, L. Korbo, N. Marcussen, A. Moller, K. Nielsen, J.R. Nyengaard, B. Pakkenberg, F.B. Sorensen, A. Vesterby, and M.J. West, "The New Stereological Tools: Disector, Fractionator and Point Sampled Intercepts and Their Use in Pathological Research and Diagnosis," *Acta Pathol. Microbiol. Immunol. Scand.* **96**, 857-881 (1988).

41. R.T. DeHoff, "Quantitative Serial Sectioning Analysis: Preview," *J. Microsc.* **131**, 259-263 (1983).

42. D.C. Sterio, "The Unbiased Estimation of Number and Sizes of Arbitrary Particles Using the Disector," *J. Microsc.* **134**, 127-136 (1984).

43. M.J. West, L. Slomianka, and H.J.G. Gundersen, "Unbiased Stereological Estimation of the Total Number of Neurons in the Subdivisions of Rat Hippocampus Using the Optical Fractionator," *Anat. Rec.* **231**, 482-492 (1991).

44. L.M. Cruz-Orive, "Particle Number Can Be Estimated Using a Dissector of Unknown Thickness: The Selector," *J. Microsc.* **145**, 121-142 (1987).

45. V. Howard, S. Reid, A.J. Baddeley, and A. Boyde, "Unbiased Estimation of Particle Density in the Tandem Scanning Reflected Light Microscope," *J. Microsc.* **138**, 203-212 (1985).

46. E. Thomson, "Quantitative Microscopic Analysis," *J. Geol.* **38**, 193 (1930).

47. E.R. Weibel, *Stereological Methods*, Vol. 2. Theoretical Foundations, (Academic Press, New York, NY, 1980).

48. C.S. Smith and L. Guttman, "Measurement of Internal Boundaries in Three-Dimensional Structures by Random Sectioning," *Trans AIME* **197**, 81-111 (1953).

49. S.L. Tomkeieff, "Linear Interceps, Areas and Volumes," *Nature* **155**, 24 (1945).

50. A.M. Gokhale, "Unbiased Estimation of Curve Length in 3D Using Vertical Slices," *J. Microsc.* **159**, 133-141 (1990).

51. A.M. Gokhale, "Estimation of Length Density L_V from Vertical Slices of Unknown Thickness," *J. Microsc.* **167**, 1-8 (1992).

52. L.M. Cruz-Orive, "Best Linear Unbiased Estimators for Stereology," *Biometrics* **36**, 595-605 (1980).

53. H. Elias and D.M. Hyde, "A Guide to Practical Stereology," (S. Karger A.G., Basel, Switzerland, 1983), pp. 16-24, 57-82.

54. H.J.G. Gundersen and R. Osterby, "Optimizing Sampling Efficiency of Stereological Studies in Biology, or "Do More Less Well!", *J. Microsc.* **121**, 65-73 (1981).

55. L. Chang, R.R. Mercer, K. Pinkerton, and J.D. Crapo, "Quantifying Lung Structure, Experimental Design and Biologic Variation in Various Models of Lung Injury," *Am. Rev. Respir. Dis.* **143**, 625-634 (1991).

56. E.E. Schneeberger-Keeley and M.J. Karnovsky, "The Ultrastructural Basis of Alveolar-Capillary Membrane Permeability to Peroxidase Used as a Tracer," *J. Cell Biol.* **37**, 781-793 (1968).

57. D. Sandoz, G. Nicolas, and M. Laine, "Two Mucous Cell Types Revisited After Quick-Freezing and Cryosubstitution," *Biol. Cell* **54**, 79-88 (1985).

58. O. Mathieu, H. Claassen, and E.R. Weibel, "Differential Effect of Glutaraldehyde and Buffer Osmolarity of Cell Dimensions: A Study on Lung Tissue," *J. Ultrastruc. Res.* **63**, 20-34 (1978).

59. C.G. Plopper, A.T. Mariassy, and L.O. Lollini, "Structure as Revealed by Airway Dissection: A Comparison of Mammalian Lungs," *Amer. Rev. Respir. Dis.* **128**, S4-S7 (1983).

60. J. Gil and E.R. Weibel, "Extracellular Lining of the Bronchioles After Perfusion Fixation of Rat Lungs for Electron Microscopy," *Anat. Rec.* **169**, 185-200 (1971).

61. R.F. Phalen, H.C. Yeh, G.M. Schum, and O.G. Raabe, "Application of an Idealized Model to Morphometry of the Mammalian Tracheobronchial Tree," *Anat. Rec.* **190**, 167-76 (1978).

62. D.M. Hyde, W.C. Hubbard, V. Wong, R. Wu, and C.G. Plopper, "Ozone-Induced Tracheobronchial Epithelial Injury: Relationship to Granulocyte Emigration in the Lung," *Am. J. Respir. Cell Mol. Biol.* **6**, 481-49 (1991).

63. C.G. Plopper and D. L. Dungworth, "Structure, Function, Cell Injury and Cell Renewal of Bronchiolar and Alveolar Epithelium," in E. M. McDowell (ed.), *Lung Carcinoma* (Churchill Livingstone, London, 1987) pp. 29-44.

The Role of Modulated Gap Junctional Intercellular Communication in Epigenetic Toxicology

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ABSTRACT

The normal development and health of all multicellular organisms, including the human being, depend on the adaptive maintenance of the integrity of the genetic information (e.g., DNA protective and repair mechanisms), as well as of the homeostatic and cybernetic regulatory systems within and between tissues. The primary focus of the past and current toxicological studies and risk assessment practices has been to ascertain and predict the "genotoxicity" of various physical and chemical agents. The paradigm of "carcinogen as mutagen," although valuable for stimulating studies of the detection of mutagens and of their potential role in "causing" somatic and germ line diseases, has tended to blunt research on the role of nongenotoxic mechanisms in disease causation.

This brief analysis will emphasize the need to consider the role of modulated gap junctional intercellular communication (GJIC) in any biological risk assessment model. It is based on the following assumptions and facts. Because gap junctions exist in all metazoans, they have been associated with the regulation of cell proliferation, development, differentiation, and the adaptive function of both excitable and nonexcitable coupled cells. A highly evolutionarily conserved family of genes codes for proteins (connexins), which, as hexameric units (connexons), form membrane-associated channels of gap junctions. Cells coupled by gap junctions will have their ions and small regulatory molecules equilibrated. Regulation of GJIC can be at the transcriptional, translational, or posttranslational levels. Transient down or up regulation of GJIC can be induced by endogenous or exogenous chemicals via many mechanisms at any of these three levels. Stable abnormal regulation has been associated with activated oncogenes, and normal regulation has been associated with several tumor-suppressor genes.

The dysfunction of these gap junctions might play a role in the actions of various toxic chemicals that have cell type/tissue/organ specificity. This could bring about distinct clinical consequences, such as embryo lethality or teratogenesis, reproductive dysfunction in the gonads, neurotoxicity of the central nervous system, hyperplasia of the skin, and tumor promotion of initiated tissue. Modulation of GJIC should be viewed as a scientific basis of "epigenetic toxicology" because the alteration of intercellular communication would alter the internal physiological state of the cell. The inhibition of GJIC is a necessary component of mitogenesis (a necessary component of the multistage carcinogenic process). The modulation of GJIC can have both toxicological, as well as therapeutic potential.

> "During evolution, long-lived multicellular organisms must have developed defense mechanisms to protect them against the carcinogenic and other deleterious effects of spontaneous mutations....Protagonists of the theory that cell replication leads to cancer do not deal with this aspect or explain how this barrier might be broken during tumor development."
>
> I.B. Weinstein (1)

INTRODUCTION

Biological Basis for Risk Assessment: What Factors are Involved?

Implicit in the acute and chronic exposure to radiations and chemicals is a risk to normal short- and long-term functioning of a multicellular organism and to its offspring. Predictions of the potential "toxicities" are being made on the bases of mathematical models based on incomplete empirical or epidemiological data, known or suspected mechanisms of action of the agent on a single level of biological organization, and extrapolations from a variety of laboratory animal studies (2). In lieu of complete understanding of how these toxic agents might act in the human being (which will never be possible), we are left with the challenge to develop risk assessment models that, at least, begin to approach having a biological basis (3).

The quotation of I.B. Weinstein serves to illustrate the point that, as toxicologists, we have a long way to go. One part of the problem we must recognize is that a multicellular organism, such as the human being, is not just a collection of 10^{14} independent cells in a hairy-covered bag made of skin. We are the result of the hierarchical process (4) of cybernetically interacting elements (5). Fundamentally, the health and adaptive ability (homeostasis) of a multicellular organism is based on a number of systems on the molecular, biochemical, cellular, tissue, organ, system (physiological, immune, nervous) levels (4). Biological organisms, both single and multicellular, have developed a series of adaptive mechanisms at each of these levels to survive the constant exposure, either acute or chronic, of radiations and

chemicals. The toxic end point at the cellular level could be mutation of the genome, cell death, or epigenetic alteration of the phenotype (5) (Figure 30).

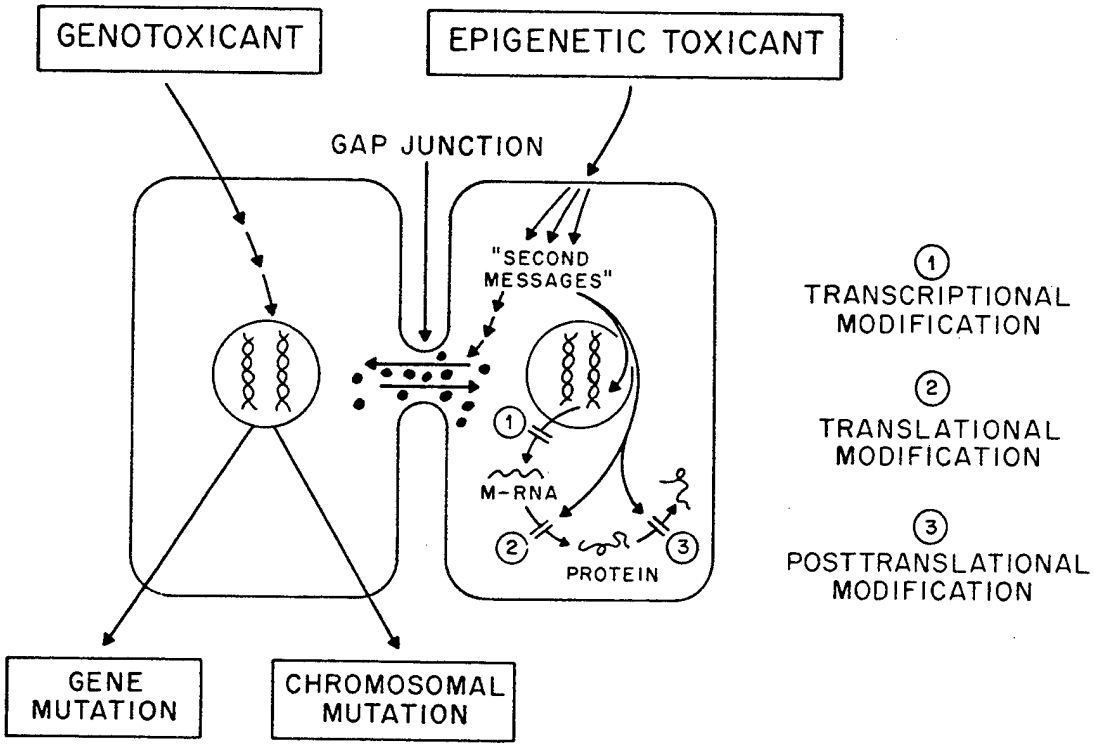


Figure 30. Diagram Illustrating the Difference Between Genotoxic and Epigenetic Chemicals. Those that alter the *quality* or *quantity* of genetic information are *genotoxic*, while those that affect the *expression* of the genetic information, at the transcriptional, translational, or posttranslational levels are *epigenetic* chemicals. (Reprinted from Trosko et al, in *In Vitro Methods of Toxicology*, G. Jolles and A. Cordier, eds, Academic Press, CA, 1992; used with permission).

The presence of melanin in the skin tissue or drug-metabolizing enzymes in the cell could protect the DNA from ultraviolet light or chemical-induced damage, respectively. DNA repair enzymes have the potential to either restore the genome to its original condition or, if not repaired or repaired in an error-prone fashion, can produce viable or nonviable mutations. Depending on the nature of the mutation, the

gene mutated, whether the mutated gene is expressed, or whether the mutated cell is replicated, the mutation might have little biological significance because of cellular redundancy or it might have devastating somatic or hereditary consequences (6). At the tissue level, cell death might or might not affect the homeostatic or adaptive status of the multicellular organism, depending on the number of cells, which cells die, and at what stage of development. For example, the death of one heptocyte might not even elicit a regenerative response in the liver. On the other hand, the death of the single stem or progenitor cell of a critical organ during development could have lethal or devastating teratogenic effects. The death of many cells could induce compensatory or regenerative hyperplasia, which, depending on the cause of the death (apoptosis [7] or induced cytotoxicity), could lead to natural tissue restructuring, wound healing, scar tissue formation, or tumor promotion (2). At the organ and system levels, control of both cell growth and differentiation is mediated by extracellular communication mechanisms (immune systems, neuroendocrine systems) (8). In other words, even if a viable mutation occurs in a single cell, if tissue, organ, and system suppression mechanisms prevent the product of that abnormal cell from disrupting homeostasis or prevent the mutated cell from clonally increasing, then these systems act as another protective barrier to maintain health and homeostasis.

Although the details of each of these barriers at the molecular, biochemical, cellular, and physiological levels have been recognized and studied to various degrees, one seems to have been largely ignored in the field of toxicology and risk assessment. How the multicellular organism suppresses the potential toxicological consequences of abnormal cell proliferation/differentiation of either normal or mutated cells will be the topic of this analysis. Specifically, the role of intercellular communication will be examined in the context of acting as a barrier to cell proliferation and how the modulation of intercellular communication could contribute to the disruption of homeostasis. This new concept will form the basis of the emerging field of epigenetic toxicology (9).

Current Problems in Risk Assessment

Fundamentally, extrapolations from data derived from *in vitro* and *in vivo* tests, as well as from human epidemiological data, are dependent on the theories of the disease end points in which one is interested, the limitations of each test system (assuming for the moment the validity of the design and execution of the experiment), and the assumptions of the extrapolations from the nonhuman test data to the human population/individual. Limitations of *in vitro* tests, especially those designed to detect genotoxic chemicals (10), and of *in vivo* bioassays (11-15) have been noted. Epidemiological approaches

are characterized by poor sensitivity and the inability to determine underlying mechanisms. Compounding the problem are the facts of interactions of physical and chemical exogenous agents with endogenous factors having genetic, developmental, and sex elements (16).

Recently, the new concept of molecular epidemiology has emerged (17). It is based on the assumption that if a given causative agent leaves a unique molecular "fingerprint" in the diseased tissues/cells/macromolecules/DNA, then one might predict and prevent these diseases. Although there exists some promise to this new approach, some of the same problems will plague this approach because it is based on some of the same assumptions and limitations of the previous approaches.

In order to limit this discussion, the end point of cancer will be used to illustrate the objective of this analysis. However, it should be obvious from this analysis that other disease end points, such as teratogenesis, reproductive dysfunction, immune dysfunction, neurotoxicity, cardiovascular diseases, and other disease states, will be involved.

Mutagenesis and Mitogenesis in Carcinogenesis

Many of the problems of risk assessment to cancer after exposure to radiation or chemicals come from the underlying assumption of the mechanism of carcinogenesis. It is now abundantly clear that no one thing "causes" cancer (18). Pathology information and epidemiological data show that human cancer is the result of multiple steps during the evolution of a normal cell to a metastatic cell (19). Although the role of mutations in cancer was well known to geneticists via the various hereditary predispositions to cancer (xeroderma pigmentosum, Downs, retinoblastoma, Wilms, Fanconi's, etc.), it took some time before a more general acceptance of the somatic mutation theory of cancer became commonly accepted. The introduction of the concept, carcinogens as mutagens (20), helped to spur the development of new interests, assays, and experiments into the detection of carcinogens. Identification of DNA lesions, such as pyrimidine dimers, being correlated with higher mutations and cancers in cells of sunlight-induced skin cancer-prone syndromes, xeroderma pigmentosum (21), together with the identification of electrophilic-induced DNA lesions in tissues capable of metabolizing chemicals (22), helped to shape the idea that mutations were responsible for cancer.

With the relatively recent identification of oncogenes (23), and more recently the tumor suppressor genes (24), more evidence was supplied to bolster the somatic mutation theory of cancer. With

modern molecular technology, it became clear that these important oncogenes and tumor suppressor genes in tumor cells were often mutated. However, as with most exciting new scientific theories, the beginning euphoria began to wear thin and the new paradigm was forced to address challenges. These challenges included the animal experiments showing the multistep nature of carcinogenesis (25) being conceptualized by the initiation, promotion, and progression stages. In addition, many of the so-called carcinogens in the animal bioassay test were shown to be nonmutagens in various *in vitro* assays presumably designed to detect mutagens (26). Furthermore, most of these animal tumor promoters also were shown to be nonmutagenic in these *in vitro* genotoxicity assays (10).

Recently, the idea emerged that because initiation of the carcinogenic process started in a single cell (evidenced by the irreversibility of the event in a single cell and the monoclonal nature of most tumors [27]), the ultimate appearance of a multicelled tumor from this single initiated cell must have been the result of the promotion process (28). In other words, promotion must be, at least, a mitogenic process for the initiated cell (9). Because many tumor promoters and promoting conditions (i.e., surgery, cell killing [29]) seemed to be associated with hyperplasia and cell proliferation, it seemed plausible to assume that mitogenesis is a necessary component of carcinogenesis (30). The idea was further supported by the findings that many tumor promoters acted as nongenotoxic agents in several *in vitro* tests for genotoxicity (31) and many were known to be mitogenic in either *in vitro* or *in vivo* systems, such as growth factors and hormones. In addition, if multiple genetic "hits" were needed for the carcinogenic process to occur, mitogenesis was necessary to complete the process because, by definition, a mutation is the hereditary transmission of an alteration of a change in the genome. Both spontaneous and induced mutations depend on mitogenesis to "fix" the alteration in DNA. However, as correctly pointed out by Weinstein (1), excessive cell proliferation, in and of itself, is probably not the causative factor in most cancers. The observations that additional exposures to initiating agents are necessary to convert promoted benign tumors to carcinomas also supports the notion that, although mitogenesis is a necessary component of carcinogenesis (32,33), it is an insufficient causative agent (34).

Gap Junctional Communication in Mitogenesis and Tumor Promotion

In order to explore the role of mitogenesis in carcinogenesis, particularly as to how the tumor promotion concept relates to it, the potential role of gap junctional communication in mitogenesis will be examined. The concept of contact inhibition (35) was created to explain the fact that most normal cells stop proliferating, *in vitro*, when they come in direct contact with each other. Cancer cells have been

characterized by their inability to inhibit their growth by cell-to-cell contact (36). With the demonstration that most, if not all, cancer cells exhibit some form of dysfunctional gap junctional intercellular communication (GJIC) (37), it seemed logical to link GJIC with the control of cell proliferation. In addition, many known endogenous and exogenous tumor-promoting chemicals have been shown to reversibly down-regulate GJIC, either at the transcriptional, translational, or posttranslational levels (31). Even physical tumor-promoting conditions, such as partial hepatectomy (38), have been associated with the down-regulation of GJIC during the regenerative phase (39). Cell killing, which does release extracellular mitogenic stimulating chemicals needed for regeneration, could indirectly cause the down-regulation of GJIC and bring about a tumor-promoting effect. Prostaglandins and their metabolites have been associated with the lysis of dead cells and the down-regulation of GJIC (40,41). Genotoxic agents, depending on the dose or concentration (42), could be either initiators or complete carcinogens. At noncytotoxic levels, these agents might primarily initiate. At cytotoxic levels, they would initiate as well as promote the surviving initiated cell. There also are nongenotoxic chemicals, such as alcohol or heptocytotoxic viruses, which could kill cells, thereby forcing compensatory hyperplasia of any surviving spontaneous or induced initiated cell. Many alcohols have been shown to inhibit GJIC near or at cytotoxic levels (43).

Although the evidence rigorously supporting the role of GJIC in contact inhibition and growth control has not yet been generated, there is strong inferential support for this hypothesis. Few, if any, cells that are gap junctionally coupled have been demonstrated to be dividing. In addition, strong correlations with the lack of GJIC have been shown with cells that are not gap junctionally coupled (see 34). Also, as previously noted, tumor-promoting chemicals, growth factors, or physical conditions, such as cell removal or killing, have been associated with both mitogenesis of the initiated cell and reduction of GJIC (31). The observations that some mitogens or mitogenic conditions are not promoters (44), are, in and of themselves, not rigorous proof that GJIC is not involved. Unless it can be shown that the mitogenic stimuli is both sustained (45) and influencing the initiated cell, mitogenic stimuli that are only transient and affecting only the noninitiated cell will not be a promoter. In addition, any mitogenic assay that indicated that a given chemical did not act as a mitogen in a given tissue/organ does not prove that the chemical might not be a tumor promoter because it might not act as a mitogen for normal cells but only for the few initiated cells in the tissue. The observation that phenobarbital can be a promoter in the liver is not determined by the observation that there exists a sustained hyperplasia in the liver, but by the selective clonal expansion of the initiated cells. The fact that apoptosis is inhibited by several agents that

are tumor promoters raises an interesting concept that the selective accumulation of initiated cells may well be the end result of the blockage of GJIC needed for controlled or programmed cell death (46). In other words, in closed organs, such as the liver, apoptosis is a necessary means to control the volume of the organ. A balance between cell birth and cell death might be necessary to maintain the stable number of cells (47). The inhibitions of GJIC could affect either or both the control of cell division or cell death.

Another line of evidence linking mitogenesis with GJIC comes from the field of oncogenes and tumor-suppressor genes. Protooncogenes and their activated counterparts, oncogenes, are defined as those genes controlling cell proliferation and differentiation (23). On the other hand, tumor-suppressor genes are those normal genes which, by definition, prevent cell proliferation (24). A number of oncogenes have now been associated with the down-regulation of GJIC and cell proliferation (48-54). Furthermore, several tumor-suppressor genes have been associated with the up-regulation of GJIC (55,56).

In recent years, several antagonists to promoters or anticarcinogens also have been associated with the up-regulation of GJIC. Retinoids (57,58), c-AMP (59,60), carotenoids (61), and lovastatin (62) have been linked with increased GJIC and decreased cell growth and restoration of a normal phenotype.

If, in fact, the lack of GJIC is causative of a lack of growth control and cancer, then it would be predicted that a restoration of GJIC in noncommunicating tumor cells should lead to growth control and a normal phenotype. Transfection of several noncommunicating tumor cells has led to the restoration of GJIC and, at least, partial growth control *in vitro* and *in vivo* (63-65).

Stem Cells – Gap Junctional Communication, Differentiation and Carcinogenesis

One of the critical assumptions to be made in cancer risk assessment involves the problem, Which cells are the target cells for the carcinogenic process? Is any cell in all tissues potentially capable of being converted to a cancer cell? Or, are there only a few special types of cells capable of tumorigenic transformation? One theory, namely, oncogeny as partially blocked ontogeny (66), supported by the theory of cancer as a disease of differentiation (67), leads one to hypothesize that stem cells are the target cells, since stem cells are defined as those giving rise to one cell that goes down a differentiation pathway and another cell retaining stem cell properties.

Evidence showing that only certain cells are transformable came from the observations by T'so and colleagues (68). They showed that only a few contact-insensitive cells were the ones that could be transformed by carcinogens. These contact-insensitive cells appear to be stem cells, which when transformed, have the ability to proliferate but are unable to control their growth or to differentiate. The ultimate stem cell, the fertilized egg or zygote, appears to lack GJIC. Only after the early stages of development do these cells express various gap junction genes (69), thereby creating a cellular mechanism for growth control, tissue compartmentalization, and differentiation. Based on these observations, a strategy to isolate normal kidney and breast epithelial stem cells from human tissue has been developed (70,71). The question now arises that if normal stem cells do not have GJIC and cancer cells do not have GJIC, then why aren't normal stem cells cancerous? The answer appears to be that normal stem cells can be induced to express GJIC very easily and they then become progenitor and differentiated cells. Moreover, they probably control their cell proliferative activity by extracellular communication mechanism, that is, extracellular negative growth regulators, such as TGF-β from the differentiated daughter cells, might suppress stem cell growth (72). If either the negative growth regulator is reduced or mutated, or the receptor on the stem cell is reduced or mutated, the stem cell could grow in an uncontrolled manner. If the stem cell cannot regulate its GJIC, it probably cannot differentiate properly. These might be the biological control points affected by the carcinogenic process.

A very significant observation has been made recently, in that tumorigenic and noncommunicating glioma cells could be growth arrested when cocultured with sister glioma cells transfected with a connexin43 gene (73). These transfected glioma cells, with the expressed connexin43, were able to establish GJIC. However, the growth arrest of the tumorigenic and noncommunicating glioma cells was via a soluble factor, produced by the newly communicating, transfected glioma cells, not by GJIC between the tumorigenic glioma cells (which do not have GJIC) and the transfected glioma cells. In other words, growth arrest was via an extracellular communication mechanism (negative growth regulator) between heterologous cells, produced by intercellular communication between homologous cells.

The implication of this study could explain (a) the selective nature of metastatic cells, and (b) the growth control of noncommunicating stem cells by communicating differentiated daughter cells. In the former example, if a noncommunicating tumor cell lands in a distal tissue which produces an effective negative growth regulator, the tumor cell would not grow. On the other hand, if the metastatic cell

invades a tissue that lacks a negative extracellular growth regulator, it would continue to proliferate. This could explain the "seed and soil" concept of tumor metastasis (74).

In the latter case, communicating differentiated cells could produce a negative growth regulator which restricts the stem cell from dividing. Removal or blockage of the source of the negative extracellular growth regulator would allow the stem cells to proliferate and differentiate.

If the preceding hypothesis is correct, then the relative number of stem cells in a given tissue during the developmental and aging process might change. The question that needs to be answered is, Are the number of stem cells in all tissues the same during development and aging? For example, open-ended tissues, such as the skin and lining of the gastrointestinal tract, have their stem cells constantly proliferating until death. On the other hand, organs, such as the lung and testes, must maintain their volume as well as replace lost cells. Other organs, such as the liver and kidney, must primarily maintain the volume of the organ once they finish their growth. The human breast might provide the evidence linking the stem cell as the target cell for carcinogenesis. Evidence for this comes from the observation that risk for breast cancer seemed to be highest in the young Japanese women exposed to the A-bomb radiation (75). One explanation might be that the number of stem cells of the breast tissue is highest in these women, particularly if they have never conceived. Pregnancy would be expected to deplete the stem cell pool by virtue of converting these stem cells to milk-producing terminally differentiated cells. Again, if this hypothesis is true, then there might be a need to consider the number of stem cells available for transformation in each tissue during each developmental stage of the individual. This would influence the initiation phase of carcinogenesis. One would predict that as one ages, the number of stem cells in some tissues would decrease, yet at the same time, as we age, the number of initiated stem cells in these tissues would increase (spontaneously or by induction). The critical issue to be considered then, would be the amount of promotion that occurs after initiation.

SUMMARY

Implications to Risk Assessment

Regulation of cell growth, development/differentiation, homeostasis, and adaptive responses to physical, chemical, and biological agents in a metazoan can occur at all levels of the biological hierarchy. At the cell level, extra-, intra-, and intercellular communication mechanisms help maintain homeostatic regulation of these important functions (Figure 31). These communication mechanisms have

evolved via evolution to be intimately integrated, such that perturbations of one communication mechanism will affect the other communication processes (76). In other words, hormonal-type extracellular communication can modulate GJIC via alterations in intracellular communication second messages, such as increases in c-AMP. The normal homeostatic control of these integrated communication processes can be disrupted by exogenous factors that either mimic, in part, the endogenous extracellular communicating signals or interfere with their ability to act. Stable interference of this integrative communication process also can occur when the genetic information coding for any of the three communication networks is either mutated, eliminated by cell removal/cell death, or epigenetically altered.

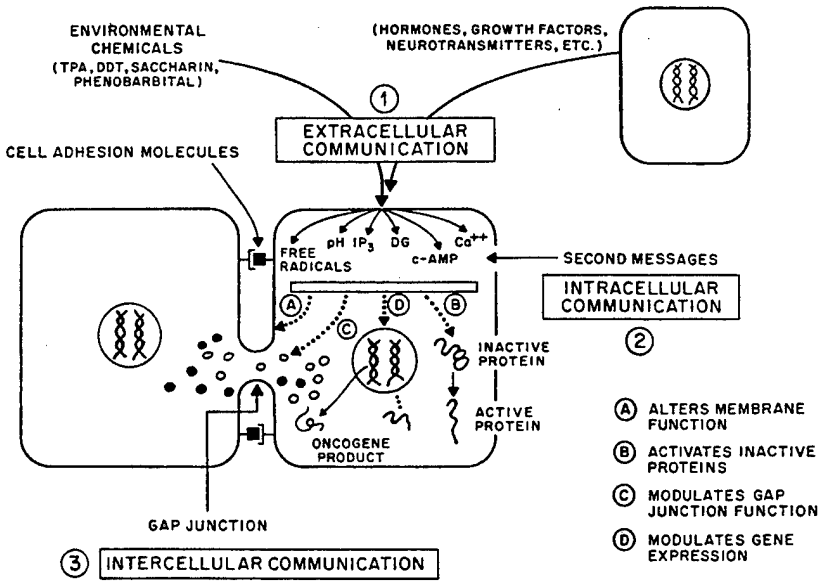


Figure 31. The Heuristic Schemata Characterized the Postulated Link Between Extracellular Communication and Intercellular Communication Via Various Intracellular Transmembrane Signalling Mechanisms. It provides an integrating view of how the neuroendocrine-immune system (mind or brain/body connection) and other multisystem coordinations could occur. Although not shown here, activation or altered expression of various oncogenes (antioncogenes) also could contribute to the regulation of gap junction function. (Reprinted from J.E. Trosko and C.C. Chang, *Toxicology Letters* 49:283-295, 1989, Elsevier Science Publisher; used with permission.)

The question of thresholds or lack of thresholds needed to alter the homeostasis at any of these levels (molecular to systems), which could bring about a disease state, needs to be answered. Resolution of this problem will not be easy. On one hand, protective systems, redundant genetic information and DNA repair systems seem to suggest that both mutagenesis and cell killing, as cellular end points, would demonstrate threshold responses. As to whether epigenetic events could occur with nonthreshold kinetics,

one can only speculate. If the homeostatic state of a cell in a multicellular organism is one in which the cell is either in the G state with a given set of genes expressed or a differentiated cell which is not responding to an adaptive stimuli, one can assume that the intercellular communication network has not been perturbed. Therefore, if intercellular communication is modulated, either up or down, one could expect the cells to respond by turning on or off the genes necessary for cell proliferation, cell differentiation, or adaptive differentiation responses. Evidence that gene expression is altered by alteration of intercellular communication is almost self evident. There is even evidence that threshold levels are needed both for tumor promotors and for modulators of GJIC (54,77-79).

To complicate the matter of the relevance of GJIC to risk assessment after exposure to toxic agents is the role of stem cells to various disease states that are the result of not one dysfunctional cell, but the consequence of a dysfunctional cell having been amplified in a given tissue so as to make its presence known to the whole body by its ability to disrupt homeostasis at the systems level. The number of stem cells in various tissue during aging is one crucial parameter and the accumulation of those stem cells that have been blocked in their ability to terminally differentiate, but not in the ability to proliferate, will be potential determinants of future disease states.

The end point of GJIC should be seriously considered in the assessment of toxic agent exposure. Abnormal GJIC has been associated with a wide variety of disease states. Because GJIC has been associated with development, differentiation, and wound healing (39,69,80), it should not be surprising to note that their dysfunction has been associated with teratogenesis (81), neurotoxicity (82), reproductive dysfunction (83), cardiovascular diseases (84), cataract formation (85), ischemia, hypertension (86), cholestasis (80), hereditary mucoepithelial dysplasia (87), as well as Chagas disease (88). These gap junctions exist in all tissues of the body. Each type of gap junction protein is probably regulated differently and in different cell types, the same gap junction might be regulated differently because of the physiological states of the different cell types. The interaction of multiple endogenous and/or exogenous chemicals could, by the net result of that interaction, synergistically, additively, or antagonistically modulate GJIC. To ignore this fundamental structure in the maintenance of homeostasis of health of the human being will be to ignore one of the parameters of a biologically based risk assessment model.

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REFERENCES

1. I.B. Weinstein, "Mitogenesis Is Only One Factor in Carcinogenesis," *Science* **251**, 387-388 (1991).

2. J.E. Trosko and C.C. Chang, "Implications for Risk Assessment of Genotoxic and Non-genotoxic Mechanisms in Carcinogenesis," in V.B. Vouk, G.C. Butler, D.G. Hoel, and D.B. Peakall (eds.), *Methods for Estimating Risk of Chemical Injury: Human and Non-Human Biota Ecosystems* (John Wiley and Sons, Chichester, England, 1985), pp. 181-200.

3. J.E. Trosko and C.C. Chang, "The Role of Inhibited Intercellular Communication in Carcinogenesis: Implications for Risk Assessment from Exposure to Chemicals," in C.C. Travis (ed.), *Biologically Based Methods for Cancer Risk Assessment* (Plenum Press, New York, 1989), pp. 165-179.

4. H. Brody, "A Systems View of Man: Implications for Medicine, Science and Ethics," *Perspect. Biol. Med.*, **17**, 71 (1973).

5. V.R. Potter, "Probabilistic Aspects of the Human Cybernetic Machine," *Perspect. Biol. Med.* **17**, 164 (1974).

6. J.E. Trosko and C.C. Chang, "The Role of DNA Repair Capacity and Somatic Mutations in Carcinogenesis and Aging," in H.T. Blumenthal (ed.), *Handbook of Diseases of Aging* (Van Nostrand Reinhold Co., New York, 1983), pp. 252-295.

7. A.H. Wyllie, "Apoptosis and the Regulation of Cell Numbers in Normal and Neoplastic Tissues," *Cancer Metastasis Rev.* **11**, 95-103 (1992).

8. J.E. Trosko and C.C. Chang, "Role of Intercellular Communication in Modifying the Consequences of Mutations in Somatic Cells," in D.M. Shankel, P.D. Hartman, T. Kada, and A. Hollaender (eds.), *Antimutagenesis and Anticarcinogenesis Mechanisms* (Plenum Publ., New York, 1986), pp. 439-456.

9. J.E. Trosko, C.C. Chang, and B.V. Madhukar, "*In Vitro* Analysis of Modulators of Intercellular Communication: Implications for Biologically Based Risk Assessment Models for Chemical Exposure," *Toxicol. In Vitro* **4**, 635-643 (1990).

10. J.E. Trosko, "A Failed Paradigm: Carcinogenesis Is More Than Mutagenesis," *Mutagenesis* **3**, 363-366 (1988).

11. G.R. Douglas, D.H. Blakey, and D.B. Clayson, "Genotoxicity Tests as Predictors of Carcinogens: An Analysis," *Mutat. Res.* **196**, 83-93 (1988).

12. C.C. Travis, S.A. Pichter Pack, A.W. Saulsbury, and N.W. Yambert, "Prediction of Carcinogenic Potency from Toxicological Data," *Mutat. Res.*, **241**, 21-36 (1990).

13. J.I. Goodman, Letter to Editor. *Molec. Carcinogenesis*, **5**, 247-248 (1992).

14. S.M. Cohen and L.B. Ellwein, "Cell Proliferation in Carcinogenesis," *Science* **249**, 1007-1011 (1990).

15. B.N. Ames and L.S. Gold, "Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis," *Science* **249**, 970-971 (1990).

16. J.E. Trosko, "Towards Understanding Carcinogenic Hazards: A Crisis in Paradigms," *J. Am. Coll. Toxicol.* **8**, 1121-1132, (1989).

17. P.G. Shields and C.C. Harris, "Molecular Epidemiology and the Genetics of Environmental Cancer," *J. Am. Med. Assoc.*, **266**, 681-687 (1991).

18. J.E. Trosko, "Does Radiation Cause Cancer?" in *RERF Update*, **4**, 3-5 (1992).

19. J. Cairns, "Mutation and Selection and the Natural History of Cancer," *Nature* **225**, 197-200 (1975).

20. B.N. Ames, W.E. Durston, E. Yamasaki, and F.D. Lee, "Carcinogens Are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection," *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2281-2285 (1973).

21. V.M. Maher and J.J. McCormick, "Effect of DNA Repair on the Cytotoxicity and Mutagenicity of UV Irradiation and of Chemical Carcinogens in Normal and Xeroderma Pigmentosum Cells," in J.M. Yuhas, R.W. Tennant and J.D. Regan (eds.), *Biology of Radiation Carcinogenesis*, (Raven Press, New York, 1976), pp.129-145.

22. M.W. Lieberman, R.N. Baney, R.E. Lee, S. Sell, and E. Farber, "Studies on DNA Repair in Human Lymphocytes Treated with Ultimate Carcinogens and Alkylating Agents," *Cancer Res.* **3**, 1292-1306 (1971).

23. R.A. Weinberg, "The Action of Oncogenes in the Cytoplasm and Nucleus," *Science* **230**, 770-776 (1985).

24. A.G. Knudson, "Hereditary Cancer, Oncogenes, and Antioncogenes," *Cancer Res.* **45**, 1437-1443 (1985).

25. H.C. Pitot, T. Goldsworthy, and S. Moran, "The Natural History of Carcinogenesis: Implications of Experimental Carcinogenesis in the Genesis of Human Cancer," *J. Supramol. Struct. Cellul. Biochem.*, **17**, 133-146 (1981).

26. R.W. Tennant, B.H. Margolin, M.D. Shelby, E. Zeiger, J.K. Haseman, J. Spalding, W. Caspary, M. Resnick, S. Stasiewicz, B. Anderson, and R. Minor, "Prediction of Chemical Carcinogenicity in Rodents from *In Vitro* Genetic Toxicity Assays," *Science* **236**, 933-941 (1987).

27. P.J. Fialkow, "Clonal Origin and Stem Cell Evolution of Human Tumors," in J.J. Mulvihill, R.W. Miller and J.F. Fraumeni, (eds.), *Genetics of Human Cancer*, (Raven Press, New York, 1977), pp. 439-453.

28. J.E. Trosko, C.C. Chang, and B.V. Madhukar, "Cell-Cell Communication: Relationship of Stem Cells to the Carcinogenic Process," in D.E. Stevenson, J.A. Popp, J.M. Ward, R.M. McClain, T.J. Slaga and H.C. Pitot (eds.), *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons*, (Alan R. Liss, Inc., New York, 1990), pp. 259-276.

29. T.S. Argyris, "Tumor Promotion by Regenerative Epidermal Hyperplasia in Mouse Skin," *J. Cutaneous Pathol.*, **9**, 1-18 (1982).

30. J.E. Trosko, C.C. Chang, and A. Medcalf, "Mechanisms of Tumor Promotion: Potential Role of Intercellular Communication," *Cancer Invest.* **1**, 511-526 (1983).

31. J.E. Trosko, and C.C. Chang, "Nongenotoxic Mechanisms in Carcinogenesis: Role of Inhibited Intercellular Communication," in R.W. Hart and F.G. Hoerger, (eds.), Banbury Report 31: *Carcinogen Risk Assessment: New Directions in the Qualitative and Quantitative Aspects* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988), pp. 139-170.

32. V.R. Potter, "Use of Two Sequential Applications of Initiators in the Production of Hepatomas in the Rat: An Examination of the Solt-Farber Protocol," *Cancer Res.*, **44**, 2733-2736, (1984).

33. H. Hennings, R. Shores, M.L. Wenk, E.F. Spangler, R. Tarone, and S.H. Yuspa, "Malignant Conversion of Mouse Skin Tumors is Increased by Tumor Initiators and Unaffected by Tumor Promoters," *Nature,* **304**, 67-69 (1983).

34. J.E. Trosko, B.V. Madhukar, and C.C. Chang, "Endogenous and Exogenous Modulation of Gap Junctional Intercellular Communication: Toxicological and Pharmacological Implications," *Life Sciences,* **53**, 1-19 (1993).

35. E.M. Levine, Y. Becker, C.W. Boone, and H. Eagle, "Contact Inhibition, Macromolecular Synthesis, and Polyribosomes in Cultured Human Diploid Fibroblasts," *Proc. Natl. Acad. Sci. U.S.A.* **53**, 350-355 (1965).

36. M. Abercrombie, "Contact Inhibition and Malignancy," *Nature* **281**, 259-262 (1979).

37. W.R. Loewenstein, "Permeability of Membrane Junctions," *Ann. NY Acad. Sci.* **137**, 441-472 (1966).

38. A.W. Pound and L.J. McGuire, "Repeated Partial Hepatectomy as a Promoting Stimulus for Carcinogenic Response of Liver to Nitrosamines in Rats," *Br. J. Cancer* **37**, 585-594 (1978).

39. S.B. Yancey, D. Easter, and J.P. Revel, "Cytological Changes in Gap Junctions During Liver Regeneration," *J. Ultrastruct. Res.* **67**, 229-242 (1979).

40. K. D. Massey, B.N. Minnich, and J.M. Burt, "Arachidonic Acid and Lipoxygenase Metabolites Uncouple Neonatal Rat Cardiac Myocyte Pairs," *Am. J. Physiol.* **263**, C494-C501 (1992).

41. R. Agarwal, and E.E. Daniel, "Control of Gap Junction Formation in Canine Trachea by Arachidonic Acid Metabolites," *Am. J. Physiol.* **250**, 495-505 (1986).

42. J.E. Trosko, C. Jone, and C.C. Chang, "The Role of Tumor Promoters on Phenotypic Alterations Affecting Intercellular Communication and Tumorigenesis," *Ann. NY Acad. Sci.* **407**, 316-327 (1983).

43. T.H. Chen, T.J. Kavanagh, C.C. Chang, and J.E. Trosko, "Inhibition of Metabolic Cooperation in Chinese Hamster V79 Cells by Various Organic Solvents and Simple Compounds," *Cell Biol. Toxicol.* **1**, 155-171 (1984).

44. A. Columbano, G.M. Ledda-Columbano, M.G. Ennas, M. Curto, A. Chelo, and P. Pani, "Cell Proliferation and Promotion of Rat Liver Carcinogenesis: Different Effect of Hepatic Regeneration and Mitogen Induced Hyperplasia on the Development of Enzyme-Altered Foci," *Carcinogenesis* **11**, 771-776 (1990).

45. E.E. Sisskin, T. Gray, and J.C. Barrett, "Correlation Between Sensitivity to Tumor Promotion and Sustained Epidermal Hyperplasia of Mice and Rats," *Carcinogenesis* **3**, 403-407 (1982).

46. G. Rodrigueztarduchy, and A. Lopezrivas, "Phorbol Esters Inhibit Apoptosis in IL-2-Dependent Lymphocytes-T," *Bioc. Biop. R.* **164**, 1069-1075 (1989).

47. Moolgavkar, S.H. "Multistage Models for Cancer Risk Assessment," in C.C. Travis (ed.), *Biologically Based Methods for Cancer Risk Assessment* (Plenum Press, New York, 1989), pp. 9-20.

48. M.M. Atkinson, A.S. Menko, R.G. Johnson, I.R. Sheppard, and J.D. Sheridan, "Rapid and Reversible Reduction of Junctional Permeability in Cells Infected with a Temperature Sensitive Mutant of Avian Sarcoma Virus," *J. Cell Biol.* **9**, 573-578 (1981).

49. R. Azarnia and W.R. Loewenstein, "Intercellular Communication and the Control of Growth: Alteration of Junctional Permeability by the SRC Gene – A Study with Temperature-Sensitive Mutant Rous Sarcoma Virus," *J. Membr. Biol.* **82**, 191-205 (1984).

50. C.C. Chang, J.E. Trosko, H.J. Kung, D. Bombick, and F. Matsumura, "Potential Role of the SRC Gene Product in Inhibition of Gap Junctional Communication in NIH 3T3 Cells," *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5360-5364 (1985).

51. M.H. El-Fouly, J.E. Trosko, C.C. Chang, and S.T. Warren, "Potential Role of the Human Ha-*ras* Oncogene in the Inhibition of Gap Junctional Intercellular Communication," *Molec. Carcinogenesis*, **2**, 131-135 (1989).

52. M.H. El-Fouly, J.E. Trosko, and C.C. Chang, "Phenotypic Transformation and Inhibition of Gap-Junctional Intercellular Communication in Epithelial and Mesenchymal Cells by the Neu Oncogene," (Abstract, 4th Annual Oncogene Meeting, Frederick, MD: July 5-9, 1988).

53. G.H. Kalimi, L.L. Hampton, J.E. Trosko, S.S. Thorgeirsson, and A.C. Huggett, "Homologous and Heterologous Gap-Junctional Intercellular Communication in V-raf-, V-myc-, and V-raf/V-myc-Transducted Rat Liver Epithelial Cell Lines," *Molec. Carcinogenesis* **5**, 301-310 (1992).

54. A.W. de Feijter, J.S. Ray, C.M. Weghorst, J.E. Klaunig, J.I. Goodman, C.C. Chang, R.J. Ruch, and J.E. Trosko, "Infection of Rat Liver Epithelial Cells with V-Ha-ras: Correlation Between Oncogene Expression, Gap Junctional Communication, and Tumorigenicity," *Molec. Carcinogenesis* **3**, 54-67 (1990).

55. G. Kalimi, C.C. Chang, P. Edwards, E. Dupont, B.V. Madhukar, E. Stanbridge, and J.E. Trosko, "Re-establishment of Gap Junctional Communication in a Non-tumorigenic Hela-normal Human Fibroblast Hybrid," *Proc. Am. Assoc. Cancer Res.* **31**, 319 (1990).

56. S.W. Lee, C. Tomasetto, and R. Sager, "Positive Selection of Candidate Tumor-Suppressor Genes by Subtractive Hybridization," *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2825-2829 (1991).

57. P.P. Mehta, J.S. Bertram, and W.R. Loewenstein, "The Actions of Retinoids on Cellular Growth Correlate with Their Actions on Gap Junctional Communication," *J. Cell Biol.* **108**,1053-1065 (1989).

58. M. Rogers, J.M. Berestecky, M.Z. Hossain, H.M. Guo, R. Kadle, B.J. Nicholson, and J.S. Bertram, "Retinoid-Enhanced Gap Junctional Communication is Achieved by Increased Levels of Connexin-43 Messenger RNA and Protein," *Molec. Carcinogenesis* **3**, 335-343 (1990).

59. R. Azarnia and T.R. Russell, "Cyclic AMP Effects on Cell to Cell Junctional Membrane Permeability During Adipocyte Differentiation of 3T3-L1 Fibroblasts," *J. Cell Biol.* **100**, 65-269 (1985).

60. A.M.G.L. Demaziere and D. W. Scheuerman, "Increased Gap Junctional Area in the Rat Liver After Administration of Dibutyl cAMP," *Cell Tiss. Res.* **239**, 651-655 (1985).

61. L.X. Zhang, R.V. Cooney, and J.S. Bertram, "Carotenoids Enhance Gap Junctional Communication and Inhibit Lipid Peroxidation in C3H/10T1/2 Cells: Relationship to Their Cancer Chemopreventive Action," *Carcinogenesis* **12**, 2109-2114 (1991).

62. R.J. Ruch, B.V. Madhukar, J.E. Trosko, and J.E. Klaunig, "Reversal of ras-Induced Inhibition of Gap Junctional Intercellular Communication, Transformation and Tumorigenesis by Lovastatin," *Molec. Carcinogenesis*, in press (1992).

63. G.I. Fishman, A.P. Moreno, D.C. Spray, and L.A. Leinwand, "Functional Analysis of Human Cardiac Gap Junction Channel Mutants," *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3525-3529 (1991).

64. D. Zhu, S. Caveney, G.M. Kidder, and C.C.G. Naus, "Transfection of C6 Glioma Cells with Connexin-43 cDNA: Analysis of Expression, Intercellular Coupling, and Cell Proliferation," *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1883-1887 (1991).

65. B. Eghbali, J.A. Kessler, and D.C. Spray, "Expression of Gap Junction Channels in Communication-Incompetent Cells After Stable Transfection with cDNA Encoding Connexin-32," *P. Nas. US.* **87**, 1328-1331 (1990).

66. V.R. Potter, "Phenotypic Diversity in Experimental Hepatomas: The Concept of Partially Blocked Ontogeny," *Br. J. Cancer* **38**, 1-23 (1978).

67. C. Markert, "Neoplasia: A Disease of Cell Differentiation," *Cancer Res.* 28, 1908-1914 (1968).

68. S. Nakano, H. Ueo, S.A. Bruce, and P.O.P Ts'o, "A Contact-Insensitive Subpopulation in Syrian Hamster Cell Cultures with a Greater Susceptibility to Chemically Induced Neoplastic Transformation," *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5005-5009 (1985).

69. C.W. Lo, "Communication Compartmentation and Pattern Formation in Development," in M.V.L. Bennett and D.C. Spray (eds.), *Gap Junctions* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1985), pp. 251-264.

70. C.C. Chang, J.E. Trosko, M.H. El-Fouly, R.E. Gibson-D'Ambrosio, and S.M. D'Ambrosio, "Contact Insensitivity of a Subpopulation of Normal Human Fetal Kidney Epithelial Cells and of Human Carcinoma Cell Lines," *Cancer Res.* **47**, 1634-1645 (1987).

71. C.C. Chang, S. Nakatsuka, G. Kalimi, J.E. Trosko, and C.W. Welsch, "Characterization of Two Types of Normal Human Breast Epithelial Cells That Are Either Deficient or Proficient in Gap Junctional Intercellular Communication," *J. Cell Biochem.* Supplement **14B**, 331 (1990).

72. J. Keski and H.C. Moses, "Growth Inhibitory Polypeptide in the Regulation of Cell Proliferation," *Med. Biol.* **65**, 13-20, (1987).

73. D.G. Zhu, G.M. Kidder, S. Caveney, and C.C.G. Naus, "Growth Retardation in Glioma Cells Cocultured with Cells Overexpressing a Gap Junction Protein," *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10218-10221 (1992).

74. S. Paget, "The Distribution of Secondary Growth in Cancer of the Breast," *Lancet* **1**, 571-573 (1889).

75. M. Tokunaga, C.E. Land, and S. Tokuoka, "Follow-Up Studies of Breast Cancer Incidence Among Atomic Bomb Survivors," *J. Radiat. Res.* (Suppl.) **32**, 201-211 (1991).

76. J.E. Trosko, C.C. Chang, B.V. Madhukar, and J.E. Klaunig, "Chemical, Oncogene and Growth Factor Inhibition of Gap Junction Intercellular Communication: An Integrative Hypothesis of Carcinogenesis," *Pathobiol.* **58**, 265-278 (1990).

77. A.K. Verma, and R.K. Boutwell, "Effects of Dose and Duration of Treatment with the Tumor Promotiong Agent, TPA, on Mouse Skin Carcinogenesis," *Carcinogenesis* **1**, 271-276 (1986).

78. T. Goldsworthy, H. Campbell, and H.C. Pitot, "The Natural History and Dose-Response Characteristics of Enzyme-Altered Foci in Rat Liver Following Phenobarbital and Diethylnitrosamine Administration," *Carcinogenesis* **5**, 67-71 (1984).

79. E. Deml and D. Oesterle, "Dose Response of Promotion by Polychlorinated Biphenyls and Chloroform in Rat Liver Foci Bioassay," *Arch. Toxicol.* **60**, 209-211 (1987).

80. O. Traub, P.M. Druge, and K. Willecke, "Degradation and Resynthesis of Gap Junction Protein in Plasma Membranes of Regenerating Liver After Partial Hepatectomy or Cholestasis," *Proc. Natl. Acad. Sci. U.S.A.* **80**, 755-759 (1983).

81. A.E. Warner, S.C. Guthrie, and N.B. Gilula, "Antibodies to Gap Junctional Protein Selectively Disrupt Junctional Communication in the Early Amphibian Embryo," *Nature* **311**, 127-131 (1984).

82. J.E. Trosko, C. Jone, and C.C. Chang, "Inhibition of Gap Junctional-Mediated Intercellular Communication *In Vitro* by Aldrin, Dieldrin, and Toxaphene: A Possible Cellular Mechanism for Their Tumor-Promotiong and Neurotoxic Effects," *Molec. Toxicol.* **1**, 83-93 (1987).

83. Y.X. Ye, D. Bombick, K. Hirst, G.X. Zhang, C.C. Chang, J.E. Trosko, and T. Akera, "The Modulation of Gap Junctional Communication by Gossypol in Various Mammalian Cell Lines *In Vitro*," *Fundam. Appl. Toxicol.* **14**, 817-832 (1990).

84. J.E. Saffitz, R.H. Hoyt, R.A. Luke, H.L. Kanter, and E.C. Beyer, "Cardiac Myocyte Interconnections at Gap Junctions - Role in Normal and Abnormal Electrical Conduction," *Trend. Cardiovasc. Med.* **2**, 56-60 (1992).

85. T. Tanaka, M. Sakai, K. Fujimoto, and K. Ogawa, "Morphometric Analysis of Gap Junctions in the Rat Lens During Cataract Formation," *Acta Histochem. Cytochem.* **23**, 781-792 (1990).

86. J.H. Smith, C.R. Green, N.S. Peters, S. Rothery, and N.J. Severs, "Altered Patterns of Gap Junction Distribution in Ischemic Heart Disease - An Immunohistochemical Study of Human Myocardium Using Laser Scanning Confocal Microscopy," *Am. J. Pathol.* **139**, 801-821 (1991).

87. C.J. Witkop, J.G. White, R.A. King, M.V. Dahl, W.G. Young, and J.J. Sauk, "Hereditary Mucoepithelial Dysplasia: A Disease Apparently of Desmosome and Gap Junction Formation," *Am. J. Hum. Genet.* **31**, 414-427 (1979).

88. A.C. Campos de Carvalho, H.B. Tanowitz, M. Wittner, R. Dermietzel, C. Roy, E.L. Hertzberg, and D.C. Spray, "Gap Junction Distribution is Altered Between Cardiac Myocytes Infected with Trypanosoma Cruzi," *Circ. Res.* **70**, 733-742 (1992).

Cell Proliferation and Formaldehyde-Induced Respiratory Carcinogenesis

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ABSTRACT

Formaldehyde is a nasal carcinogen in the rat, but the cancer risk this chemical poses for humans remains to be determined. Formaldehyde induces nonlinear, concentration-dependent increases in nasal epithelial cell proliferation and DNA-protein cross-link formation following short-term exposure. Presented in this review are results from a mechanistically based formaldehyde inhalation study in which an important end point was the measurement of cell proliferation indices in target sites for nasal tumor induction. Male Fischer 344 rats were exposed to 0, 0.7, 2, 6, 10, or 15 ppm formaldehyde for up to 2 years (6 h/day, 5 days/wk). Statistically significant increases in cell proliferation were confined to the 10 and 15 ppm groups and remained elevated throughout the study. The concentration-dependent increases in cell proliferation correlated strongly with the tumor response curve, supporting the proposal that sustained increases in cell proliferation are an important component of formaldehyde carcinogenesis. The nonlinearity observed in formaldehyde-induced rodent nasal cancer is consistent with a high-concentration effect of regenerative cell proliferation of the target organ coupled with the genotoxic effects of formaldehyde. Cell kinetic data from these studies provide important information that may be utilized in the assessment of risk for humans exposed to formaldehyde.

CELL PROLIFERATION AND CANCER

The complex process of cancer develops secondary to one or more mutational events that alter growth regulatory genes of normal cells, with subsequent clonal growth of the resulting precancerous or cancerous cells (1,2). Cell proliferation, an essential component of the multistage process of carcinogenesis, is required for both initiation and promotion of neoplasia in certain organs, and it plays an essential role in the later stages of carcinogenesis, including the progression of benign lesions to malignancy and metastasis (3,4). Each time a cell divides, there is a chance, albeit rare, that a mutational

event related to the carcinogenic process will occur (6,7). Enhanced cell proliferation may increase the frequency of these spontaneous mutations either by errors in replication or by the conversion of endogenous or exogenous DNA adducts to mutations before DNA repair can occur (8). Chemicals that induce cytotoxicity and sustained increases in cell proliferation, therefore, could enhance the likelihood of cancer development by providing additional cell divisions, each with an opportunity for spontaneous or chemically induced mutations (7, 9).

Epidemiologic evidence indicates that increased cell proliferation induced by external or internal stimulation is a common denominator in the pathogenesis of many human cancers. Prolonged irritation by physical or chemical agents may cause cell death, and the subsequent cell division that occurs during repair of the damaged tissue may eventually lead to a cancer at the irritated site (10). For example, tobacco, an established carcinogen, is a well-known irritant. Snuff users develop leukoplakia, and eventually, cancer of the buccal mucosa at the site of snuff application. Tobacco smoke is a local irritant to the epithelial tissue lining the bronchi, lungs, larynx, pharynx, oral cavity, and esophagus, sites where smoking-related cancers arise.

Many chemicals identified as carcinogenic for humans are genotoxic and also have been determined to induce cancer in laboratory animals (11). The primary biological activity of a genotoxic chemical or its metabolite, is the alteration of the genetic information encoded in the DNA, inducing a mutation in growth regulatory genes (12). A genotoxic chemical administered at a dose that is both cytotoxic and a cell proliferation enhancer, would be expected to be a more effective mutagen and carcinogen than when given at a noncytotoxic dose which does not induce cell proliferation (7,8). In addition to regenerative cell proliferation and cytotoxicity, other cellular responses such as metabolic activation and DNA repair also could greatly affect the carcinogenic response of a target tissue to a given dose of a chemical (9). Understanding the relationship between chemically induced cell proliferation and carcinogenic activity would be of value in the investigation of mechanisms of carcinogenesis, the selection of appropriate doses for cancer bioassays, and the improvement of risk assessment models (13).

Research with some respiratory carcinogens, such as formaldehyde gas, illustrates the principle that both genotoxicity and enhanced cell proliferation of the target organ should be considered in mechanistic studies and the improvement of risk assessment models. An extensive database on cell proliferation and the induction of upper respiratory tract tumors has been generated from a long-term,

mechanistically based, formaldehyde pathogenesis study in the Fischer 344 (F-344) rat (14). This review presents time-course and concentration-response data on site-specific increases in nasal epithelial cell proliferation and compares these data with the formaldehyde-induced tumor response. The application of these data in improving the risk assessment process also will be addressed.

FORMALDEHYDE

Formaldehyde is an important commodity chemical used widely in the manufacture of resins, particle board, plywood, textiles, and many other consumer products. The finding that formaldehyde is a nasal carcinogen in rats (15-17) has provoked concern that this chemical also may pose a cancer risk for humans. Although there is widespread exposure of humans to formaldehyde, epidemiological data for exposed individuals are suggestive, but still short of conclusive with respect to any causal association between formaldehyde exposure and nasal cancer incidence (18-21). This finding has stimulated a series of research projects aimed at understanding the mechanisms involved in formaldehyde-induced toxicity and carcinogenesis.

The genotoxic effects of formaldehyde have been extensively reviewed (22). Formaldehyde induces gene mutations in many organisms including bacteria, fungi, yeast, fruit flies, and in cultured mammalian cells. Formaldehyde also has been shown to induce single-strand DNA breaks, sister chromatid exchanges, and chromosomal aberrations in a variety of cultured mammalian cells, including human bronchial cells (23).

Formaldehyde induces a variety of toxic effects in experimental animals. It is a potent upper respiratory irritant and cytotoxicant that is almost entirely deposited in the anterior nasal cavity of rodents (24). Metabolized in the nasal mucosa, formaldehyde reacts covalently with DNA, RNA, and proteins. The covalent reactions of formaldehyde with macromolecules are generally accepted as the fundamental causes of its toxic effects (24).

In the chronic formaldehyde bioassay (17), the relationship between the incidence of nasal tumors in rats and the concentration of formaldehyde was distinctly nonlinear. At 2 ppm, no nasal tumors were present, whereas between 5.6 and 14.3 ppm, the tumor incidence increased 50-fold as exposure concentration rose less than 3-fold. Other concentration-dependent responses in rats exposed to

formaldehyde include inhibition of mucociliary function, cytotoxicity, inflammation, and induction of DNA-protein cross-links (24-25).

Molecular dosimetry studies in rats exposed to a range of formaldehyde concentrations using a DNA-protein cross-linking assay, have shown that formaldehyde induces cross-links in rat nasal respiratory mucosa following exposure to ≥ 2 ppm formaldehyde (26). The rate of formation of these cross-links is a nonlinear function of the airborne formaldehyde concentration, increasing more rapidly at high than at low concentrations (Figure 32). The yield of cross-links at a given exposure concentration is probably determined by the ability of host defense mechanisms, such as metabolism and DNA repair, to maintain the integrity of the DNA. Companion studies by Casanova and Heck Hd'A (27), investigating glutathione mediated metabolism of formaldehyde, have shown that saturable metabolism of formaldehyde is an important defense mechanism against the formation of cross-links. Currently, DNA-protein cross-links are used as a measure of formaldehyde dose at the site of tumor formation (28).

FORMALDEHYDE-INDUCED CELL PROLIFERATION

Increased nasal epithelial cell proliferation, another important biological response of the rat exposed to formaldehyde, is a sensitive indicator of respiratory epithelial cell toxicity (29). Increased cell proliferation in response to formaldehyde exposure also has been demonstrated in nonhuman primates (30) and xenotransplanted human nasal respiratory epithelium (31). However, little is known about the mechanisms of these proliferative responses, which may involve autocrine and paracrine growth factors, mutation in growth regulatory genes, and/or regenerative stimuli brought about by death of adjacent cells (6, 32-34).

Epithelial injury with consequent hyperplasia is a common feature of many chemically induced toxic responses, including those induced by formaldehyde gas. Following cytotoxic insult, epithelium of the upper respiratory tract, including the nasal passages, may undergo a dynamic series of alterations to include hyperplasia, metaplasia, dysplasia, carcinoma *in situ* or intraepithelial neoplasia, and carcinoma (35). These alterations are all characterized by a common feature, increased cell proliferation (35).

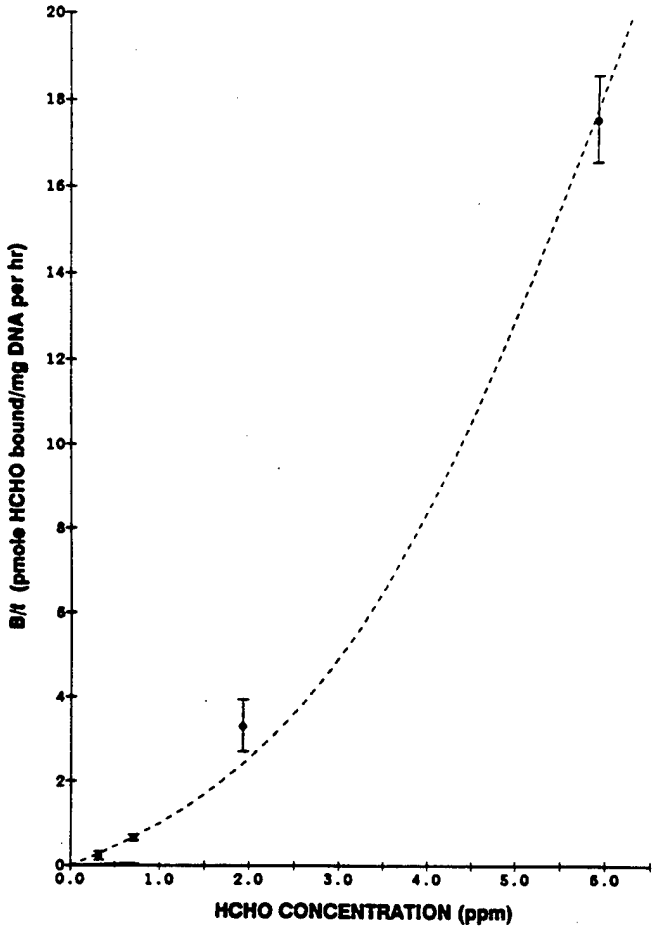


Figure 32. **The Average Concentration of DNA-Protein Cross-Links (B), Formed Per Unit Time (t), in the Anterior Nasal Mucosa of F-344 Rats Versus the Airborne Concentration of 0.7, 2, 6, or 10 ppm of [^{14}C] Formaldehyde.** (Modified from Casanova et al., *Fundam. Appl. Pharmacol.*, 12, 397, 1989)

Recent studies have demonstrated that formaldehyde-induced lesions and increases in cell proliferation in rats following acute (1, 4, or 9 days or 6 weeks), or subchronic (3 month) exposure were concentration-dependent. Nasal epithelial lesions occurred in specific regions of the anterior nasal passages, primarily the walls of the lateral meatus, mid-septum, and medial aspect of the maxilloturbinate (29, 36) (Figure 33). The increases in nasal cell proliferation were associated with formaldehyde-induced nasal lesions, which included epithelial degeneration, necrosis, hyperplasia, and squamous metaplasia.

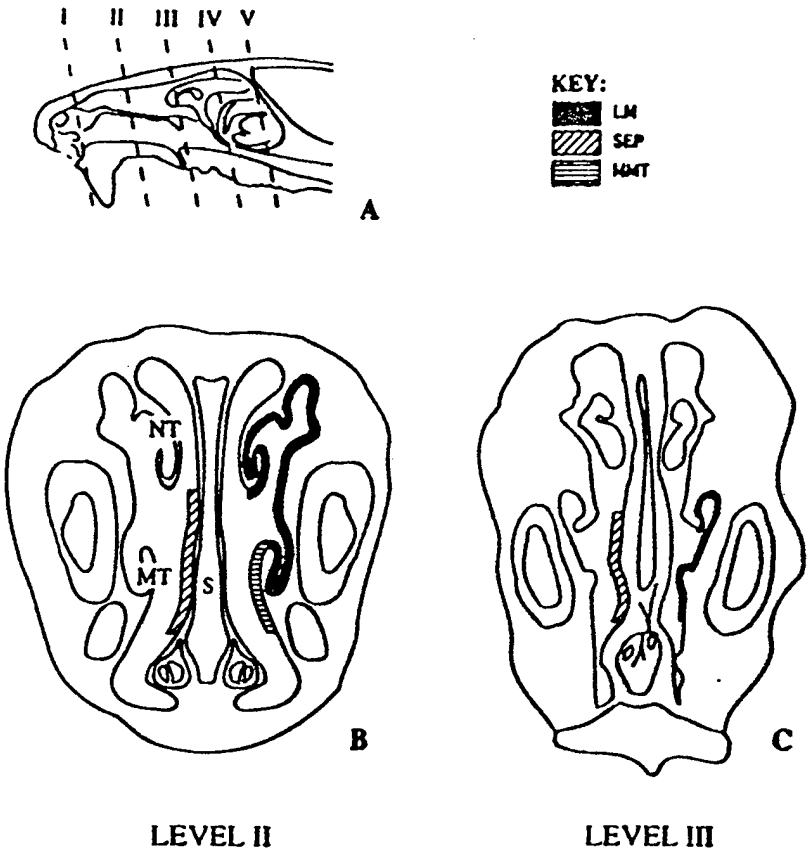


Figure 33. **Diagram Depicting (A) Lateral View of the Rat Nose and Levels I to V of the Nasal Passages. (B) Level II and (C) Level III Demonstrate the Sites Selected for Cell Proliferation Studies.** NT, nasoturbinate; MT, maxilloturbinate; S, septum. (Reprinted from Monticello et al., *Toxicol. Appl. Pharmacol.*, 111, 409, 1991, with permission.)

Increased nasal cell proliferation was present in rats exposed to 6, 10, or 15 ppm formaldehyde following up to 6 weeks of exposure (Figure 34), whereas no increases were detected in the 0.7 or 2 ppm groups (29). After 3 months of exposure, increases in cell proliferation were confined to only the 10 and 15 ppm groups (14). These short-term studies demonstrated that 0.7 and 2 ppm formaldehyde do not induce increases in nasal cell proliferation and that 6 ppm formaldehyde induces transient increases in cell proliferation that return to control levels by 3 months. The transient increase in cell proliferation observed at 6 ppm emphasizes the importance of evaluating multiple time points during a cell proliferation study. Time-course data on site-specific increases in cell proliferation provide important information that is necessary for certain biologically based risk assessment models of formaldehyde carcinogenesis (37).

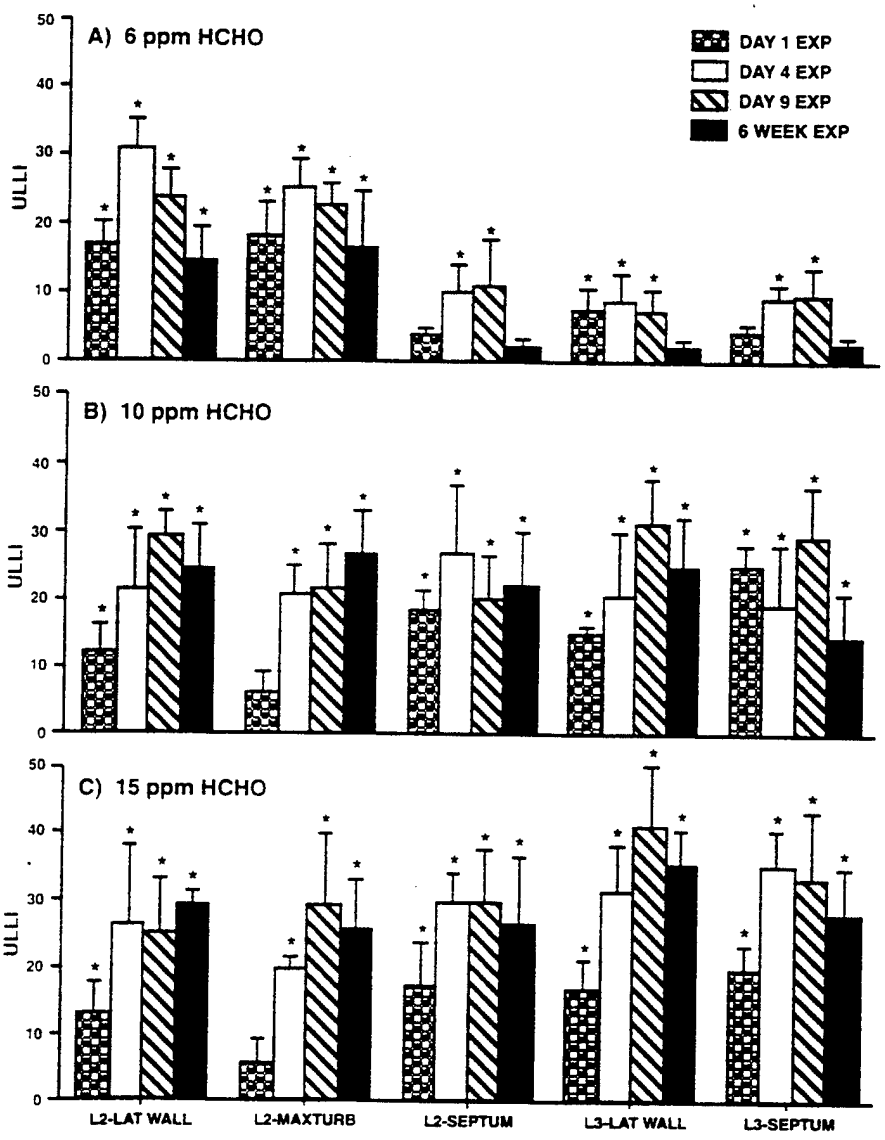


Figure 34. **Bar Graph Depicting Mean Cell Proliferation Indices Expressed as a Unit Length Labeling Index, (ULLI), for Specific Sites in the Rat Nasal Passages Following (A) 6 ppm, (B) 10 ppm, or (C) 15 ppm Formaldehyde Exposure.** No increases in cell proliferation were present in the 0.7, or 2 ppm formaldehyde groups, and control animals averaged a 1.3 ULLI. L2, level II of the nasal passages, L3, Level III of the nasal passages. LAT WALL, lateral meatus; MAXTURB, medial aspect of the maxilloturbinate. (Reprinted from Monticello et al., *Toxicol. Appl. Pharmacol.*, 111, 409, 1991, with permission.)

Various metaplastic-dysplastic or preneoplastic lesions occur in the respiratory epithelium of both laboratory animals and humans following exposure to carcinogens (38,39). Preneoplasia is generally believed to be a precursor response that has a high probability of developing into neoplasia. Formaldehyde exposure induces putative preneoplastic lesions in rat nasal epithelium following exposure to carcinogenic concentrations (15 ppm) for several months (40,41). The preneoplastic lesions were characterized by epithelial hyperplasia-metaplasia with atypia, similar to those reported in extranasal respiratory epithelium (38).

Cell proliferation rates in formaldehyde-induced preneoplastic lesions were significantly higher than those of control nasal epithelium (40). Tritiated-thymidine-labeled cells were present throughout the lesion, including the superficial layers, suggesting either a rapid migration from the basal cell population to the surface, or the capability of cells throughout the lesion to undergo replicative DNA synthesis (40). Data on formaldehyde-induced putative preneoplastic lesions, such as number of cells per lesion, cell proliferation rate per lesion, and other parameters, can be utilized in multistage models of carcinogenesis.

The tumor incidence from the long-term formaldehyde cell proliferation study (14) confirmed the previously reported bioassay tumor response (17). Similar to the bioassay (17), the majority of tumors were squamous cell carcinomas and arose from the nasal epithelium lining the walls of the anterior lateral meatus and the nasal septum (14), the same locations reported in other chronic formaldehyde studies (42,43) and the sites evaluated for alterations in cell proliferation. Nonneoplastic nasal lesions following long-term exposure were concentration-dependent, and included inflammation, epithelial hyperplasia, squamous metaplasia, and necrosis with exfoliation.

There were no detected treatment-induced responses in cell proliferation in the three lowest formaldehyde concentration groups, 0.7, 2, and 6 ppm, following exposure for up to 18 months. Increases in cell proliferation were present only in the 10 and 15 ppm groups, and were generally greater in the 15 ppm group as compared to the 10 ppm group (14). At each time point evaluated (3, 6, 12, and 18 months), a good correlation existed between the concentration-dependent increases in cell proliferation and the tumor incidence, supporting the proposal that sustained increases in cell proliferation are an important component of formaldehyde carcinogenesis (14).

Site-specific increases in cell proliferation following long-term exposure to formaldehyde were not only present at the lateral meatus and nasal septal locations, but also at the medial maxilloturbinate (MMT) site, even though the number of tumors originating from this site was disproportionately lower as compared to the other sites at risk (Figure 34). This discrepancy may be attributed to a decreased probability of cell mutation and subsequent cancer, due to the significantly smaller MMT target area and cell population at risk. Site-specific nasal responses also could be due to differences in regional susceptibility to formaldehyde, or other, as yet unidentified, factors.

The association of epithelial cytotoxicity, cell proliferation, and nasal cancer also has been demonstrated in a study where male rats with damaged or undamaged nasal mucosa were exposed to 10 ppm formaldehyde (44). The nasal damage was induced by bilateral intranasal electrocoagulation of the anterior third of the nasal passages. Rats with damaged nasal mucosa exhibited increases in formaldehyde-induced rhinitis, hyperplasia, and metaplasia of the nasal epithelium. Exposure to 10 ppm formaldehyde for 28 months produced an 8-fold increase in nasal squamous cell carcinomas in rats with damaged noses when compared to a similarly exposed group with intact noses (i.e., not pretreated with nasal electrocautery). These researchers concluded that both severe damage to the nasal mucosa and hyperproliferation are important in the development of nasal tumors in rats exposed to formaldehyde.

CELL PROLIFERATION AND FORMALDEHYDE RISK ASSESSMENT

Cell death and renewal are predominant features of most toxicologic injuries to the respiratory epithelium. Toxicant-induced cell necrosis, followed by regeneration, could, therefore, be a major determinant in chemically induced respiratory tract carcinogenesis. The studies of cell proliferation and nasal epithelium in formaldehyde-exposed rats demonstrate a good correlation of cellular injury and cell proliferation. The proliferative and tumor response are dependent on formaldehyde concentration. Induction of nasal carcinoma in rats by formaldehyde requires long-term exposure to high concentrations that result in cell death, followed by regenerative hyperplasia and metaplasia, changes associated with increases in cell proliferation. Because cell proliferation is clearly involved in chemical carcinogenesis, these concentration-responsive changes represent potentially important data that could be included in the risk assessment process.

A pharmacokinetic model, utilizing the rate of formaldehyde-induced DNA-protein cross-link formation, has been described in which the concentration of formaldehyde-induced cross-links formed

in corresponding tissues of different species can be predicted by scaling certain parameters (45). DNA-protein-cross-links are not the only biological factor affected by formaldehyde exposure, however, and a biologically based risk assessment strategy for inhaled formaldehdye has been proposed (37). The biologically based model incorporates molecular dosimetry and cell kinetic data, because both of these factors are causally involved in formaldehyde-induced rodent nasal carcinogenesis (37).

Over the past decade, mechanistic studies have provided a great deal of information on the pathogenesis of formaldehyde-induced nasal carcinogenesis. These data are important for the assessment of human risks at formaldehyde concentrations below those at which cancer develops in rodent bioassays. Quantitative risk assessment methods should improve our understanding of the shape of the formaldehyde nasal cancer exposure–response curve, and the quantitative importance of cell proliferation and mutation in this process. Moreover, the incorporation of mechanistically based data will improve low-dose and interspecies extrapolation of formaldehyde risk.

REFERENCES

1. H.C. Pitot,"Fundamentals of Oncology," (3rd ed., Marcel Dekker Publ., New York, 1986).

2. A.J. Levine, J. Momand, and C.A. Finlay, "The p53 Tumor Suppresser Gene," *Nature* **351**, 453-456 (1991).

3. J.W. Grisham, W.K. Kaufmann, and D.G. Kaufman, "The Cell Cycle and Chemical Carcinogenesis," *Surv. Syn. Pathol. Res.* **1**, 49-66 (1983).

4. E. Farber and D.R.S. Sarma, "Hepatocarcinogenesis: A Dynamic Cellular Perspective," *Lab. Invest.*, **56**, 4-22 (1987).

5. J. Russo and I.H. Russo, "Biological and Molecular Bases of Mammary Carcinogenesis," *Lab. Invest.*, 112-137 (1987).

6. L.A. Loeb, "Endogenous Carcinogenesis: Molecular Oncology into the Twenty-First Century," *Cancer Res.* **49**, 5489-5496 (1989).

7. B.N. Ames and L.S. Gold, "Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis," *Science* **249**, 970-971 (1990).

8. B.E. Butterworth and T.L. Goldsworthy, "The Role of Cell Proliferation in Multistage Carcinogenesis," *Proc. Soc. Exper. Biol. Med.* **198**, 683-687 (1991).

9. S.M. Cohen and L.B. Ellwein, "Genetic Errors, Cell Proliferation, and Carcinogenesis," *Cancer Res.* **51**, 6493-6505 (1991).

10. S. Preston-Martin, M.C. Pike, R.K. Ross, and B.E. Henderson, "Cell Division and Human Cancer," *Prog. Clin. Biol. Res.* **369**, 21-34 (1991).

11. International Agency for Research on Cancer, *Chemicals and Industrial Processes Associated with Cancer in Humans*, IARC Monographs, Suppl 1. (Lyon, France, 1979).

12. B.E. Butterworth, "Consideration of Both Genotoxic and Nongenotoxic Mechanisms in Predicting Carcinogenic Potential," *Mutat. Res.* **239**, 117-132 (1990).

13. S.R. Eldridge, L.F. Tilbury, T.L. Goldsworthy, and B.E. Butterworth, "Measurement of Chemically Induced Cell Proliferation in Rodent Liver and Kidney: A Comparison of 5-Bromo-2'-deoxyuridine and [^{3}H]Thymidine Administered by Injection or Osmotic Pump," *Carcinogenesis* **11**, 2245-2251 (1990).

14. T.M. Monticello, "Formaldehyde-Induced Pathology and Cell Proliferation," Ph.D. Dissertation (Duke University, Durham, NC, 1990).

15. J.A. Swenberg, W.D. Kerns, R.I. Mitchell, E.J. Gralla, and K.L. Pavkov, "Induction of Squamous Cell Carcinomas of the Rat Nasal Cavity by Inhalation Exposure to Formaldehyde Vapor," *Cancer Res.* **40**, 3398-3402 (1980).

16. R.E. Albert, A.R. Sellakumar, S. Lashkin, M. Kuschner, M. Nelson, and C.A. Snyder, "Gaseous Formaldehyde and Hydrogen Chloride Induction of Nasal Cancer in the Rat," *J. Natl. Cancer Inst.* **68**, 597-603 (1982).

17. W.D. Kerns, K.L. Pavkov, D.J. Donofrio, E.J. Gralla, and J.A. Swenberg, "Carcinogenicity of Formaldehyde in Rats and Mice After Long-Term Inhalation Exposure," *Cancer Res.* **43**, 4382-4392 (1983).

18. U.S. Environmental Protection Agency, *Formaldehyde Risk Assessment Update,* Final Draft, (Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, 1991).

19. Universities Associated for Research and Education in Pathology, Inc. "Epidemiology of Chronic Occupational Exposure to Formaldehyde: Report of the Ad Hoc Panel on Health Aspects of Formaldehyde," *Toxicol. Ind. Health* **4**, 77-90 (1988).

20. T.B. Starr, J.E. Gibson, C.S. Barrow, C.J. Boreiko, Hd'A. Heck, R.J. Levine, K.T. Morgan, and J.A. Swenberg, "Estimating Human Cancer Risk from Formaldehyde: Critical Issues," in V. Turoski (ed.), *Formaldehyde: Analytical Chemistry and Toxicology*, Advances in Chemistry Series No. 210 (American Chemical Society, Washington DC, 1985), pp.300-333.

21. A. Blair, P. Stewart, M. O'berg, W. Gaffey, J. Walrath, J. Ward, R. Bales, S. Kaplan, and D. Cubit, "Mortality Among Industrial Workers Exposed to Formaldehyde," *J. Natl. Cancer Inst.* **76**, 1071-1084 (1986).

22. T.H. Ma and M.M. Harris, "Review of the Genotoxicity of Formaldehyde," *Mutat. Res.* **196**, 37-59, (1988).

23. R.C. Grafstrom, A. Fornace, H. Autrup, J.F. Lechner, and C.C. Harris, "Formaldehyde Damage to DNA and Inhibition of DNA Repair in Human Bronchial Cells," *Science* **220**, 216-218 (1983).

24. Hd'A. Heck, M. Casanova, and T.B. Starr, "Formaldehyde Toxicity - New Understanding," *Critical Rev. Toxicol.* **20**, 397-426 (1990).

25. J.A. Swenberg, C.S. Barrow, C.J. Boreiko, Hd'A. Heck, R.J. Levine, K.T. Morgan, and T.B. Starr, "Nonlinear Biological Responses to Formaldehyde and Their Implications for Carcinogenic Risk Assessment," *Carcinogenesis* **4**, 945-952 (1983).

26. M. Casanova, D.F. Deyo, and Hd'A. Heck, "Covalent Binding of Inhaled Formaldehyde to DNA in Nasal Mucosa of F344 Rats: Analysis of Formaldehyde and DNA by High Performance Liquid Chromatography and Provisional Pharmacokinetic Interpretation," *Fundam. Appl. Pharmacol.* **12**, 397-417 (1989).

27. M. Casanova and Hd'A. Heck, "Further Studies on the Metabolic Incorporation and Covalent Binding of Inhaled [3H]- and [14C]Formaldehyde in F344 Rats: Effects of Glutathione Depletion," *Toxicol. Appl. Pharmacol.* **89**, 105-121 (1987).

28. U.S. Environmental Protection Agency, *Formaldehyde Risk Assessment Update*, June 11, 1991. (Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, 1991).

29. T.M. Monticello, F.J. Miller, and K.T. Morgan, "Regional Increases in Rat Nasal Epithelial Cell Proliferation Following Acute and Subchronic Inhalation of Formaldehyde," *Toxicol. Appl. Pharmacol.* **111**, 409-421 (1991).

30. T.M. Monticello, K.T. Morgan, J.I. Everitt, and J.A. Popp, " Effects of Formaldehyde Gas on the Respiratory Tract of Rhesus Monkeys," *Am. J. Pathol.* **134**, 515-527 (1989).

31. A.J.P. Klein-Szanto, H. Ura, and J. Resau, "Formaldehyde-Induced Lesion of Xenotransplanted Nasal Respiratory Epithelium," *Toxicol. Pathol.* **17**, 33-37 (1989).

32. A.B. Pardee, "Biochemical and Molecular Events Regulating Cell Proliferation," *J. Pathol.* **149**, 1-2 (1986).

33. W.K. Lutz and P. Maier, "Genotoxic and Epigenetic Chemical Carcinogenesis: One Process, Different Mechanisms," *Trends in Pharmacol. Sci.* **9**, 322-326 (1988).

34. L. Recio, S. Sisk, L. Pluta, E. Bermudez, E.A. Gross, Z. Chen, K.T. Morgan, and C. Walker, "p53 Mutations in Formaldehyde-Induced Nasal Squamous Cell Carcinomas in Rats," *Cancer Res.* **52**, 6113-6116 (1992).

35. A.J.P. Klein-Szanto, "The Role of Chemically Induced Epithelial Hyperplasia in the Development of Human Cancer," *Prog. Clin. Biol. Res.* **369**, 35-41 (1991).

36. K.T. Morgan, J.S. Kimbell, T.M. Monticello, A.L. Patra, and A. Fleishman, "Studies of Inspiratory Airflow Patterns in the Nasal Passages of the F344 Rat and Rhesus Monkey Using Nasal Molds: Relevance to Formaldehyde Toxicity," *Toxicol. Appl. Pharmacol.* **110**, 223-240 (1991).

37. R.B. Conolly, T.M. Monticello, K.T. Morgan, H.J. Clewell, and M.E. Andersen, "A Biologically-Based Risk Assessment Strategy for Inhaled Formaldehyde," *Comments Toxicol.* **4**, 269-293 (1992).

38. P. Nettesheim, A.J.P. Klein-Szanto, A.C. Marchok, V.E. Steele, M. Terzaghi, and D.C. Topping, "Studies of Neoplastic Development in Respiratory Tract Epithelium," *Arch. Pathol. Lab. Med.* **105**, 1-10 (1981).

39. A.J.P. Klein-Szanto, D.C. Topping, C.A. Heckman, and P. Nettesheim, "Ultrastructural Characteristics of Carcinogen-Induced Dysplastic Changes in Tracheal Epithelium," *Am. J. Pathol.* **98**, 83-100 (1980).

40. T.M. Monticello and K.T. Morgan, "Cell Kinetics and Characterization of "Preneoplastic" Lesions in Nasal Respiratory Epithelium of Rats Exposed to Formaldehyde," *Proc. Amer. Assoc. Cancer Res.* **30**, 195 (1989).

41. K.T. Morgan and T.M. Monticello, "Formaldehyde Toxicity: Respiratory Epithelial Injury and Repair," In D.G. Thomassen and P. Nettesheim (eds.), *Biology, Toxicology and Carcinogenesis of Respiratory Epithelium*, (Hemisphere Publ., New York, 1990), pp. 155-171.

42. K.T. Morgan, X.Z. Jiang, T.B. Starr, and W.D. Kerns, "More Precise Localization of Nasal Tumors Associated with Chronic Exposure of F344 Rats to Formaldehyde Gas," *Toxicol. Appl. Pharmacol.* **82**, 264-271 (1986).

43. R.A. Woutersen and V.J. Feron, "Localization of Nasal Tumors in Rats Exposed to Acetaldehyde or Formaldehyde," in V.J. Feron and M.C. Bosland (eds.), *Nasal Carcinogenesis in Rodents: Relevance to Human Health Risk* (Pudoc, Wageningen, 1989).

44. R.A. Woutersen, A. van Gardeen-Hoetmet, J.P. Bruijnjes, A. Zwart, and V.J. Feron, "Nasal Tumors in Rats After Severe Injury to the Nasal Mucosa and Prolonged Exposure to 10 ppm Formaldehyde," *J. Appl. Toxicol.* **9**, 39-46 (1989).

45. M. Casanova, K.T. Morgan, W.H. Steinhagen, J.I. Everitt, J.A. Popp, and Hd'A. Heck, "Covalent Binding of Inhaled Formaldehyde to DNA in the Respiratory Tract of Rhesus Monkeys: Pharmacokinetics, Rat-to-Monkey Interspecies Scaling, and Extrapolation to Man," *Fundam. Appl. Toxicol.* **17**, 409-428 (1991).

Apoptosis and Chemical Carcinogenesis

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ABSTRACT

Long recognized as a normal component of organogenesis during development, apoptosis (programmed cell death) has recently been implicated in alterations of cell growth and differentiation. Tissue homeostasis is normally maintained by a balance between cell division and cell death, with apoptosis often functioning in complement to cell growth. Thus, antithetical parallels in chemical carcinogenesis can be drawn between apoptosis and the proliferative events more commonly addressed. Whereas enhanced cell replication may contribute to an increased frequency of mutation, apoptosis within a tissue may counteract chemical carcinogenesis through loss of mutated cells. Many strong carcinogens act as tumor promoters, selectively expanding an initiated cell population advantageously over surrounding cells. Similarly, chemicals with a selective inhibition of apoptosis within an initiated population would offer a growth advantage. In contrast, chemicals causing selective apoptosis of initiated cells would be expected to have an anticarcinogenic effect. Selective apoptosis, in concert with cell-specific replication, may explain the unique promoting effects of different carcinogens such as the peroxisome proliferating chemicals, phenobarbital, and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin. Cell turnover, both cell growth and cell death, is central to the process of chemically induced carcinogenesis in animals and understanding its impact is a critical determinant of the relevance of chemically induced effects to man.

APOPTOSIS: SYSTEMATIC, GENE-DIRECTED CELL DEATH

During the development of an organism, considerable remodeling takes place, involving not only cell proliferation and differentiation, but highly organized apoptosis, or programmed cell death. Nowhere has this been more elegantly demonstrated than in the nematode *Caenorhabditis elegans* where, in route to the adult organism of 959 cells, precisely 131 cells systematically die (1,2). In this animal model, the

genetic control of apoptosis involves unique sets of controlling genes. Some apparently function in negative roles, controlled by gene products in which gain of function mutations prolong the life of the cell, and deletions of specific regions result in cell death. The targets of these inhibitors are the activators of the orderly and systematic process of cell death (1). Thus, the apoptotic process is highly active and controlled by numerous molecular events, some of which can be recognized histologically.

Apoptosis, as recognized in liver and other tissues, has been well described elsewhere (3,4). In hepatocytes, these stages histologically involve a brief period of cytoplasmic basophilia and nuclear condensation, followed by eosinophilia and cytoplasmic condensation, and finally, nuclear and cellular fragmentation and dissolution of the cell. Typically, when taking place within a solid organ, apoptosis also characteristically involves phagocytosis by neighboring cells. However, luminal sloughing or "shedding" also occurs in epithelial organs such as the kidney (5). Early cytoplasmic changes are considered to be an indicator of the active involvement of specific gene products in the orderly demise of the cell; however, to date, few definitive nonmorphological markers exist. Although active transcription of a few genes is initiated, the majority of cellular functions are diminishing, consistent with the observed nuclear and cytoplasmic condensation. Coinciding with nuclear condensation is the induction of an endonuclease, cleaving chromatin at internucleosomal DNA linker regions and generating a characteristic oligonucleosomal ladder ($\sim$185 base-pair units), observable following agarose gel electrophoresis (6). One promising new approach for identification of single apoptotic cells may be through DNA double-stranded break end labeling of terminal linker DNA (7). The appearance of cytoplasmic eosinophilia and condensation coincides with fibrin organization, arranged concentrically around the periphery to collapse the cell on itself. When phagocytosis by neighboring cells does occur, apoptotic cells may be recognized by cell-surface signals, such as the vitronectin receptor (8). Cellular blebbing, fragmentation, and packaging of detergent-insoluble structures appears to involve a tissue transglutaminase. However, the significance and generality of this marker for apoptosis remain to be established (9,10). Final degradation of the apoptotic remnant or "body" occurs within phagocytic cells and the time from the first detectable alterations consistent with apoptosis to disappearance of the apoptotic body may be as rapid as 1 to 3 h (11). Subsequently, smaller insoluble membrane products (morphologically recognizable as lipofuscin) may remain for a considerable period of time (12).

Several types of cell loss or irreversible growth arrest occur in mammalian systems in addition to apoptosis; these would include terminal differentiation, senescence, and necrosis and will only be

mentioned briefly here (9). Terminal differentiation is often associated with a cessation of cell replication and the expression of a specialized function of a tissue. In contrast, cellular senescence may involve genes that are activated or whose functions become manifested only at the end of the life span of the cell. Defects in the function of these gene products may allow cells to escape the route to senescence to become 'immortal'. Immortalization may be one mechanism by which tumor suppressor genes may operate in some neoplasias (13,14). Necrosis, in contrast to apoptosis, is not an orderly cellular process but rather the disorganized death of a cell. Loss of osmotic and ionic gradients, membrane rupture, release of cellular constituents, random dissolution of all cellular components, and often the induction of an overt inflammatory response are all characteristics of necrosis, and are not typically associated with apoptosis (15). Apoptosis, even when involving a considerable portion of an organ or tissue, is not associated with an inflammatory process (16), although apoptosis and necrosis may coexist (17). Although markedly different both morphologically and in their modes of induction and genetic control, all of these forms of cell loss effectively result in exclusion of cell(s) from the replicating population.

Despite considerable evidence that apoptosis is under tight genetic control, only a few genes have been identified. The cellular signalling and genetic control of apoptosis have been reviewed recently elsewhere (9) and will not be discussed here. Candidate genes that have been identified include *bcl*-2, and the tumor suppressor gene p53. Signalling factors that have been indicated to be involved include cytosolic Ca^{2+}, IL-2, IL-3, colony stimulating factor, and TGF-β. Paradoxically, factors such as cytosolic Ca^{2+}, and induction of *c-fos* and *c-myc* immediate early genes are shared by both the cell death and cell proliferation signalling pathways.

Impact of Apoptosis-Inhibition and Enhanced Cell Replication on Initiated Cells

Carcinogenesis has been described in risk assessment paradigms as a multistage phenomenon involving multiple, discrete genetic mutations. More recently, mathematical descriptions of this phenomenon have become increasingly biologically based, recognizing the potential contributions of both cell proliferation and cell loss (18). These models have been constructed to describe cancers involving two heritable changes (μ1 and μ2; Figure 35), recognizing that although useful for this discussion, it is likely to be an oversimplification.

Enhanced cell replication has been implicated as a risk factor in several cases of chemically induced carcinogenesis (19,20). In these instances, it is stated or implicitly implied that background

errors in DNA replication or other undetectable forms of mutagenesis resulted in "spontaneous" initiation of cells (21). Theoretically, in cases where either the replicating cell population (N; Figure 35) or the replication rate of this population (R0) exceeds baseline levels, the normally rare probability of a intermediate cell with a critical gene alteration (I; Figure 35) to form and persist is increased. Although this probability-mutagenesis is often discussed in relation to cell proliferation, it is often neglected that cell loss may play a complementary role. Thus, inhibition of apoptosis may similarly increase mutational frequencies by increasing the population at-risk (N or I populations). Although this decreased cell loss may impact on carcinogenesis, apoptosis-inhibition may play a more critical direct role by its impact on mutational frequencies (μ1 and/or μ2), as discussed below.

Inhibition of Apoptosis is Reversible

One important characteristic of the inhibition of apoptosis by chemicals is reversibility. A synchronized wave of apoptosis occurs within the liver following the withdrawal of numerous hepatic mitogens/hepatocarcinogens, indicative of homeostatic reversal of apoptosis-inhibition (16,22-25). Induction of apoptosis following withdrawal of endogenous hormones or growth factors has similarly been demonstrated (9,26,27). Inhibition of apoptosis, counterbalanced by apoptosis-controlled regression is now considered a normal homeostatic mechanism and these observations suggest that only proper sampling intervals will detect evidence of these events. Within the context of chemical carcinogenesis, these observations also suggest that chemicals may exert important yet transient effects. Critical alterations occurring secondary to a transient, chemically induced inhibition of apoptosis may be untraceable by examination of the end product, the tumor.

APOPTOSIS AND INITIATION

The blockade of cell death becomes even more intriguing when one considers that aborted cell death may itself lead to initiation of the carcinogenic process. If apoptosis-resistance is due to blockade of a stage of apoptosis after DNA fragmentation has begun (6), outcomes of genomic instability may increase. Inappropriate activation and cleaving of genomic DNA by the apoptotic endonuclease may directly give rise to such heritable alterations as deletions, frame shift mutations, and genetic recombinants. Thus, apoptosis-inhibited cells may not only have a growth advantage over their neighboring cells, but possess an error-prone mutator phenotype, hypothesized to be a critical event in some forms of chemical carcinogenesis (21). As mentioned above, apoptosis-inhibition may be through a reversible alteration of signal transduction. In these instances chemicals could act as indirect initiators,

with no demonstrable form of DNA reactivity. Indeed, a common feature of many diverse hepatocarcinogens is their lack of DNA reactivity. These chemicals are not only strong promoters (28,29), but are also carcinogenic in long-term feeding studies in the absence of an initiator (30,31). Cells normally programmed to die, due to an accumulation of spontaneous or chemically induced genetic damage, may persist and even replicate if apoptosis is inhibited.

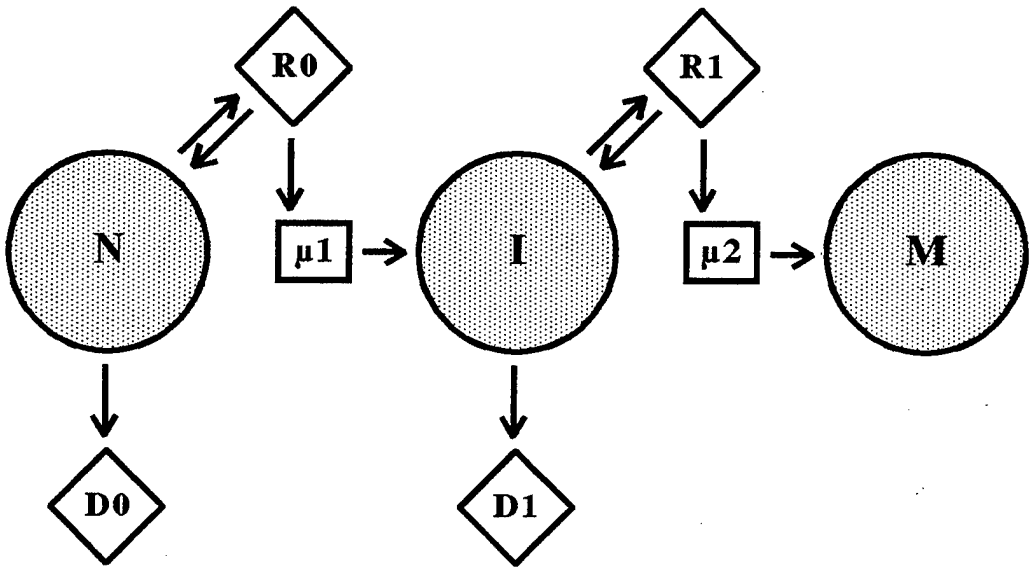


Figure 35. Biologically Based Cancer Model. In this quantitative cell growth model of carcinogenesis, a normal (N) cell is required to undergo two, step-wise mutational events (μ1 and μ2) to become a malignant cell (M). In addition, this model is structured to incorporate the impact of cell replication and death within both the N and initiated (I) cell compartments over time. Thus, cell replication (R0) within the N cell population, in the excess of cell death (D0), will increase the size of the N cell population. Similarly, the size of the I cell population is directly dependent upon R1 and D1. Generation of the first I cell may be accelerated by directly increasing μ1. Also, as μ1 is considered a probabilistic event dependent on the number of replicating cells, increasing the number of N cells (via relative increases in R0 or decreases in D0) would generate a larger population at risk of mutation. Finally, increased generation of I cells could occur when the N cell population is constant, in cases where cell replication (R0) is high but equivalent to cell death (D0). A similar scenario of interrelationships would be expected at later stage(s) of carcinogenesis (i.e., M cells dependent on relative values of μ2, I, R1, and D1).

Promotional Factors in the Context of a Single Mutated Cell

Following the mutation of a single cell, tumor promotional factors (R1 and D1; Figure 35) become critical to the survival of any subsequent initiated cell population (I). Survival of a single initiated cell, assuming that cell loss is comparable to the surrounding tissue, can be described as D1=D0. Clearly, apoptosis inhibition directed at the initiated cell (D1<D0) would have a significant impact; an incremental decrease in D1 results in an incremental increase in the number of surviving initiated cells and I-clones. Enhanced cell replication within a proliferating tissue (R0) may similarly contribute to tumor promotion. Experimentally, this presumably occurs for mutagenic liver carcinogens such as diethylnitrosamine during the regenerative period following a necrogenic dose or when low doses are administered to young, growing animals (32). These studies have shown that despite the high DNA reactivity of diethylnitrosamine, cell replication is instrumental in fixing the genetic damage. However, the continuing regenerative response likely ensures survival of a genetically altered population by encouraging additional rounds of replication within these initiated cells. This nonspecific promotional contribution can be described mathematically in the context of the model as an enhancement of R1 equal to the enhancement of R0. Cell growth model simulations are consistent with the hypothesis that the impact of random cell death (D0=D1) on the loss of an entire altered cell population, such as an altered hepatic focus, quickly diminishes as the size of the intermediate clone (I) increases.

Apoptosis and Promotion

In many growth-selection carcinogenesis experiments, the clonal growth of altered cells is hypothesized to occur through their selective resistance to a mitoinhibitory effect of the chemical (33). This would result in a passive growth advantage to the subpopulation of altered cells. Similarly, cell death may simply be overwhelmed in an initiated cell population by the activation of growth signals and thus a heritable proliferation advantage. However, selective cellular resistance to apoptosis may also generate the emergence of subpopulations of altered cells with a selective growth advantage over surrounding cells. Enhanced hepatocyte apoptosis has been observed in the livers of rats fed the strong rodent hepatocarcinogen, Wy-14,643 (12). In cases such as this, apoptosis-resistant hepatocytes would have dual growth advantage: a passive resistance to excess cell loss, and an accelerated growth advantage as these resistant cells may be recruited for cell proliferation due to the surrounding cell loss.

Cell-Specific Apoptosis and Lesion Regression

Elevated apoptosis is central to the regression of liver hyperplasia following the removal of a mitogenic carcinogen. However, rapid induction of apoptosis is also observed within proliferative hepatic adenomas (23-25). A hopeful line of research in cancer chemotherapy is to exploit the induction of apoptosis, directed at specific cell types. Enhanced apoptosis within a tumor would directly decrease tumor size, rather than simply inhibit further growth. High selectivity would still be highly desirable for such an agent however, as complete loss of the tumor will not likely result unless the tumor is very small (i.e., the size of the I population near 1; Figure 35), or the efficacy is very high (D1 significantly greater than R1).

In peroxisome proliferator-treated rats, many of the common histologic markers indicative of preneoplasia are negative, with numbers of hepatic foci often less in treated animals than in control (34,35). Although this may be simply due to selective expression of certain phenotypes, the relative decrease of individual hepatic foci may actually represent loss of initiated cell populations. As noted above, unless apoptosis was specifically directed at these cell types, only small populations of initiated cells would likely be eliminated. Cell-directed specificity is not unlikely however, with many rodent liver tumor promoters (such as the dioxins and the peroxisome proliferators) now thought to exert all or some of their effects through receptor mediated events (36, 37). Where this selective promotional activity is targeted to a subpopulation of cells, promoters may take on a very distinct pattern of phenotypic expression. For instance, $D1 > D0$ may eliminate some phenotypes, whereas $R1 > R0$ and $D1 < D0$ within another altered cell population would give this second population a clear growth advantage.

APOPTOSIS AND TUMOR PROGRESSION

A hallmark of tumor progression in chemical carcinogenesis is the persistence and autonomous growth of a tumor, despite the removal of the inciting agent. As mentioned above, escape from cellular senescence or apoptotic inhibition may lead to unrestrained growth of cells or immortality (9). In hepatocellular tumors induced by the peroxisome proliferating chemical, Wy-14,643, tumor progression is particularly evident (38). Despite the induction of numerous large hepatocellular adenomas by Wy-14,643, withdrawal of this carcinogen results in few persistent tumors (Figure 36). In contrast, hepatocellular carcinomas induced by 52 weeks of treatment are no longer dependent on Wy-14,643 for either growth or metastasis. Either escape from senescence or loss of apoptotic control may be critical in the progression of these hepatocellular adenomas to hepatocellular carcinomas.

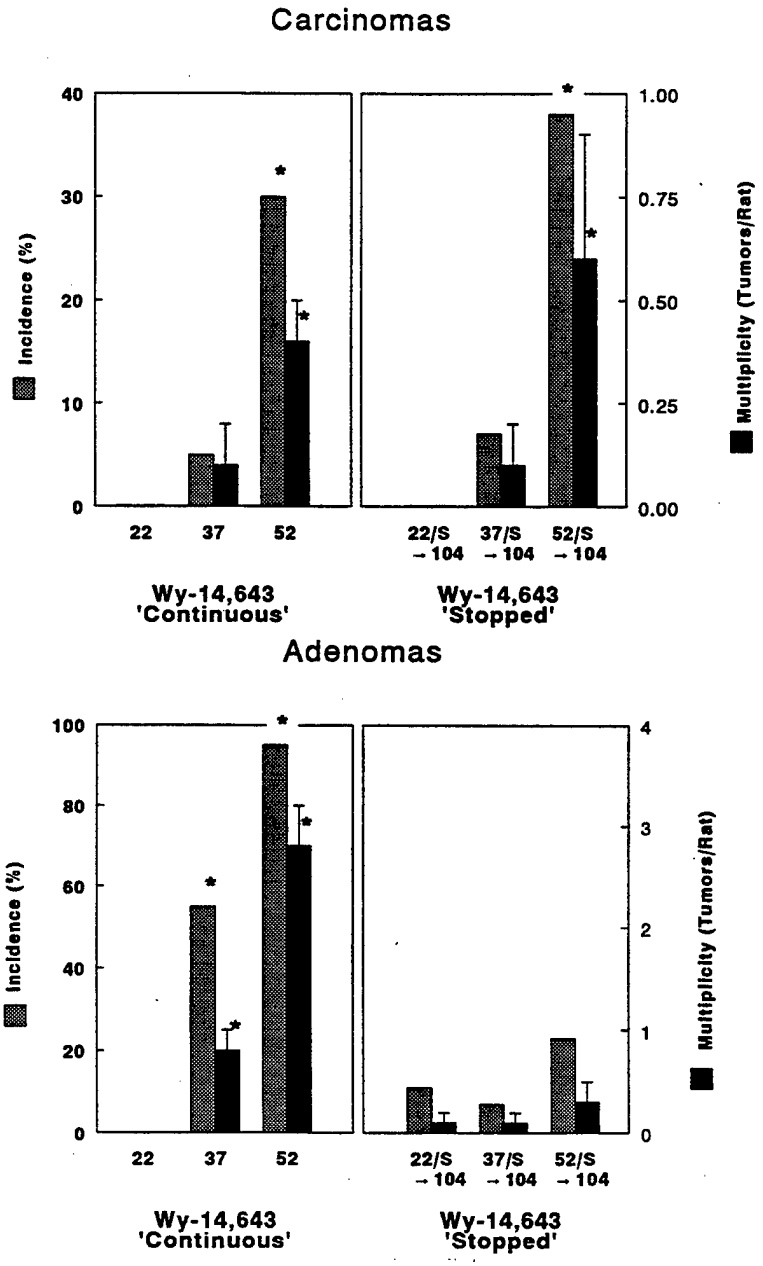


Figure 36. Biological Potential of Hepatocellular Adenomas and Carcinomas Induced by Wy-14,643. Incidence (cross-hatched bars) and multiplicity (solid bars) of tumors (ADENOMAS and CARCINOMAS) detected in male F-344 rats (n=20/group) fed Wy-14,643 (0.1%) for 22, 37, or 52 weeks, with additional animals returned to control diet until sacrifice at 104 weeks (STOP groups). No tumors were detected in control animals. Asterisks (*) denote incidence and multiplicity values significantly greater than control values ($p < 0.05$). Adenomas were not significantly increased in stop animals over age matched controls.

CONCLUSION

Human health assessments of cancer risk associated with exposure to environmental chemicals are most reliable when a full understanding of the carcinogenic mechanism in animals and the relevance of this response to man are known. To this end, understanding the chemical induction and inhibition of apoptosis becomes a necessary component in our understanding of the carcinogenic mechanism and extrapolation of the animal data to humans.

REFERENCES

1. R.E. Ellis, J.Y. Yuan, and H.R. Horvitz. "Mechanisms and Functions of Cell Death," *Annu. Rev. Cell Biol.* **7**, 663-698 (1991).

2. M.C. Raff, "Social Controls on Cell Survival and Cell Death," *Nature* **356**, 397-400 (1992).

3. W. Bursch, H.S. Taper, B. Lauer, and R. Schulte-Hermann, "Quantitative Histological and Histochemical Studies on the Occurrence and Stages of Controlled Cell Death (Apoptosis) During Regression of Rat Liver Hyperplasia," *Virchows Arch. [Cell Pathol.]*, **50**, 153-166 (1985).

4. M.J. Arends, and A.H. Wyllie, "Apoptosis: Mechanisms and Roles in Pathology," in International Review of Experimental Pathology (Academic Press, New York, Vol. **32**, 1991), pp. 233-254.

5. G.M Ledda-Columbano, A. Columbano, P. Coni, G. Faa, and P. Pani, "Cell Deletion by Apoptosis During Regression of Renal Hyperplasia," *Amer. J. Pathol.* **135**, 657-662 (1989).

6. M.J. Arends, R.G. Morris, and A.H. Wyllie, "Apoptosis: The Role of the Endonuclease," *Amer. J. Pathol.* **136**, 593-608 (1990).

7. Y. Gavrieli, Y. Sherman, and S.A. Ben-Sasson, "Identification of Programmed Cell Death *In Situ* Via Specific Labeling of Nuclear DNA Fragmentation," *J. Cell Biol.* **119**, 493-501 (1992).

8. J. Savill, I. Dransfield, and N. Hogg, "Vitronectin Receptor-Mediated Phagocytosis of Cells Undergoing Apoptosis," *Nature* **343**, 170-173 (1990).

9. J.C. Barrett, "Cell Senescence and Apoptosis," in Molecular Genetics of Nervous System Tumors (Wiley-Liss, Inc., 1993), pp. 61-72.

10. M. Piacentini, F. Autuori, and L. Dini, "Tissue" Transglutaminase Is Specifically Expressed in Neonatal Rat Liver Cells Undergoing Apoptosis Upon Epidermal Growth Factor-Stimulation," *Cell Tissue Res.* **263**, 227-235 (1991).

11. W. Bursch, S. Paffe, B. Putz, G. Barthel, and R. Schulte-Hermann, "Determination of the Length of the Histological Stages of Apoptosis in Normal Liver and in Altered Hepatic Foci of Rats," *Carcinogenesis* **11**, 847-853 (1990).

12. D.S. Marsman, T.L. Goldsworthy, and J.A. Popp, "Contrasting Hepatocytic Peroxisome Proliferation, Lipofuscin Accumulation, and Cell Turnover for the Hepatocarcinogens Wy-14,643 and Clofibric Acid," *Carcinogenesis* **13**, 1011-1017 (1992).

13. J.C. Barrett and W.F. Fletcher, "Cellular and Molecular Mechanisms of Multistep Carcinogenesis in Cell Culture Models," in J.C. Barrett (ed.), Mechanisms of Environmental Carcinogenesis: Multistep Models of Carcinogenesis (CRC Press, Boca Raton, Vol. **2**, 1987), pp. 73-116.

14. R. Sager, "Genetic Suppression of Tumor Formation: A New Frontier in Cancer Research," *Cancer Res.* **46**, 1573-1580 (1986).

15. A.H. Wyllie, J.F.R. Kerr, and A.R. Currie, "Cell Death: The Significance of Apoptosis," *Int. Rev. Cytol.* **68**, 251-306 (1980).

16. W. Bursch, B. Düsterberg, and R. Schulte-Hermann, "Growth, Regression and Cell Death in Rat Liver as Related to Tissue Levels of the Hepatomitogen Cyproterone Acetate," *Arch. Toxicol.* **59**, 221-227 (1986).

17. G.M. Ledda-Columbano, P. Coni, M. Curto, L. Giacomini, G. Faa, S. Oliverio, M. Piacentini, and A. Columbano, "Induction of Two Different Modes of Cell Death, Apoptosis and Necrosis," in Rat Liver After a Single Dose of Thioacetamide, *Amer. J. Pathol.* **139**, 1099-1109 (1989).

18. S.H. Moolgavkar, "Biologically Motivated Two-Stage Model for Cancer Risk Assessment," *Toxicol. Lett.* **43**, 139-150 (1988).

19. P. Grasso, M. Sharratt, and A.J. Cohen, "Role of Persistent, Non-Genotoxic Tissue Damage in Rodent Cancer and Relevance to Humans," *Annu. Rev. Pharmacol. Toxicol.* **31**, 253-287 (1991).

20. B.N. Ames and L.S. Gold, "Chemical Carcinogenesis: Too Many Rodent Carcinogens," *Proc. Natl. Acad. Sci. USA* **87**, 7772-7776 (1990).

21. L.A. Loeb, "Mutator Phenotypes May Be Required for Multistage Carcinogenesis," *Cancer Res.* **51**, 3075-3079 (1991).

22. L.D. Tomei, P. Kanter, and C.E. Wenner, "Inhibition of Radiation-Induced Apoptosis *In Vitro* by Tumor Promoters," *Biochem. Biophys. Res. Comm.* **155**, 324-331 (1988).

23. W. Bursch, B. Lauer, I. Timmermann-Trosiener, G. Barthel, J. Schuppler, and R. Schulte-Hermann, "Controlled Death (Apoptosis) of Normal and Putative Preneoplastic Cells in Rat Liver Following Withdrawal of Tumor Promoters," *Carcinogenesis* **5**, 453-458 (1984).

24. U. Gerbracht, W. Bursch, P. Kraus, B. Putz, M. Reinacher, I.Timmermann-Trosiener, and R. Schulte-Hermann, "Effects of Hypolipidemic Drugs Nafenopin and Clofibrate on Phenotypic Expression and Cell Death (Apoptosis) in Altered Foci of Rat Liver," *Carcinogenesis* **11**, 617-624 (1990).

25. R. Schulte-Hermann, I. Timmermann-Trosiener, G. Barthel, and W. Bursch, "DNA Synthesis, Apoptosis, and Phenotypic Expression as Determinants of Growth of Altered Foci in Rat Liver During Phenobarbital Promotion," *Cancer Res.* **50**, 5127-5135 (1990).

26. R.J. Rotello, M.B. Hocker, and L.E. Gerschenson, "Biochemical Evidence for Programmed Cell Death in Rabbit Uterine Epithelium," *Am. J. Pathol.* **134**, 491-495 (1989).

27. T.I. Azmi and J.D. O'Shea, "Mechanism of Deletion of Endothelial Cells During Regression of the Corpus Luteum," *Lab. Invest.* **51**, 206-217 (1984).

28. R.C. Cattley and J.A. Popp, "Differences Between the Promoting Activities of the Peroxisome Proliferator Wy-14,643 and Phenobarbital in Rat Liver," *Cancer Res.* **49**, 3246-3251 (1989).

29. H.C. Pitot, T. Goldsworthy, H.A. Campbell, and A. Poland, "Quantitative Evaluation of the Promotion by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin of Hepatocarcinogenesis from Diethylnitrosamine," *Cancer Res.* **40**, 3616-3620 (1980).

30. D.S. Marsman, R.C. Cattley, J.G. Conway, and J.A. Popp, "Relationship of Hepatic Peroxisome Proliferation and Replicative DNA Synthesis to the Hepatocarcinogenicity of the Peroxisome Proliferators Di(2-ethylhexyl)phthalate and [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic Acid (Wy-14,643) in Rats," *Cancer Res.* **48**, 6739-6744 (1988).

31. J.E. Huff, A.G. Salmon, N.K. Hooper, and L. Zeise, "Long-Term Carcinogenesis Studies on 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and Hexachlorodibenzo-*p*-dioxins," *Cell Biol. Toxicol.* **7**, 67-94 (1991).

32. F.H. Deal, F.C. Richardson, and J.A. Swenberg, "Dose Response of Hepatocyte Replication in Rats Following Continuous Exposure to Diethylnitrosamine," *Cancer Res.* **49**, 6985-6988 (1989).

33. D.B. Solt, A. Medline, and E. Farber, "Rapid Emergence of Carcinogen-Induced Hyperplastic Lesions in a New Model for the Sequential Analysis of Liver Carcinogenesis," *Am. J. Pathol.* **88**, 595-618 (1977).

34. W. Stäubli, P. Bentley, F. Bieri, E. Fröhlich, and F. Waechter, "Inhibitory Effect of Nafenopin Upon the Development of Diethylnitrosamine-Induced Enzyme-Altered Foci Within the Rat Liver," *Carcinogenesis* **5**, 41-46 (1984).

35. M.S. Rao, N.D. Lalwani, D.G. Scarpelli, and J.K. Reddy, "The Absence of γ-Glutamyl Transpeptidase Activity in Putative Preneoplastic Lesions and in Hepatocellular Carcinomas Induced in Rats by the Hypolipidemic Peroxisome Proliferator Wy-14,643," *Carcinogenesis* **3**, 1231-1233 (1982).

36. A. Poland, E. Glover, and A.S. Kende, "Stereospecific, High Affinity Binding of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin by Hepatic Cytosol," *J. Biol. Chem.* **251**, 4936-4946 (1976).

37. I. Issemann and S. Green, "Activation of a Member of the Steroid Hormone Receptor Superfamily by Peroxisome Proliferators," *Nature* **347**, 645-650 (1990).

38. D.S. Marsman and J.A. Popp, "Biological Potential of Basophilic Hepatocellular Foci and Hepatic Adenoma Induced by the Peroxisome Proliferator, Wy-14,643," *Carcinogenesis*, in press.

Implications of the Two-Stage Clonal Expansion Model for Interaction Between Two Carcinogens

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ABSTRACT

Both toxicological and epidemiological studies have provided evidence of interactive effects between two or more carcinogens. In this article, the effects of exposure to two carcinogens are examined within the context of the two-stage clonal expansion model of carcinogenesis. This biologically based model provides a useful framework for the quantitative description of laboratory and human data on carcinogenesis, and for defining carcinogenic agents acting as initiators, promoters, or completers. Depending on the mechanism of action of the two agents involved, the two-stage model predicts a spectrum of joint effects leading to additive, supra-additive, multiplicative, and even supra-multiplicative age-specific relative risks. Similar findings are presented in terms of cumulative lifetime risk rather than age-specific relative risk.

Reducing Conservatism in Risk Estimation for Mixtures of Carcinogens

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ABSTRACT

The excess cancer risk that might result from exposure to a mixture of chemical carcinogens usually must be estimated using data from experiments conducted with individual chemicals. In estimating such risk, it is commonly assumed that the total risk due to the mixture is the sum of the risks of the individual components, provided that the risks associated with individual chemicals at levels present in the mixture are low. This assumption, although itself not necessarily conservative, has led to the conservative practice of summing individual upper-bound risk estimates in order to obtain an upper bound on the total excess cancer risk for a mixture. Less conservative procedures are described here and are illustrated for the case of a mixture of four carcinogens.

INTRODUCTION

In the absence of appropriate human data, animal bioassay data often are used to estimate (predict) human cancer risk that might result from exposure to chemical carcinogens. In the case of individual chemical exposures, this involves both extrapolation (within animals) of risks observed at high experimental doses to low exposure levels usually more relevant to the human experience, and extrapolation of those low-dose risks predicted in animals to risks that might be expected in humans.

Because several chemicals may be present in air, water, food, and commercial products, frequently humans are exposed to many carcinogens simultaneously. In most situations, data on multiple-chemical exposures are not available, even in animals. Thus, predictions of cancer risk due to exposure to several carcinogens simultaneously must be based on the results of single-carcinogen experiments. This represents, in effect, a third type of extrapolation (i.e., from single-chemical to multiple-chemical exposure situations).

The principal assumption that has provided the basis for cancer risk estimation for mixtures is that the total risk due to the mixture is simply the sum of the risks of the individual components, provided

that the risks associated with individual carcinogens at levels present in the mixture are low. The Environmental Protection Agency (EPA) (1) has discussed the implications of this assumption in the context of low-dose linearity. The National Research Council (NRC) (2) identified conditions under which the additivity assumption applies theoretically, and indicated that it is reasonable in a wide variety of situations, including cases for which joint effects are far from additive at high-exposure levels. Although empirical validation of the NRC's conclusions might not be feasible, in the absence of specific information on chemical interactions (e.g., synergism or antagonism), the principle of low-dose additivity of risks continues to offer the best approach to cancer risk assessment for mixtures.

It was long ago recognized that various mathematical models that all fit a given set of observed experimental tumor data reasonably well could give extrapolated doses corresponding to low-risk levels that differed by several orders of magnitude (3). Thus, reliance solely on model-based point estimates of risk at low doses has been avoided. Instead, statistical upper-confidence limits (UCLs) corresponding to specific exposure (dose) levels generally have been used to characterize the experimental low-dose risk (4-7). In order to avoid model dependency, it is also desirable to characterize the cancer risk for mixtures in terms of statistical UCLs.

In the absence of a formal procedure for calculating upper bounds for mixtures, regulatory agencies have adopted the practice of summing upper-bound risk estimates for individual components. For example, the Food and Drug Administration (FDA) has stated that in the absence of specific information on the interactions among carcinogenic impurities in color or food additives, estimated upper-bound risks should be summed (8). Likewise, the EPA has stated that, assuming low-exposure levels and no synergistic or antagonistic interactions, the substance-specific upper-bound cancer risks should be summed to estimate the cancer risk due to exposure to mixtures found at Superfund sites (9). As a case in point, the latter procedure has been followed by the Army Environmental Hygiene Agency in its health risk assessment for Kuwait oil fires (10).

This paper addresses the calculation of statistical UCLs on total cancer risk for mixtures of carcinogens, assuming the additivity of excess cancer risks at low doses. For the calculation of these UCLs, usually a single experimental dataset will be selected to represent each chemical in the mixture, based on criteria such as sensitivity of species and relevance of end point. In addition, various conversions (e.g., species, route, dose units, duration) will be made to translate the administered animal

dose to a human-equivalent dose in the appropriate metric. In the single-chemical case, such UCLs on excess risk that are derived from selected experimental data and converted to human-equivalent UCLs often are referred to as "plausible upper bounds."

The problem is not as simple as summing statistical UCLs (or plausible upper bounds) for individual components in the mixture. In fact, such a procedure is generally conservative in the sense that it tends to overestimate the true underlying risk when such risk is truly additive. Thus, formal procedures for calculating upper limits on cancer risk for mixtures that are less conservative than simply summing upper-bound estimates could have an important impact on the regulation of chemical carcinogens.

PROBLEM DEFINITION

The problem is most easily visualized for a mixture of two carcinogens. Suppose, for example, that $R(D_1)$ and $R(D_2)$ define excess risks above background risk for dose levels D_1 of chemical 1 and D_2 of chemical 2, and that $R(D_1) <= R_1$ and $R(D_2) <= R_2$ with 95% confidence. R_1 and R_2 might be upper-bound estimates of risk calculated using GLOBAL86 (11). What is needed is a procedure for calculating a 95% UCL on $R(D_1,D_2)$ which is not so conservative as simply using R_1+R_2, the sum of upper bounds.

The reverse problem to that just defined is also encountered in practice. In this case, the objective is to fix the total excess risk at a particular value, for example R_0, where already $R(D_1) <= R_0$ and $R(D_2) <= R_0$ with 95% confidence. That is, values $d_1 < D_1$ and $d_2 < D_2$ are needed, such that $R(d_1,d_2) <= R_0$ with 95% confidence. Assuming low-dose linearity, the conservative approach for this reverse application that is equivalent to summing upper bounds is to select d_1 and d_2 to satisfy the equation $d_1/D_1 + d_2/D_2 = 1$. In practice, solutions for d_1 and d_2 using this equation have been found by choosing a particular mixing proportion, p, such that $d_1=pd$ and $d_2=(1-p)d$, where d represents the total mixture dose. The value of d that satisfies the equation gives unique solutions for d_1 and d_2, for the given value of p (12).

The problem for a mixture of $K>2$ carcinogens is defined similarly as for $K=2$. As would be expected, the reduction in conservatism that can be achieved by using a direct method rather than summing upper bounds increases as the number of carcinogens in the mixture increases. This reduction

will be most apparent for mixtures whose components have individual upper-bound risk estimates of approximately the same order of magnitude.

QUANTITATIVE METHODS FOR SINGLE CARCINOGENS

The approach to developing strategies for the mixture problem will be to extend procedures that have been used for the case of a single carcinogen. An experiment for a single carcinogen consists of g dose groups, including an untreated control. The i^{th} group contains n_i animals, each of which is subjected to a constant dose rate d_i throughout its lifetime. At the end of the experiment, x_i of the n_i animals are tumor-bearing. The binomial likelihood function for these data is

$$\prod_{i=1}^{g} [n_i!/(x_i!(n_i-x_i)!)][P(d_i,\theta)]^{x_i} [1-P(d_i,\theta)]^{n_i-x_i}$$

where $P(d,\theta)$ is a dose−response function (e.g., generalized multistage model) representing the probability of a carcinogenic response at dose d, and θ is a vector of parameters of the dose−response function.

The excess risk due to a carcinogen may be defined either as the "additional risk," $R(d,\theta)=P(d,\theta)-P(0,\theta)$, or as the "extra risk," which is additional risk divided by $1-P(0,\theta)$. A virtually safe dose is defined as a low level of the carcinogen, for example d_0, that satisfies $R(d_0,\theta)=\pi$, where π is a preselected small number (e.g., $\pi=10^{-6}$).

Procedures for obtaining UCLs on excess risk (lower-confidence limits [LCLs] on dose) for single carcinogens include likelihood-based methods and bootstrap techniques (13). To calculate a $100(1-\alpha)\%$ UCL on $R(d_0,\theta)$ for fixed d_0 using the likelihood approach, one solves

$$\max_\theta\{R(d,\theta):d=d_0,\ 2[L(\theta^*)-L(\theta)]=X^2_{1,1-2\alpha}\},$$

where L is the loglikelihood function, θ^* is the maximum likelihood estimate of θ, and $X^2_{1,1-2\alpha}$ is the $(1-2\alpha)^{th}$ percentage point of a Chi- square distribution with one degree of freedom.

To obtain UCLs on excess risk using bootstrap resampling, one may first obtain the maximum likelihood estimate of $P(d,\theta)$, i.e., $P(d,\theta^*)$, based on the given experimental data. Then, Monte Carlo simulation methods are used to generate a large number (e.g. 1000) of similar datasets using binomial sampling at each dose level, with $n=n_i$ and $p=P(d_i,\theta^*)$. For each of these generated datasets, the dose−response model is reestimated and $R(d_0,\theta')$ is calculated, where θ' is the new maximum likelihood

estimate of θ. The $1000\alpha^{th}$ largest value of $R(d_0,\theta')$ out of the 1000 would represent the bootstrapped upper-$100(1-\alpha)\%$-confidence limit on excess risk at d_0. This method is referred to as a parametric bootstrap resampling method. Alternatively, the bootstrap procedure may be implemented nonparametrically by randomly resampling the original data with replacement using Monte Carlo techniques, instead of generating samples based on $P(d,\theta^*)$. Lower-confidence limits on virtually safe doses for fixed excess risks may be obtained by reversing the likelihood and bootstrap procedures.

METHODS FOR TWO OR MORE CARCINOGENS

Suppose now that experimental data are available from K separate experiments on K components of a mixture. Chen *et al.* (14) have proposed a formal likelihood-based method to obtain an upper bound on the total excess risk for such a situation. As stated above, the risk-addition procedure assumes that the total excess risk due to $\underline{d}=(d_1, d_2,\ldots, d_K)$ is $R(\underline{d},\theta)=\Sigma_k R_k(d_k,\theta_k)$, where $\theta=(\theta_1, \theta_2,\ldots,\theta_K)$. To calculate a $100(1-\alpha)\%$ UCL on $R(\underline{d}_0,\theta)$ for fixed $\underline{d}_0=(d_{10}, d_{20},\ldots, d_{K0})$, one solves

$$\max_\theta\{R(\underline{d},\theta):\underline{d}=\underline{d}_0,\ 2\Sigma_k[L_k(\theta_k^*)-L_k(\theta_k)]=X^2_{1,1-2\alpha}\},$$

where $L_k(\theta_k)$ is the loglikelihood function for the k^{th} dataset.

A computational algorithm for implementing the procedure of Chen *et al.* (14) is outlined in the following steps.

1. Fix $\underline{d}_0 = (d_{10}, d_{20},\ldots, d_{K0})$.
2. Partition $X^2_{1,1-2\alpha}$ into K components, C_k (i.e., $\Sigma_k C_k=X^2_{1,1-2\alpha}$).
3. For the given partition, and for each k, solve $\max_{\theta_k}\{R_k(d_k,\theta_k):d_k=d_{k0},\ 2[L_k(\theta_k^*)-L_k(\theta_k)]=C_k\}$.
4. Calculate $R(\underline{d}_0,\theta)=\Sigma_k \max R_k(d_{k0},\theta_k)$ for the given partition.
5. Repeat steps 2-4 iteratively to find the partition of $X^2_{1,1-2\alpha}$ such that $R(\underline{d}_0,\theta)$ is maximum.

This algorithm may be implemented for the generalized multistage model using either GLOBAL82 or GLOBAL86 (11) sequentially, iteratively.

Use of the bootstrap method to generate upper bounds on the total excess cancer risk for a mixture of carcinogens involves a straightforward extension of the method for a single carcinogen. For each of a large number, N, of simulations (e.g., N=1000), a representative dataset is randomly generated for each of the K chemicals. For each such dataset at each simulation, $P_k(d_k,\theta_k)$ is estimated (k=1, 2,

..., K), and $R(\underline{d}_0,\theta)$ is calculated. The $N\alpha^{th}$ largest value of $R(\underline{d}_0,\theta)$ out of the N values would represent the bootstrapped upper-100(1-α)%-confidence limit on total excess risk at $\underline{d}_0$.

The inverse problem of finding an LCL on the virtually safe dose for a mixture is slightly more complicated than finding a UCL on total excess risk. For this problem, not only is the total excess risk preselected, but also the dose levels of K-1 chemicals in the mixture are fixed in advance. Then, an LCL on the remaining dose level is found using an algorithm similar to the one outlined above. Of course, the K-1 dose levels must be selected in such a way that the total excess risk for the K-1 carcinogens does not already exceed the preselected total excess risk for all K carcinogens. An alternative approach to the inverse problem is to fix the proportions $p_1, p_2,..., p_K$ of each component in the mixture, where $\Sigma_i p_i = 1$, and then solve for the total mixture dose that gives the preselected risk.

For the inverse problem, the bootstrap method is much easier to implement than the likelihood method, particularly for K>2. A brief description of the bootstrap method for K=2 is as follows. Let the value of d_1 be fixed at d_{10} and the total excess risk, $R(\underline{d}_0,\theta)$, be fixed at the value π, where $R(d_{10}) < \pi$. A lower-100(1-α)%-confidence limit, d_{20}, on d_2 is desired. For each of N (e.g., N=1000) simulations, generate a representative dataset for each of the two chemicals. For Chemical 1, fit the model and calculate $R(d_{10},\theta_1)$. For Chemical 2, fit the model and solve for the value of d_2 such that $R(d_2,\theta_2) = \pi - R(d_{10},\theta_1)$. The LCL, d_{20}, will be the $N\alpha^{th}$ smallest value of d_2 calculated this way.

LOW-DOSE LINEAR EXTRAPOLATION

For the case of a single carcinogen, Gaylor and Kodell (15) recommended calculating confidence limits below the lowest experimental dose level using linear extrapolation, rather than by direct calculation of such confidence limits. The purpose of the recommendation, which was later clarified to encourage linear extrapolation below the dose corresponding to a predicted 1% excess risk level (16), was to minimize model dependency at extremely low dose (low risk) levels. A similar approach may be used in the case of a mixture of K carcinogens. For purposes of illustration, the case in which K=2 is described below.

Asssume that an upper 100(1-α)% confidence limit on the total risk is desired for a mixture containing levels d_{10} and d_{20} of Chemicals 1 and 2, respectively. Assume further that $d_{10} < ED_{01,1}$ and $d_{20} < ED_{01,2}$, where $ED_{01,k}$ represents the predicted dose level at 1% excess risk for Chemical k. Using

the bootstrap method, a large number, N, of representative datasets for Chemicals 1 and 2 are randomly generated. For each pair of datasets, the generalized multistage model (or some other model) is fitted, and the predicted $ED_{01,k}$ (k=1,2) is calculated; this involves an iterative routine for models such as the multistage model. The excess risk at dose level d_{k0} of Chemical k (k=1,2) is calculated by linear extrapolation as

$$R(d_{k0},\theta_k) = (0.01)d_{k0}/ED_{01,k}.$$

The $N\alpha^{th}$ largest value of $R(d_{10},\theta_1)+R(d_{20},\theta_2)$ calculated this way will be the linearly extrapolated 100(1-α)% UCL on the total excess risk. Lower-confidence limits on dose may also be calculated using this linear-extrapolation approach.

ILLUSTRATION OF LIKELIHOOD AND BOOTSTRAP APPROACHES

The health effects of 21 chemicals found in drinking water have been evaluated by the NRC (17,18). In the evaluation, the multistage model was used to calculate cancer risk for chemicals that showed evidence of carcinogenicity in animal bioassays. Various conversion factors were used to translate the UCLs on risk calculated for the administered animal doses to upper bounds on lifetime human risk associated with concentrations (in μg/L) in drinking water. Four chemicals from the 21 evaluated by the NRC are used for illustration of the confidence-limit methodology. These chemicals, their associated relevant bioassay data identified by the NRC (18), and their estimated multistage model coefeicients are given in Table 35.

Individual 95% UCLs on lifetime excess cancer risk for each chemical, calculated using the bioassay data and conversion factors identified by the NRC (18), and assuming daily consumption of 1 L of water containing the chemical at a concentration of 1 μg/L, are given in Table 36 for both the likelihood-based and nonparametric bootstrap methods. For the likelihood approach, UCLs were calculated based only on direct model predictions, while for the bootstrap approach, UCLs were calculated based both on direct model predictions and on extrapolation below the ED_{01}s of the individual chemicals. Calculations were made on the basis of extra risk, as defined above, with 1000 simulations being used for the bootstrap method. The results in Table 36 suggest that upper bounds on risk produced by the bootstrap method tend to be lower than those produced by the likelihood method. There is no difference between the UCLs for individual chemicals computed by the two different bootstrap methods; for mixtures, UCLs based on linear extrapolation below the ED_{01} tend to be slightly larger.

TABLE 35. BIOASSAY TUMOR DATA AND MULTISTAGE MODEL PARAMETER ESTIMATES FOR FOUR CHEMICALS IN DRINKING WATER[a]

Chemical	Sex/ Strain/ Species	Doses (mg/kg/d)	Tumor Site	Tumor Rates	Parameter Estimates
Chlorobenzene	Male	0	Liver	2/50	q_0=4.08E-2
	F-344	60		4/49	q_1=3.33E-4
	Rat	120		8/49	q_2=6.77E-6
Hexachlorobenzene	Male	0	Liver	0/47	q_0=0
	Swiss	6		0/30	q_1=2.04E-3
	Mouse	12		3/29	q_2=2.47E-4
		24		7/44	q_3=0
Trichloroethylene	Male	0	Liver	8/48	q_0=1.82E-1
	$B6C3F_1$	1,000		30/50	q_1=7.34E-4
1,1,1-Trichloroethane	Male	0	Liver	16/50	q_0=4.38E-1
	$B6C3F_1$	1,500		24/50	q_1=4.93E-5
	Mouse	3,000		20/50	Q_2=0

[a] Tabulated data were selected for risk assessment by the NRC (18).

TABLE 36. UPPER-95%-CONFIDENCE LIMITS ON EXTRA CANCER RISK FOR SELECTED CHEMICALS AT CONCENTRATIONS OF 1 μG/L IN DRINKING WATER

		Statistical Method for UCL		
Chemical		Direct Likelihood	Direct Bootstrap	ED_{01} Bootstrap
A. Chlorobenzene		2.14×10^{-7}	1.91×10^{-7}	1.91×10^{-7}
B. Hexachlorobenzene		1.92×10^{-6}	1.50×10^{-6}	1.50×10^{-6}
C. Trichloroethane		2.74×10^{-7}	2.79×10^{-7}	2.79×10^{-7}
D. 1,1,1-Trichloroethane		3.70×10^{-8}	3.78×10^{-8}	3.78×10^{-8}
Mixture	**Mixture Method**	**Direct Likelihood**	**Direct Bootstrap**	**ED_{01} Bootstrap**
A. and B.	Sum UCLs	2.14×10^{-6}	1.69×10^{-6}	1.69×10^{-6}
	Proposed	2.01×10^{-6}	1.63×10^{-6}	1.65×10^{-6}
A. and C.	Sum UCLs	4.88×10^{-7}	4.70×10^{-7}	4.70×10^{-7}
	Proposed	4.30×10^{-7}	4.01×10^{-7}	4.03×10^{-7}
A., B., C.	Sum UCLs	2.41×10^{-6}	1.97×10^{-6}	1.97×10^{-6}
	Proposed	---	1.83×10^{-6}	1.86×10^{-6}
A., B., C., D.	Sum UCLs	2.45×10^{-6}	2.01×10^{-6}	2.01×10^{-6}
	Proposed	---	1.84×10^{-6}	1.88×10^{-6}

Comparison of upper bounds in Table 36 computed by summing individual UCLs to those based on the method proposed in this paper demonstrates the reduction in conservatism that can be achieved using the proposed method. Note that the reduction in conservatism of 12 to 15% for water containing only chlorobenzene and trichloroethylene (A and C) is considerably greater than the reduction of only 2 to 6% for water containing only chlorobenzene and hexachlorobenzene (A and B). This is because the individual upper bound for chlorobenzene (A) is of the same order of magnitude as that for trichloroethylene (C), but is an order of magnitude smaller than the upper bound for hexachlorobenzene (B). This illustrates the property that the greatest reduction in conservatism is achieved when individual UCLs are roughly of the same order of magnitude. When they are not, the chemical with the highest individual UCL tends to dominate the UCL for the mixture. This is also illustrated in Table 36 by the addition of trichloroethylene (C) to water already containing chlorobenzene (A) and hexachlorobenzene (B), because hexachlorobenzene continues to dominate. It is further shown by the insignificant change in the UCL for the mixture after the addition of 1,1,1-trichloroethane (D) to water already containing the other three chemicals.

DISCUSSION

The calculation of UCLs on excess carcinogenic risk due to mixtures of chemicals does not have to be as conservative as simply summing upper-bound risks for individual components of the mixture. If carcinogenesis data on individual chemicals are available, then it is fairly easy to implement the methods described in this paper. Although, in principle, the likelihood-based method that is popular for individual chemicals can be extended to apply to a mixture of K chemicals, developing a computational algorithm for $K>2$ is somewhat difficult. Because it will involve considerable iteration, it might not be all that less computer-intensive than the bootstrap method. The bootstrap method is easy to implement, and the fact that it is computationally intensive is not a major concern in light of the speed of modern computers.

Large differences between the likelihood and bootstrap UCLs are seen in Table 36 for certain individual chemicals and certain mixtures, with the bootstrap values being less than the corresponding likelihood values. In general, the more linear the data and corresponding fitted model (Table 35), the closer the agreement between the methods. This is in line with expectations, because for highly nonlinear data, the bootstrap approach would be expected to overcome some of the conservatism for which the likelihood approach has been criticized. However, this reduction in conservatism is meaningful only if

the bootstrap-based confidence limits actually maintain appropriate coverage. Smith and Sielken (19) conducted a Monte Carlo simulation study to compare the behavior of the likelihood approach to various bootstrap procedures, including the simple parametric approach described above. Their study was directed at the inverse problem, namely, finding an LCL on the dose corresponding to an excess risk of 1/100,000. Smith and Sielken (19) concluded that the simple bootstrap procedure offers improvements over the likelihood-ratio-based confidence limit procedure with virtually no undesirable side effects. However, for multistage models that had only linear and cubic terms, the parametric bootstrap procedure did not maintain nominal coverage in their study. None of the fitted models for the chemicals in Table 35 are of this type. Smith and Sielken did not simulate the simple nonparametric bootstrap approach used in this paper.

One interpretation of the results in Table 36 is that the conservatism associated with adding upper-bound risk estimates does not appear to be of great concern, at least for a mixture with a small number of components. In fact, using the ED_{01}-bootstrap approach, the proposed method leads to only a 7% reduction in the UCL for the four-chemical mixture (Table 36). However, as has been stated above, for a mixture of equipotent chemicals, the reduction in conservatism will be more striking. Furthermore, because the proposed method is easy to implement, there is no compelling reason not to use it to reduce unnecessary conservatism. Of course, the validity of the procedure rests on the assumption of additivity of risks at low doses. To evaluate this assumption, it would be desirable, and perhaps not infeasible, to have low-dose experimental data on a mixture of a small number of chemicals.

Whereas in this paper, attention has centered on the generalized multistage model for fitting tumorigenicity data, other models, such as the two-stage clonal expansion model, may just as appropriately be used to implement the bootstrap procedure. The main consideration is having a suitable model-fitting routine. The entries in Table 36 were generated using GLOBAL86 (11) in conjunction with a FORTRAN routine for resampling and sorting. Calculation of the bootstrapped UCL for the four-chemical mixture in Table 36 took approximately 4½ min. for the direct method and just over 5 min. for the linear extrapolation method, using a 486-33 machine with OS/2. The authors are considering the development of a computational algorithm for the general user.

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REFERENCES

1. U.S. Environmental Protection Agency, "Guidelines for the Health Risk Assessment of Chemical Mixtures," *Federal Register* **51** (185), 34014-34025 (1986).

2. National Research Council, *Complex Mixtures: Methods for In Vivo Toxicity Testing* (National Academy Press, Washington, 1988), pp. 195-198.

3. FDA Advisory Committee on Protocols for Safety Evaluation, Panel on Carcinogenesis, "Report on Cancer Testing in the Safety Evaluation of Food Additives and Pesticides." *Toxicol. Appl. Pharmacol.* **20**, 419-438 (1971).

4. E.L. Anderson and the Carcinogen Assessment Group of the U.S. Environmental Protection Agency, "Quantitative Approaches in Use to Assess Cancer Risk," *Risk Anal.* **3**, 277-295 (1983).

5. W.G. Flamm and J.S. Winbush, "Role of Mathematical Models in Assessment of Risk and in Attempts to Define Management Strategy," *Fundam. Appl. Toxicol.* **4**, S395 (1984).

6. Food and Drug Administration, "Sponsored Compounds in Food-Producing Animals: Proposed Rule and Notice," *Federal Register* **50**, 45530-45556 (1985).

7. U.S. Environmental Protection Agency, "Guidelines for Carcinogen Risk Assessment," *Federal Register* **51** (185), 33993-34003 (1986).

8. Food and Drug Administration, "D&C Red No. 33: Final Rule," *Federal Register* **53** (168), 33110-33121 (1988).

9. U.S. Environmental Protection Agency, *Risk Assessment Guidance for Superfund*, Volume I - Human Health Evaluation Manual (Part A), Interim Final, EPA 540 1-89 002 (NTIS, Springfield, VA, 1989).

10. Army Environmental Hygiene Agency, *Interim Kuwait Oil Fire Health Risk Assessment*, AEHA No. 39-26-L192-91 (1991).

11. Clement International Corp., Risk Assessment Software for Cancer Endpoints, Ruston, LA 71270 (1986).

12. M.L. Dourson and J.M. Clark, "Fish Consumption Advisories: Toward a Unified, Scientifically Credible Approach," *Reg. Toxicol. Pharmacol.* **12**, 161-178 (1990).

13. K.S. Crump and R.B. Howe, "A Review of Methods for Calculating Confidence Limits in Low Dose Extrapolation," in D.B Clayson, D.R. Krewski and I.C. Munroe (eds.), *Toxicological Risk Assessment*, Vol.1, *Biological and Statistical Criteria* (CRC Press, Boca Raton, 1985), pp.187-203.

14. J.J. Chen, D.W. Gaylor, and R.L. Kodell, "Estimation of the Joint Risk from Multiple-Compound Exposure Based on Single-Compound Experiments," *Risk Anal.* **10**, 285-290 (1990).

15. D.W. Gaylor and R.L. Kodell, "Linear Interpolation Algorithm for Low Dose Risk Assessment of Toxic Substances," *J. Environ. Pathol. Toxicol.* **4**, 305-312 (1980).

16. J.H. Farmer, R.L. Kodell, and D.W. Gaylor, "Estimation and Extrapolation of Tumor Probabilities from a Mouse Bioassay with Survival/Sacrifice Components," *Risk Anal.* **2**, 27-34 (1982).

17. National Research Council, *Drinking Water and Health*, Vol. 3, *Report of the Safe Drinking Water Committee*, Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences (National Academy Press, Washington, DC, 1980).

18. National Research Council, *Drinking Water and Health*, Vol.5, *Report of the Safe Drinking Water Committee*, Board on Toxicology and Environmental Health Hazards, Commission of Life Sciences (National Academy Press, Washington, DC, 1983).

19. L.A. Smith and R.L. Sielken, "Bootstrap Bounds for 'Safe' Doses in the Multistage Cancer Dose-Response Model," *Communications in Statistics - Simulations* **17**, 153-175 (1988).

Modeling for Risk Assessment of Neurotoxic Effects

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ABSTRACT

The regulation of noncancer toxicants, including neurotoxicants, has usually been based upon a reference dose (allowable daily intake). A reference dose is obtained by dividing a no-observed-effect level by uncertainty (safety) factors to account for intraspecies and interspecies sensitivities to a chemical. It is assumed that the risk at the reference dose is negligible, but no attempt generally is made to estimate the risk at the reference dose. A procedure is outlined that provides estimates of risk as a function of dose. The first step is to establish a mathematical relationship between a biological effect and the dose of a chemical. Knowledge of biological mechanisms and/or pharmacokinetics can assist in the choice of plausible mathematical models. The mathematical model provides estimates of average responses as a function of dose. Secondly, estimates of risk require selection of a distribution of individual responses about the average response given by the mathematical model. In the case of a normal or lognormal distribution, only an estimate of the standard deviation is needed. The third step is to define an adverse level for a response so that the probability (risk) of exceeding that level can be estimated as a function of dose. Because a firm response level often cannot be established at which adverse biological effects occur, it may be necessary to at least establish an abnormal response level that only a small proportion of individuals would exceed in an unexposed group. That is, if a normal range of responses can be established, then the probability (risk) of abnormal responses can be estimated. In order to illustrate this process, measures of the neurotransmitter serotonin and its metabolite 5-hydroxyindoleacetic acid in specific areas of the brain of rats and monkeys are analyzed after exposure to the neurotoxicant methylenedioxymethamphetamine. These risk estimates are compared with risk estimates from the quantal approach in which animals are classified as either abnormal or not, depending upon abnormal serotonin levels.

INTRODUCTION

Most disease risk estimation techniques have focused on quantal data in which an animal can be classified with or without a biological effect, (e.g., tumor or birth defect). For nonquantal (continuous) data, different procedures are needed. Examples of continuous data are organ weights and hematological measurements. Crump (1) utilized several dose–response models to estimate the doses corresponding to various changes, (e.g., 1, 5, and 10%), from the control means. No attempt was made to define an adverse change or to estimate the risk for continuous end points.

Gaylor and Slikker (2) proposed a technique for estimating risk of an adverse effect for continuous data. In the absence of a specified level defined to be adverse, the risk of an abnormal level was estimated. Abnormal was defined as outside the normal range (e.g., below the 0.1 percentile or above the 99.9 percentile), for a continuous end point in untreated control animals. Unless a large number of animals are available, extreme percentiles cannot be determined directly and must be estimated from a sample of animals. This requires establishing a distribution of the end point about the average value. For a normal or lognormal distribution, estimates of the mean and standard deviation provide estimates of percentiles. The estimate of the mean, as a function of dose, is provided by fitting a dose–response curve to bioassay data. Then the risk (probability of being outside the normal range for a biological end point) can be estimated as a function of dose.

Let y denote the measurement of a biological effect in an individual animal, $\bar{y}$ the sample average, and s the sample standard deviation. If y is normally distributed, then the proportion of measurements estimated to be below ($\bar{y}$ - Z_ps) is approximately p, where Z_p can be obtained from statistical tables (e.g., Beyer [3]). For example, the estimate of the 0.1 percentile for control animals is $y_{.001}$ = ($\bar{y}$ - 3.09s), where $\bar{y}$ is the average and s is the standard deviation for the controls. This value can be used to define the abnormal range. Let $\bar{y}$ (d) represent the estimate of the average at a dose (d). As given by Gaylor and Slikker (2), the probability of an abnormal level at dose (d) is estimated by calculating Z = ($y_{.001}$- $\bar{y}$ (d))/s(d), where s(d) is the estimated standard deviation at dose (d). The estimated probability of an abnormal level at dose (d) is p(d), which is the probability corresponding to Z obtained from standard tables, (e.g., Beyer [3]). The excess risk above background is estimated to be (p(d) - 0.001). Obviously, a different percentile could be used to define an abnormal level.

If there is concern about large values of an end point, values above the 99.9 percentile ($y_{.999} = \bar{y} + 3.09s$) may be considered abnormal. The probability of an abnormal level at a dose (d) is estimated by the probability that Z is greater than $(y_{.999} - \bar{y}(d))/s(d)$.

If the measurements are described by a lognormal distribution, then $\bar{y}$ is the average of the logarithms of the ys (i.e., the geometric mean) and s is the standard deviation of the logarithms of the ys. A detailed example of the calculation of risk is given by Gaylor and Slikker (4).

In the above calculations of risk, it is necessary to establish a dose–response function to provide an estimate of the average response $\bar{y}(d)$ at dose (d). For most bioassay data, there generally will be several dose–response models that will describe the data equally well. For predictions of risk within the experimental dose range, the choice of the dose–response model generally has little effect. However, for risk estimation below the experimental dose range, the choice of the dose–response model is critical. To the extent possible, the dose–response model should be based upon knowledge of pharmacokinetics and the biological mechanisms of toxicity. In this paper, the risk estimation procedure proposed by Gaylor and Slikker (2) will be reviewed and illustrated with data on rats and monkeys. A comparison will be made between the analysis based on continuous data and converting the data to a quantal analysis.

Benchmark Dose

The regulation of noncancer toxicants generally is based upon establishing a no-observed-adverse-effect level (NOAEL) and dividing by uncertainty (safety) factors to obtain a reference dose (allowable daily intake). A comprehensive description of the choice of uncertainty factors and calculation of reference doses is given by Barnes and Dourson (5). It is recognized that the NOAEL is ill-defined, depends on the dose levels tested, and does not make full use of dose–response data. More importantly, use of the NOAEL does not encourage good experimentation. In fact, just the opposite is true. The poorer experiment that has less power to detect biological effects may result in higher NOAELs and correspondingly higher reference doses. Further, the risk at the NOAEL is not necessarily zero and varies from case to case. Gaylor (6) showed from published data on 93 developmental toxicants that the risk of malformations or dead/resorbed fetuses at the NOAEL exceeded 1% in about one-fourth of the cases.

Because of the shortcomings of the NOAEL, various authors (e.g., Crump [1] and Kimmel and Gaylor [7]), have suggested that the NOAEL be replaced by a dose corresponding to a low level of risk on the order of 1 to 10%. These levels of risk generally can be estimated with adequate precision from bioassay data. Specifically, the benchmark dose (BMD) is defined as a lower confidence limit estimate corresponding to a low level of risk. For example, if the effective dose corresponding to a risk of 5% is chosen (ED05), the BMD would be designated as its lower 95% confidence limit (LED05). Now, a better experiment with tighter confidence limits will result in a larger BMD. Uncertainty (safety) factors would still be applied to the BMD to account for variation in sensitivity among individuals, animal to human extrapolation, and different exposure conditions.

The BMD approach will be illustrated later for neurochemical effects in the brains of rats and monkeys.

Neurochemical Examples

Neurochemical effects measured in experiments conducted at the National Center for Toxicological Research reported by Slikker *et al.* (8,9) will be used to illustrate methods of risk assessment for continuous data. In these studies, methylenedioxymethamphetamine (MDMA) was administered orally to male rats and female monkeys for 4 consecutive days. Neurochemical measurements were made in various areas of the brain 2 and 4 weeks later. For purposes of illustration, concentrations of the neurotransmitter serotonin (5-HT) and its metabolite 5-hydroxindoleacetic acid (5-HIAA) in the hippocampus and frontal cortex will be used. Gaylor and Slikker (2) showed that 5-HT and 5-HIAA are approximately lognormally distributed. Thus, all calculations were performed on the logarithms of 5-HT and 5-HIAA. Also, the standard deviation of the logarithm of 5-HT and 5-HIAA were approximately equal across doses. That is, the coefficient of variation of 5-HT and 5-HIAA measurements are nearly constant for all dose levels. Thus, pooled estimates of the standard deviation were used.

Based on pharmacokinetic and mechanistic considerations, Slikker and Gaylor (10) used a saturation-type model to describe the dose−response. The concentrations (y) of 5-HT and 5-HIAA in the brain relative to the background level in control animals was modeled by

$$y = \frac{1 + Ad}{1 + Bd}$$

where A and B are constants estimated from bioassay data and d is dose. At the background level d=0, y=1. At large doses, the relative concentration approaches A/B. The low-dose slope is (A-B). The SAS procedure NLIN (11) was used to fit the nonlinear saturation-type model to the bioassay data.

The geometric means (based on the averages of the logarithms of 5-HT and 5-HIAA) are displayed in Tables 37 and 38 for male rats and female monkeys, respectively. Doses are expressed as milogram per kilogram body weight per day. The fitted dose−response curves are given in Tables 39 and 40 for rats and monkeys, respectively. The results for 5-HT in the hippocampus are also displayed in Figure 37. The estimated proportions (risk) of abnormal concentrations of 5-HT in the hippocampus are shown in Figure 38.

The abnormal levels were based on the 0.1 percentile of the lognormal distribution of the control animals. To illustrate the benchmark dose process, the dose corresponding to a risk of 5% (i.e., the ED05) was calculated. Chen and Gaylor (12) and Kodell and West (13) provide procedures for calculating confidence limits for continuous data. Here, approximate variances (V) of estimates of the logarithm of relative concentrations (y) were obtained for the Taylor's Series expansion of the dose-response model

$$V(\ln y) \approx d^2 \left[\frac{V(A)}{(1+Ad)^2} + \frac{V(B)}{(1+Bd)^2} - \frac{2\,Cov(A,B)}{(1+Ad)(1+Bd)} \right]$$

V(A) is the variance of the estimate of A, similarly for V(B), and Cov (A,B) is the covariance of the estimates of A and B.

The asymptotic upper 95% confidence limit on the relative concentration is $U = \exp(\ln \bar{y} + 1.645 \sqrt{V(\ln y)})$. Solving $U = (1+Ad)/(1+Bd)$ for d gives the approximate LED05 which could be used as a BMD in place of the NOAEL. A "safe" or "reference" dose is calculated by dividing the NOAEL or BMD by appropriate safety or uncertainty factors. The results are shown in Tables 39 and 40 for male rats and female monkeys, respectively.

TABLE 37. GEOMETRIC MEAN RESPONSE EXPRESSED AS PROPORTION OF THE CONTROL MEAN 30 DAYS AFTER ORAL ADMINISTRATION OF MDMA IN MALE RATS ON 4 CONSECUTIVE DAYS

Dose (mg/kg/day)	5-HT in Hippocampus	5-HT in Frontal Cortex	5-HIAA in Hippocampus	5-HIAA in Frontal Cortex
0	1.00 (13)[a]	1.00 (13)	1.00 (13)	1.00 (13)
5	0.88 (5)	0.80 (5)	0.75 (5)	0.89 (5)
10	0.79 (4)	0.66 (4)	0.56 (4)	0.74 (4)
80[b]	0.46 (8)	0.39 (8)	0.41 (7)	0.62 (7)
160[b]	0.54 (6)	0.44 (6)	0.42 (6)	0.64 (6)
160	0.53 (5)	0.44 (7)	0.41 (7)	0.62 (7)

[a]Number of animals examined.
[b]Animals examined 14 days after last administration of MDMA.

TABLE 38. GEOMETRIC MEAN RESPONSE EXPRESSED AS PROPORTION OF THE CONTROL MEAN 30 DAYS AFTER ADMINISTRATION OF MDMA IN FEMALE MONKEYS ON 4 CONSECUTIVE DAYS

Dose (mg/kg/day)	5-HT in Hippocampus	5-HT in Frontal Cortex	5-HIAA in Hippocampus	5-HIAA in Frontal Cortex
0	1.00 (7)[a]	1.00 (7)	1.00 (7)	1.00 (6)
2.5	0.87 (4)	0.95 (4)	0.78 (4)	0.92 (4)
5.0	0.69 (3)	0.60 (4)	0.84 (3)	0.74 (4)
10.0	0.26 (3)	0.23 (3)	0.38 (3)	0.42 (3)
20.0	0.17 (3)	0.11 (2)	0.20 (3)	0.05 (2)
40.0	0.09 (4)	0.19 (4)	0.15 (4)	0.42 (4)

[a]Number of animals examined.

TABLE 39. SUMMARY OF NEUROCHEMICAL RESPONSES 30 DAYS AFTER ORAL ADMINISTRATION OF MDMA IN MALE RATS ON 4 CONSECUTIVE DAYS (ADAPTED FROM SLIKKER AND GAYLOR [10])

	5-HT in Hippocampus	5-HT in Frontal Cortex	5-HIAA in Hippocampus	5-HIAA in Frontal Cortex
Saturation model[a]	$\frac{1+0.031d}{1+0.068d}$	$\frac{1+0.043d}{1+0.114d}$	$\frac{1+0.069d}{1+0.179d}$	$\frac{1+0.078d}{1+0.129d}$
Standard deviation[b]	0.318	0.312	0.354	0.250
Abnormal level[c]	0.37	0.38	0.33	0.46
ED05[d]	30.9	13.0	11.1	28.0
LED05[e]	17.3	6.6	5.4	4.6
Quantal ED05[f]	61.1	17.3	19.9	h
Quantal LED05[g]	23.1	9.8	10.8	h

[a]Geometric mean relative to controls at a dose of mg/kg/day of MDMA.
[b]Standard deviation of $\log_e$ (level of 5-HT or 5-HIAA).
[c]0.1 percentile of control levels expressed as proportion control mean.
[d]Estimated dose from saturation model corresponding to excess risk of 5% abnormal animals.
[e]Lower 95% confidence limit on estimated ED05.
[f]ED05 estimated from binomial incidence of abnormal animals.
[g]Lower 95% confidence limit on the binomial estimate of the ED05.
[h]Inadequate number of abnormal animals for binomial analysis.

TABLE 40. SUMMARY OF NEUROCHEMICAL RESPONSES 30 DAYS AFTER ORAL ADMINISTRATION OF MDMA IN MALE RATS ON 4 CONSECUTIVE DAYS (ADAPTED FROM SLIKKER AND GAYLOR [10])

	5-HT in Hippocampus	5-HT in Frontal Cortex	5-HIAA in Hippocampus	5-HIAA in Frontal Cortex
Saturation model[a]	$\frac{1}{1+0.137d}$	$\frac{1}{1+0.100d}$	$\frac{1}{1+0.099d}$	$\frac{1+0.027d}{1+0.127}$
Standard deviation[b]	0.35	0.65	0.41	0.40
Abnormal level[c]	0.34	0.13	0.28	0.29
ED05[d]	4.8	16.5	8.3	10.0
LED05[e]	2.0	1.7	3.9	4.2
Quantal ED05[f]	3.0	8.0	5.9	5.3
Quantal LED05[g]	0.6	2.2	0.9	1.2

[a]Geometric mean relative to controls at dose of mg/kg/day of MDMA.
[b]Standard deviation of $\log_e$ (level of 5-HT or 5-HIAA).
[c]0.1 percentile of control levels expressed as proportion control mean.
[d]Estimated dose from saturation model corresponding to excess risk of 5% abnormal animals.
[e]Lower 95% confidence limit on estimated ED05.
[f]ED05 estimated from binomial incidence of abnormal animals.
[g]Lower 95% confidence limit on the binomial estimate of the ED05.

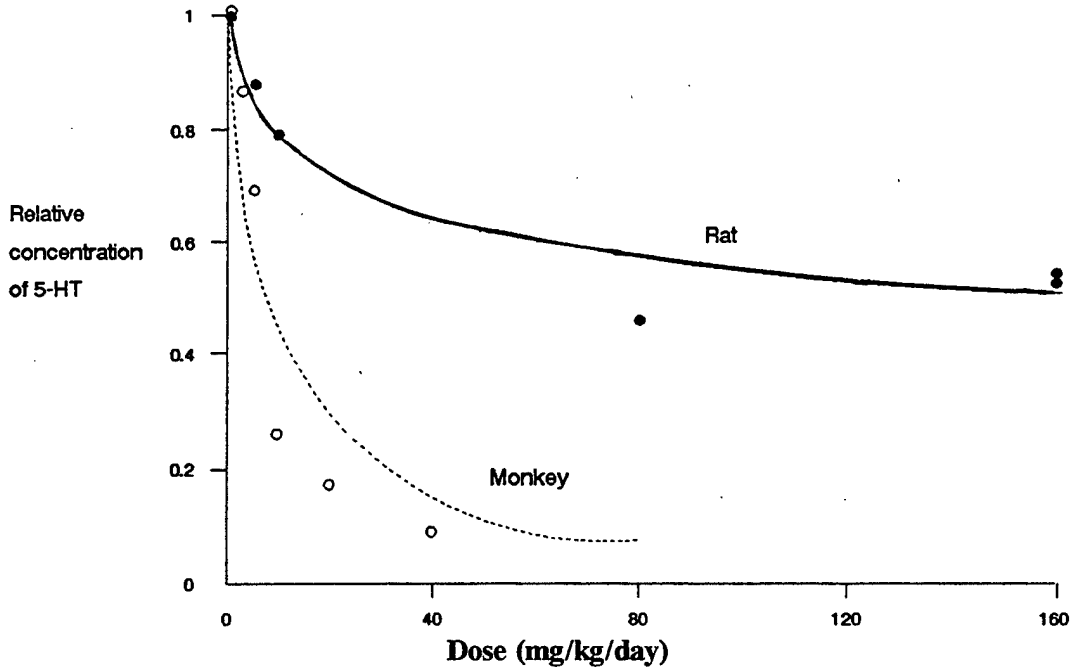


Figure 37. Relative Concentration of 5-HT in the Hippocampus of Rats and Monkeys.

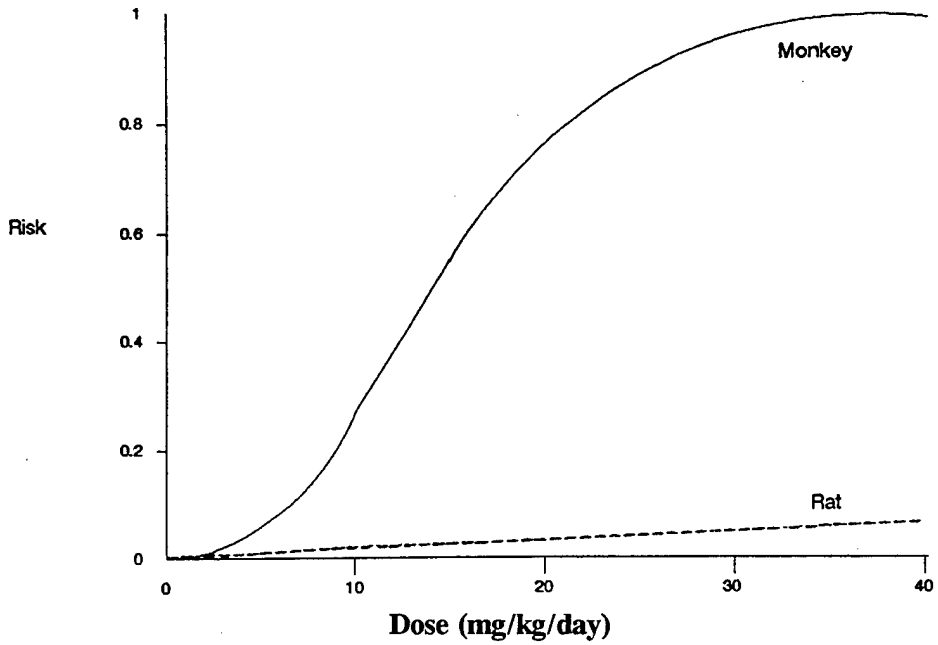


Figure 38. Estimated Proportion of Animals with an Abnormal Concentration of 5-HT in the Hippocampus as a Function Dose of MDMA.

An alternative approach to calculating a BMD is to classify the measurements as abnormal or normal and then proceed with a quantal analysis of the binomial proportion of abnormal animals. The values below the 0.1 percentile of the control animals were classified as abnormal. The proportion P of abnormal animals was assumed to be described by an exponential-polynomial of dose (d)

$$P = 1 - \exp[b_0 + b_1 d + b_2 d^2 + \ldots] .$$

Procedures given by Howe and Crump (14) were used to estimate the LED05 for a BMD. These results are given in Tables 39 and 40 for rats and monkeys, respectively.

DISCUSSION

These analyses demonstrate that female monkeys are more sensitive to MDMA than male rats when compared on the basis of the concentrations of 5-HT and 5-HIAA in the hippocampus and frontal cortex relative to the concentrations in unexposed control animals.

For the male rats, the lowest dose of 5 mg/kg/day giving a mean of 88% of the controls (Table 37) might not be considered as showing an adverse effect; although, it is not clear if 5 mg/kg/day would be selected as the NOAEL. If the LED05 were used as a BMD in place of the NOAEL, a larger value of 17.3 mg/kg/day would be used to set allowable doses based upon 5-HT in the hippocampus (Table 39). For the other end points, the LED05 is close to the purported NOAEL.

For female monkeys, the lowest dose of 2.5 mg/kg/day giving a mean of 87% of the controls (Table 38), might not be considered as showing an adverse effect. Again, it is not clear if this would be considered the NOAEL. The LED05 is close to 2.5 mg/kg/day for each of the four end points (Table 40).

Further use of these techniques is needed to determine what level of risk should be used for the BMD.

For the rats, the LEDs obtained from the continuous data analysis and the quantal analysis were similar (Table 39). For the monkeys, the LED05s were often considerably much lower when the quantal analysis was used. This may be a result, in part, of the small number of animals per dose in the monkey

studies. Again, a large simulation study could probably resolve the relative efficiency of the continuous analysis compared to the quantal analysis.

The continuous data analysis requires an estimate of the standard deviation. Estimates of the standard deviation can become quite imprecise for small numbers of animals. Fortunately, compensating errors tend to reduce the effects of imprecision of the estimate of the standard deviation. For example, if the estimate of the standard deviation is too large, the estimated spread of the distribution is too large, but the estimate of the difference from the mean for the abnormal level also will be too large. Again, an examination of the effects of imprecision of estimates of the standard deviation could be studied by computer simulations. This could also lead to recommendations for the number of dose levels and number of animals per dose to use in bioassays.

In the analyses conducted to date, it is encouraging that reasonable results appear to be obtainable from the continuous data approach even with a relatively small number of animals.

REFERENCES

1. K.S. Crump, "A New Method for Determining Allowable Daily Intakes," *Fundam. Appl. Toxicol.* **4**, 854-871 (1984).

2. D.W. Gaylor and W. Slikker, Jr., "Risk Assessment for Neurotoxic Effects," *Neurotoxicology* **11**, 211-218 (1990).

3. W.H. Beyer, *Handbook of Tables for Probability and Statistics*, 2nd ed. (The Chemical Rubber Co., Cleveland, OH, 1968).

4. D.W. Gaylor and W. Slikker, Jr., "Risk Assessment for Neurotoxicants," in H. Tilson and C. Mitchell (eds.), *Neurotoxicology* (Raven Press, Ltd., NY, 1992).

5. D.G. Barnes and M. Dourson, "Reference Dose (RfD): Description and Use in Health Risk Assessments," *Reg. Toxicol. Pharmacol.* **8**, 471-486 (1988).

6. D.W. Gaylor, "Incidence of Developmental Defects at the No Observed Adverse Effect Level (NOAEL)," *Reg. Toxicol. Pharmacol.* **15**, 151-160 (1992).

7. C.A. Kimmel and D.W. Gaylor, "Issues in Qualitative and Quantitative Risk Analysis for Developmental Toxicology," *Risk Anal.* **8**, 15-20 (1988).

8. W. Slikker, Jr., S.F. Ali, A.C. Scallet, C.H. Frith, G.D. Newport, and J.R. Bailey, "Neurochemical and Neurohistological Alterations in the Rat and Monkey Produced by Orally Administered Methylenedioxymethamphetamine (MDMA)," *Toxicol. Appl. Pharmacol.* **94**, 448-457 (1988).

9. W. Slikker, Jr., R.R. Holson, S.F. Ali, M.G. Kolta, M.G. Paule, A.C. Scallet, D.E. McMillan, J.R. Bailey, J.S. Hong, and F.M. Scalzo, "Behavioral and Neurochemical Effects of Orally Administered MDMA in the Rodent and Non-Human Primate," *Neurotoxicology* **10**, 529-542 (1989).

10. W. Slikker, Jr. and D.W. Gaylor, "Biologically-Based Dose-Response Model for Neurotoxicity Risk Assessment," *Korean J. Toxicol.* **6**, 205-213 (1990).

11. *SAS User's Guide: Statistics*, Version 5 ed. (SAS Institute, Inc., Cary, NC, 1985).

12. J.J. Chen and D.W. Gaylor, "Dose-Response Modeling of Quantitative Response Data for Risk Assessment," *Communications Statistics - Theory Meth.* **21**, 2367-2381 (1992).

13. R.L. Kodell and R.W. West, "Upper Confidence Limits on Excess Risk for Quantitative Responses," *Risk Anal.* **13**, 177-182 (1993).

14. R.B. Howe and K.S. Crump, "GLOBAL82: A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses," (ICF Clement Assoc., K.S. Crump Div., Ruston, LA (1982).

Lactational Transfer of Tetrachloroethylene in Rats

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ABSTRACT

Tetrachloroethylene (PCE) is a commonly used organic solvent and a suspected human carcinogen, reportedly transferred to human breast milk following inhalation exposure. Transfer of PCE to milk may represent a threat to the nursing infant. A physiologically based pharmacokinetic (PBPK) model was developed to quantitatively assess the transfer of inhaled PCE into breast milk and the consequent exposure of the nursing infant. The model was validated in lactating rats. Lactating Sprague-Dawley female rats were exposed via inhalation to PCE at concentrations ranging from 20 ppm to 1000 ppm, and then returned to their nursing, 10- to 11-day-old pups. Tetrachloroethylene concentrations in the air, blood, milk, and tissue were determined by gas chromatography and compared to model predictions. The model described the distribution of inhaled PCE in maternal blood and milk, as well as the nursed pup's gastrointestinal tract, blood, and tissue. Several computer simulations of PCE distribution kinetics in exhaled air, blood, and milk of exposed human subjects were run and compared with limited human data available from the literature. It is concluded that the PBPK model successfully described the concentration of PCE in both lactating rats and humans. Although predictions *versus* observation were good, the model slightly underpredicted the peak whole pup PCE concentration and underpredicted systemic clearance of PCE from the pup.

INTRODUCTION

Transfer of toxic chemicals via mother's milk represents an important, although not widely recognized health risk to the infant. An evaluation of the hazards of exposure to occupational chemicals transferred from mother to baby must include qualitative and quantitative determinations of chemicals that contaminate breast milk.

A review of the literature conducted by Cone *et al.* (1) for the U.S. Environmental Protection Agency (EPA) revealed that many chemical compounds may be transferred with breast milk during feeding. Most of these chemicals were either environmental pollutants or drugs. An extensive list of the chemicals detected or excreted in human milk (about 150 compounds) has been published recently by Giroux *et al.* (2). The lactational transfer of both environmental pollutants and drugs was reviewed in two recent publications (3, 4).

Volatile organic solvents deserve special attention because exposures to these chemicals occur very often in industrial facilities. Inhaled volatile organic chemicals quickly transfer to systemic circulation where they are likely to partition into fat stores, including breast milk. The residence time for volatile organic chemicals in the body (including breast milk) is not long when compared to persistent environmental contaminants such as polychlorinated biphenyls, but the levels achieved in the fat stores and breast milk may be substantial. For instance, tetrachloroethylene (synonym: perchloroethylene or PCE) was detected in milk from one Canadian woman who regularly visited her husband during his lunch hour at a dry-cleaning factory (5). The concentration of PCE in breast milk was 10 ppm/1 h after the visit and over the next 24 h the PCE concentration in breast milk decreased to 3 ppm. Her infant developed jaundice at the age of 6 weeks, but recovered quickly after cessation of breast-feeding (5). Although the disease was attributed to the contamination of breast milk with PCE, it is still not clear whether the association with obstructive jaundice was causal or spurious.

Tetrachloroethylene is a volatile, nonflammable liquid widely used in the dry-cleaning industry and in metal degreasing operations. Acute inhalation of PCE vapor by humans has produced central nervous system depression ranging from lightheadedness and muscular incoordination at low concentrations, to loss of consciousness and respiratory paralysis at higher doses (6-9). The development of minor, reversible hepatic dysfunction several days following accidental human exposure to anesthetic concentrations of PCE also has been noted (6, 7).

There was a limited amount of information in the literature on tissue concentrations of PCE in rats or mice, resulting from test exposures, from which a physiologically based pharmacokinetic (PBPK) model describing the pharmacokinetics of PCE was developed and validated by Ward *et al.* (10). Using this generic model, without milk compartment, Schreiber (11) has estimated human breast milk concentrations of PCE and excess cancer risk for infants exposed to PCE in breast milk. These estimates

by Schreiber (11) were based on solubility of PCE in fat but not in milk. Thus, without the actual measurements of breast milk PCE concentrations in either occupational or experimental exposure, this kind of estimate remains rather speculative and has limited value for appropriate risk management until its predictions can be validated experimentally.

In this report, a PBPK model for lactational transfer of PCE is described and validated in nursing rats. Several computer simulations and predictions for the long-term PCE distribution in exhaled air, blood, and milk of exposed human subjects were done and compared with available human data from the literature. The model predictions appear to be in good agreement with the measured values. The computer simulation of the kinetics of lactational transfer of PCE may aid a quantitative assessment of the dose passed by the exposed mother to the nursing infant.

MATERIAL AND METHODS

Gas Uptake

The amount of PCE metabolized by animals was estimated from a sustained decrease of PCE concentration in a closed gas-uptake chamber (7.9 L), measured by gas chromatographic (GC) analysis as described by Gargas *et al.* (12). The amount of PCE in the sampled air was measured by the Hewlett-Packard 5890 Series II GC with flame ionization detector. A standard curve was prepared by injecting known amounts of PCE into empty chambers and sampling the chamber atmosphere immediately after equilibrium was reached. At each PCE concentration level, a loss run was performed for 6 h in an empty chamber to quantify any unspecific absorption, leakage, or other loss of PCE from chamber atmosphere (the determined loss rate was 0.05^{-1}). For each gas-uptake run, three lactating female Sprague-Dawley rats were used.

The decrease in PCE chamber concentration was indicative of the rate of metabolism of the chemical by the animal (12). Analogous to the description of kinetics for isolated enzymes by Michaelis and Menten theory, a "pseudo V_{max}" and an "apparent K_m" were determined by PBPK modeling.

Blood and Milk Analysis

Samples of blood or milk were collected in triplicate from each animal into glass capillary tubes of known volume, transferred directly to autosampler vials, and extracted using *n*-hexane. The extracts were analyzed by a GC equipped with a Vocol™ fused silica column and an electron capture detector.

Calibration curves were prepared and evaluated statistically for the best fit. Concentrations in blood and milk were corrected for appropriate extraction efficiency, determined by spiking blood and milk with PCE (90.0% ±2.4 and 95.5% ±2.8, respectively; n=9). The standards also were processed with each series of samples.

Tissue Analysis

Tissues of pups, euthanitized with CO_2 and bled, were placed either in sample bags and frozen in liquid N_2, or in jars with *n*-hexane and processed fresh. Thawed or fresh samples were homogenized and extracted with *n*-hexane. The extracts were analyzed by GC in a manner analogous to that used for blood and milk extracts. The difference between extraction efficiency calculated for frozen tissues (56.0% ±6; n=3) and fresh tissues (57.0% ±9; n=3) was insignificant. Calibration curves were prepared using tissues of control pups spiked with known amounts of PCE.

Determination of Partition Coefficients

A smear method was used for determination of tissue, milk, and blood partition coefficients. The fresh or frozen tissues were homogenized and approximately 0.1 g of muscle, liver, kidney, or pup; 0.05 g of adipose tissue; or 250 μL of blood or milk were smeared on the walls of tared, 25 mL, borosilicate glass scintillation vials. The vials were weighed, sealed, and then injected with known amounts of PCE vapors from an equilibrated standard bag. The vials were then incubated with vortexing for 3 h at 37 °C. Aliquots of 1 mL of head space were injected automatically onto the GC and analyzed as described above for blood and milk. The blood/air and tissue/air partition coefficients were then calculated, essentially as described by Gargas *et al.* (13).

Animal Exposure

Lactating female Sprague-Dawley rats (body weight 232 to 352 g) were used as test animals. After delivery, litters were reduced to 8 pups per dam and kept undisturbed for 10 to 11 days (the range of body weight for 10- to 11-day-old pups was 16.2 to 27.9 g). On Day 10 or 11 *post partum*, the lactating females were exposed to PCE either in the closed gas-uptake chamber (3 rats per 7.9-L chamber) for up to 6 h or in an open inhalation chamber (5 rats per 30.0-L chamber) for 1 to 6 h. Numbers of rats included in the inhalation exposures are reported in figure captions.

Inhalation was selected as the route of administration most relevant to occupational exposure of women. Concentrations for inhalation exposures of rats were set between 20 and 1000 ppm for 1 to 6 h. At a selected exposure level (600 ppm), the measurements were performed in dam's milk and blood after 1-, 2-, 3-, 4-, and 5-h exposures to PCE. An additional group of dams, exposed to 600 ppm of PCE for 2 h, was returned to their nursing pups after the exposure, and at selected times blood and tissue levels of PCE were measured in pups.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institutes of Health, Publication No. 86-23, 1985; and the Animal Welfare Act of 1966, as amended.

Computer Simulations

A PBPK model was written in SIMUSOLV, a Fortran-based continuous simulation language, and simulations were performed using SIMUSOLV software package with optimization capabilities (DOW Chemical Co., Concord, MA) on a VAX/VMS mainframe computer (VAX8530, Digital Equipment Corp., Maynard, MA).

RESULTS

PBPK Model Construction

Figure 39 shows the scheme of PBPK model, essentially as described by Ramsey and Andersen (14). Additional compartments were added to describe milk (15) and nursing pups (16). Initially, for simplicity, the pups were described by the lungs, arterial and venous blood, and the "other tissues" compartment (17). However, this simplified model did not describe adequately the kinetics of PCE in pup blood and tissues. Thus, milk was retained in the gastrointestinal tract of nursing pups, apparently causing delayed absorption of PCE for several hours. To describe this phenomenon, an additional compartment was added simulating the pup gastrointestinal tract (Figure 39).

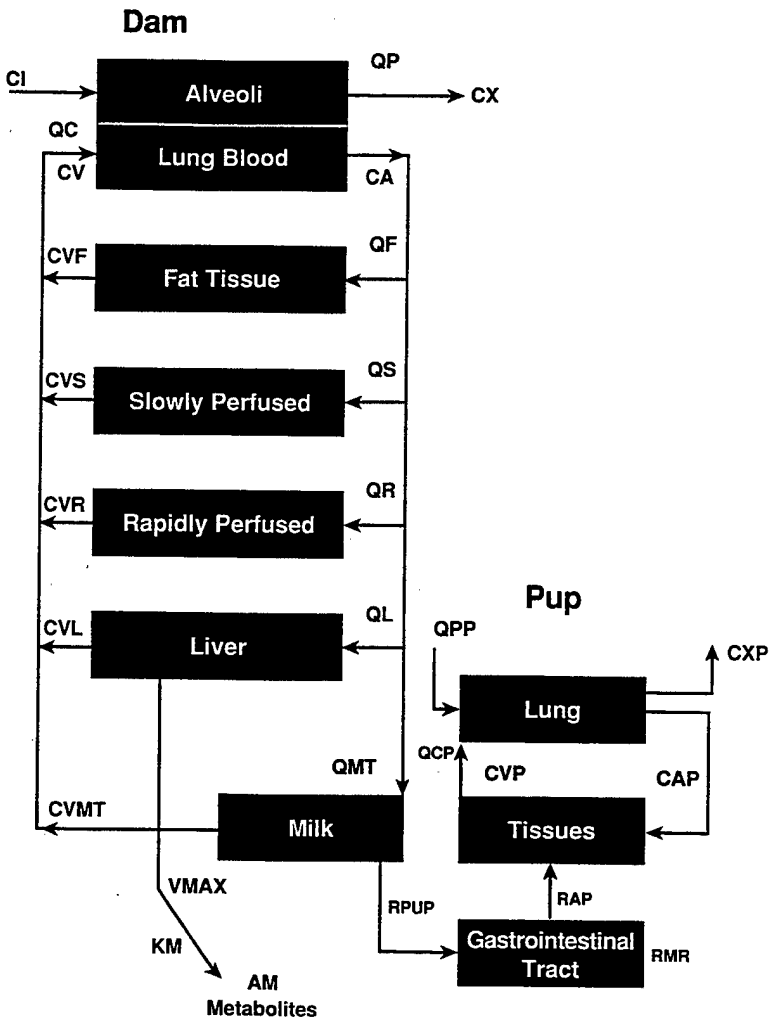


Figure 39. Scheme of physiologically based pharmacokinetic (PBPK) model used to simulate lactational transfer of PCE in nursing rats and humans. Abbreviations: CI = concentration in inhaled air (mg/L); QP = alveolar ventilation rate adjusted for body weight (L/h); CX = concentration in exhaled air (mg/L); QC = cardiac output adjusted for body weight (L/h); CVF = venous concentration leaving the fat tissue (mg/L); QF = blood flow to fat (L/h); CVS = venous concentration leaving the slowly perfused tissues (mg/L); QS = blood flow to slowly perfused tissues (L/h); CV = concentration in mixed venous blood (mg/L); CA = concentration in arterial blood (mg/L); CVR = venous concentration leaving the rapidly perfused tissues (mg/L); QR = blood flow to rapidly perfused tissues (L/h); CVL = venous concentration leaving the liver tissue (mg/L); QL = blood flow to liver (L/h); QPP = alveolar ventilation rate in pups adjusted for body weight (L/h); CXP = concentration in air exhaled by pups (mg/L); CVMT = venous concentration leaving the mammary glands tissue (mg/L); QCP = cardiac output in pups adjusted for body weight (L/h); CVP = concentration in venous blood in pups (mg/L); CAP = concentration in arterial blood in pups (mg/L); RPUP = elimination rate for PCE from milk to pups (mg/h); VMAX = pseudo-maximal velocity of PCE metabolism (mg/h); KM = apparent Michaelis-Menten constant for PCE metabolism (mg/L); AM = amount of PCE metabolized (mg); RMR = the rate of gastrointestinal tract loading with PCE in pups (mg/h); RAP = the rate of gastrointestinal absorption of PCE in pups (mg/h).

The Differential Equations

Mass transfer differential equations describing each compartment building the PBPK model for lactational transfer of PCE (schematically shown in Figure 39) are described below.

For well-stirred compartments without metabolism or other losses (fat tissue, slowly perfused and rapidly perfused tissues, pup tissues), the amount change (dA*i*) over time was described as follows:

$$dAi/dt = Qi(CA - CVi)$$

where A represents amount, subscript *i* represents "i-th" compartment; Q*i* represents the blood flow through the "i-th" compartment; CA represents the arterial concentration; CV*i* represents the venous concentration leaving the "i-th" compartment (CV*i* = C*i*/P*i*; where C*i* is a concentration in the tissue in "i-th" compartment and P*i* is the tissue/blood partition coefficient for "i-th" compartment. C*i* = A*i*/V*i*, where V*i* represents the volume of the "i-th" compartment).

For the liver compartment, a loss term (RAM) was added to the well-stirred compartment description to account for metabolism (RAM = VMAX*CVL/(KM + CVL) + KF*CVL*VL; where * is multiplication, VMAX is pseudo-maximal velocity rate of PCE metabolism, CVL is venous concentration leaving the liver, VL is liver volume, KM is apparent Michaelis-Menten constant, KF is the first-order rate of metabolism):

$$dAL/dt = QL(CA - CVL) - RAM$$

where dAL is change of amount in the liver.

Analogously, for the mammary glands compartment, the change of amount (dAMAT), described as above, contained a loss term for elimination for PCE from milk to pups, RPUP (RPUP = CMAT*OUTX; where CMAT is concentration in milk, OUTX is periodic zero order milk yield *per* dam):

$$dAMAT/dt = QMT(CA - CVMT) - RPUP$$

where QMT represents mammary blood flow, CVMT represents venous concentration leaving the mammary glands. CMAT = AMAT/VMILK, where CMAT is concentration and VMILK represents volume of milk. It was assumed that the milk compartment is in intimate contact with the arterial blood perfusing the mammary tissue, and that PCE rapidly equilibrates with the milk.

The rate of change in the amount of PCE in the pup gastrointestinal tract (dAGIT) was described as a difference between the rate of ingesting of PCE with mother's milk (RPUP) and the rate of absorption from the gastrointestinal tract, RAP (RAP = MR*KAP; where MR is the amount remaining in the pup gastrointestinal tract, KAP is absorption constant for pup, determined to be equal to 0.5 hr^{-1}):

$$dAGIT/dt = RPUP - RAP$$

The concentration of PCE in the pup gastrointestinal tract (CGIT) was calculated as CGIT = MR/GIW, where GIW represents weight of gastrointestinal tract of pup, adjusted for the pup's weight.

Closed Chamber Exposure

The closed chamber gas-uptake data were used to estimate and optimize the metabolism constants (VMAXC = 0.03 mg/kg/hr; and KM = 0.32 mg/L). These values suggested a very slow metabolism rate of PCE by lactating rats (17). Kinetic constants and physiological parameters are listed in Table 41.

Computer simulations were conducted of 6-h gas-uptake exposures to an initial air concentration of 670 ppm of PCE. The predictions of blood and milk levels were compared to the results of measurement of respective concentrations at the end of exposure (CV = 6.14±0.29 mg/L; CMAT = 89.18±12 mg/L; n = 3; results not shown here). Model predictions appeared to be in good agreement with the measured values, supporting further the validity of the estimated metabolism constants (17).

Open Chamber Exposure

Further validation of the PBPK model was completed using lactating rats exposed for 2 h to constant concentrations of PCE (ranging from 20±2 to 1000±47 ppm). The dependence of PCE concentration in milk *versus* concentration in air (CI) is shown in Figure 40. The data for blood and milk collected from rats exposed to these different concentrations of PCE were compared to the simulated levels by PBPK model. Similarly, the data collected from lactating rats exposed for different times to constant concentrations of 600 ppm PCE were compared to the levels in blood and milk simulated by PBPK model (results not shown). In both cases, the dose- and time-dependent courses of PCE concentrations were in agreement with those predicted by the PBPK model (17).

TABLE 41. KINETIC CONSTANTS AND PHYSIOLOGICAL PARAMETERS USED IN PBPK MODELING OF LACTATIONAL TRANSFER OF PCE IN RATS AND HUMANS

Description	[Units]		Rat	Human
Tissue Volumes	[Fraction of Body Weight:BW]			
Maternal				
Liver	VLC	=	0.04	0.04
Fat	VFC	=	0.1	0.2
Mammary	VMATC	=	0.044	0.05
Perinatal				
Pup (Infant) Tissue	VTCP	=	0.9	0.9
Maternal	[L]			
Slowly Perfused	VS	=	0.79*BW-VF-VMAT	
Rapidly Perfused	VR	=	0.12*BW-VL	
Milk Volume	VMILK	=	0.00233	0.03542
Flow Rates	[L/h/kg]			
Maternal				
Alveolar Ventilation	QPC	=	14.5	19.7
Cardiac Output	QCC	=	14.3	18.0
Perinatal				
Alveolar Vent. Pup (Inf.)	QPCP	=	30.0	25.2
Cardiac Output Pup (Inf.)	QCCP	=	22.0	22.0
Maternal	[Fraction of Cardiac Output]			
Liver	QLC	=	0.25	0.25
Fat	QFC	=	0.07	0.05
Partition Coefficients	[Ratio of Solubility]			
Maternal				
Blood/air	PB	=	33.5	19.8
Liver/blood	PL	=	1.9	6.83
Fat/blood	PF	=	42.35	159.03
Slowly Perf./blood	PS	=	0.93	7.77
Rapidly Perf./blood	PR	=	1.67	6.83
Milk/blood	PMILK	=	12.0	2.8
Perinatal				
Blood/air Pup (Inf.)	PPB	=	24.3	8.0
Other Tiss./bld.Pup (Inf.)	PPT	=	4.54	6.596
Metabolism				
Maternal	[mg/L]			
Apparent Michaelis-Menten	KM	=	0.32	0.32
	[mg/kg/h]			
Pseudo Maximal Velocity	VMAXC	=	0.03	0.151

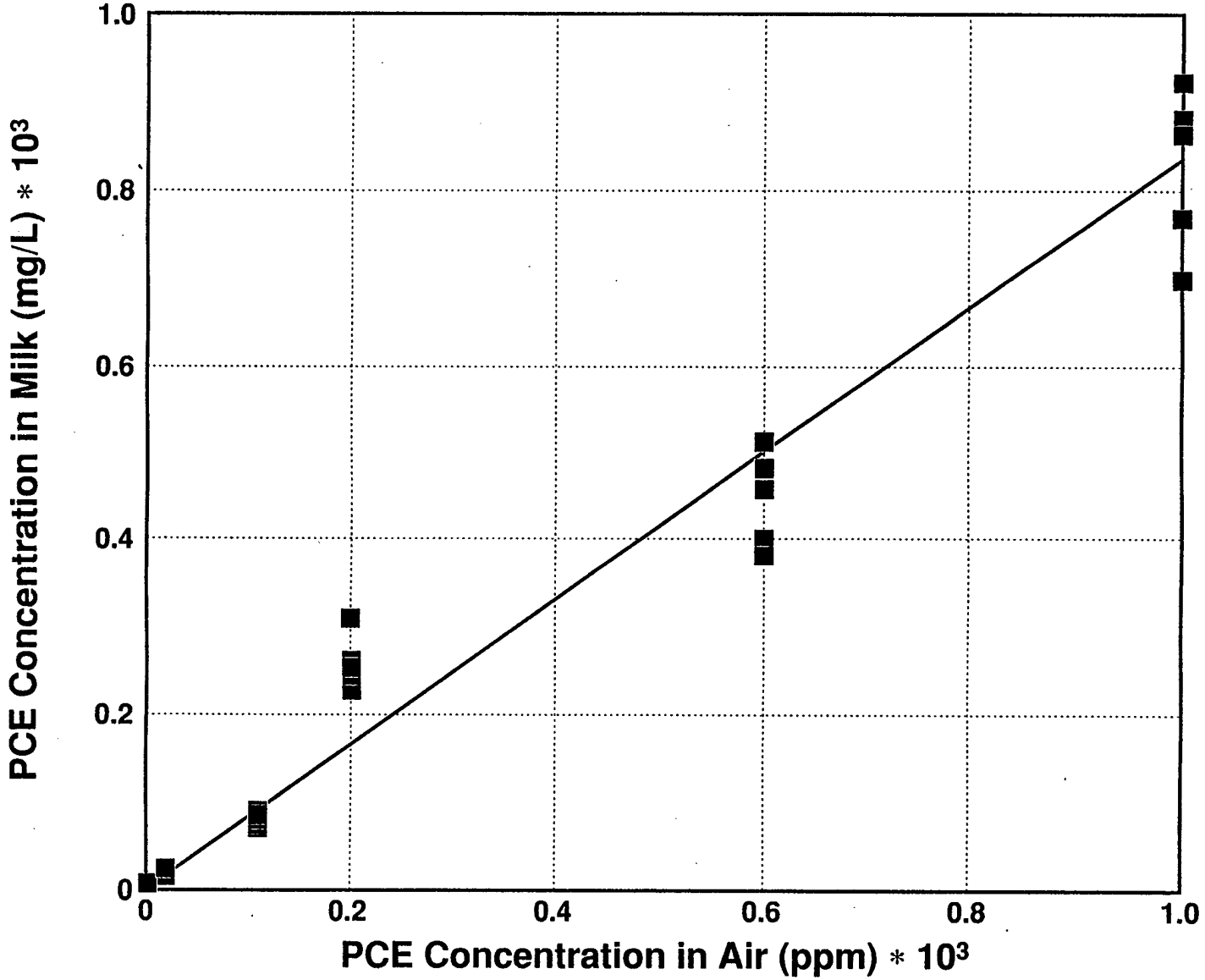


Figure 40. Relationship Between Concentration of PCE Measured in Milk Shortly After Exposure, and the Time-Weighted Average PCE Concentration in Inhaled Air for Rats Exposed for 2 h to 20 ppm to 1000 ppm of PCE (n=5 for Each PCE Air Concentration).

Exposure of Pups via Mother's Milk

Another group of 20 lactating rats was exposed for 2 h using the same exposure concentration of 600 ppm of PCE. The dams then were returned to their nursing pups and concentrations of PCE were followed for the next 24 h in blood and milk. The time-dependent model predictions appeared to be in good agreement with the measured values.

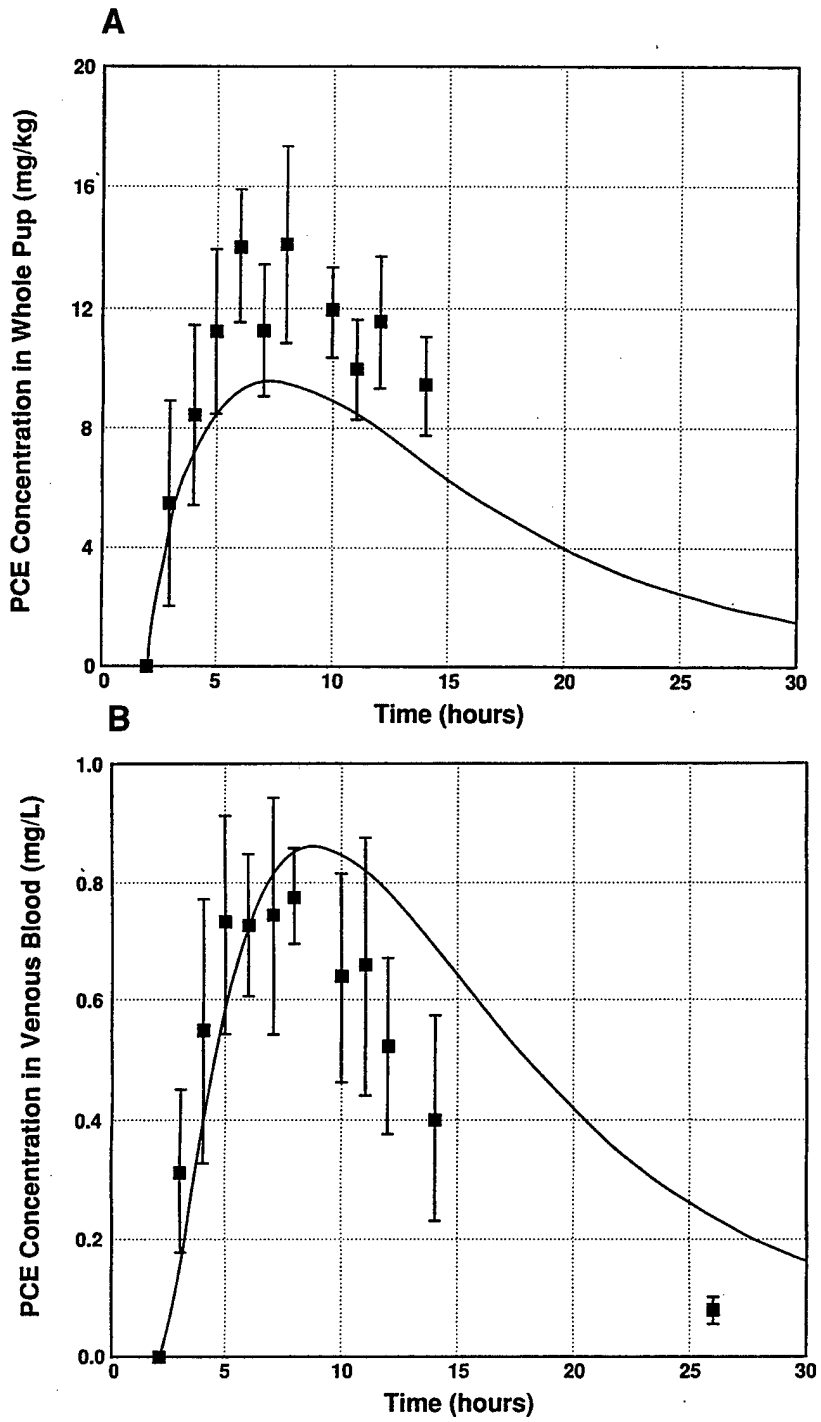
Concentrations of PCE were measured for up to 24 h in 10- to 11-day old pups nursing from exposed dams. The time-dependent model predictions for pups were compared with measured values. The nursing pup whole body burdening of PCE was slightly underpredicted by the model (Figure 41a). On the other hand, PCE concentrations in pup venous blood were slightly overpredicted for times longer than 6 h (Figure 41b).

DISCUSSION

PBPK Models in Lactational Transfer of Chemicals

The implementation of PBPK models by Shelley *et al.* (15, 18) represented significant progress in estimating the infant's exposure to chemicals transferred with breast milk, and in assessment of the overall risk to the infant. Their approach involved a physiologically based mathematical simulation capable of modeling, for instance, the transfer of volatile organic solvents from mother's breathing zone to the nursing infant. Such PBPK models of lactational transfer of chemicals may be scaled up or down, according to algorithms for the body weight, and can be validated using laboratory animals, such as lactating rats (16).

We have used the same approach in the present study. However, PCE distribution in the dam was described better by five compartments rather than three, as in the general PBPK model presented by Shelley *et al.* (15, 18). On the other hand, the pup was at first described by one, and finally by two compartments only, without incorporating a negligible rate of metabolism. This was in contrast to the PBPK model for well metabolized trichloroethylene by Fisher *et al.* (16). Elimination of PCE in the pup was assumed to occur by exhalation. The physiological parameters, partition coefficients, and metabolism parameters estimated or determined by experiments are shown in Table 41. Using these parameters, the PBPK model fairly accurately described both blood and milk concentrations of PCE in lactating rats exposed to different concentrations of this chemical for different periods of time (17).



Figures 41a,b. Validation of PBPK Model Predictions (Solid Lines) of Time-Dependent PCE Concentrations in Tissues of Whole Pup (A) and Venous Blood (B) of Pups Fed by the Dams Exposed to 600 ppm of PCE for 2 h. The small rectangle and vertical bars show measured mean value ± standard deviation (n=3 or 6 for each recovery time).

On the other hand, differences between the kinetics of loading of a pup's gastrointestinal tract and blood required explicit description of the gastrointestinal tract as a virtual initial compartment that was loaded with PCE several hours prior to the venous blood and solid tissues (Figure 41a). Using these assumptions, the PBPK model reliably predicted the distribution of PCE inhaled by dams and then passed onto their nursing pups *via* breast milk.

Computer Simulation of Repetitive Exposures in Rats

Assuming 2-h exposures to 600 ppm of PCE, five times per week, a simulation was run to predict the time-course of PCE concentration in rat milk for up to one month (Figure 42a). The total dose of PCE received by pups was estimated to reach as much as 600 mg/kg during this time (Figure 42b). Under these exposure conditions, the model predicted that the concentration of PCE retained in pup blood may reach 1 mg/L, which is a concentration referred to by the American Conference of Governmental Industrial Hygienists (ACGIH) as the index of biological exposure (BEI) to the threshold limit concentration (TLV-TWA = 50 ppm) for PCE inhaled by an adult human subject (19).

Computer Simulations and Predictions of PCE Distribution in Humans

The attempt was made to scale-up the PBPK model and to test its predictions versus available data for humans. The blood/air and milk/air partition coefficients for PCE were measured by our laboratory in the samples collected from volunteer donors (20). Initially, a set of physiological parameters and kinetic constants, pertinent to PCE in humans, was adopted from Ward *et al.* (10). The other values describing human milk, infant, and mammary glands compartments were calculated from data published for "Reference Man" (21), and finally the constants were optimized using the SIMUSOLV software package, over the experimental data from literature describing inhalation exposures of human subjects to PCE (7-9, 22, 23). The final set of parameters and constants is listed in Table 41.

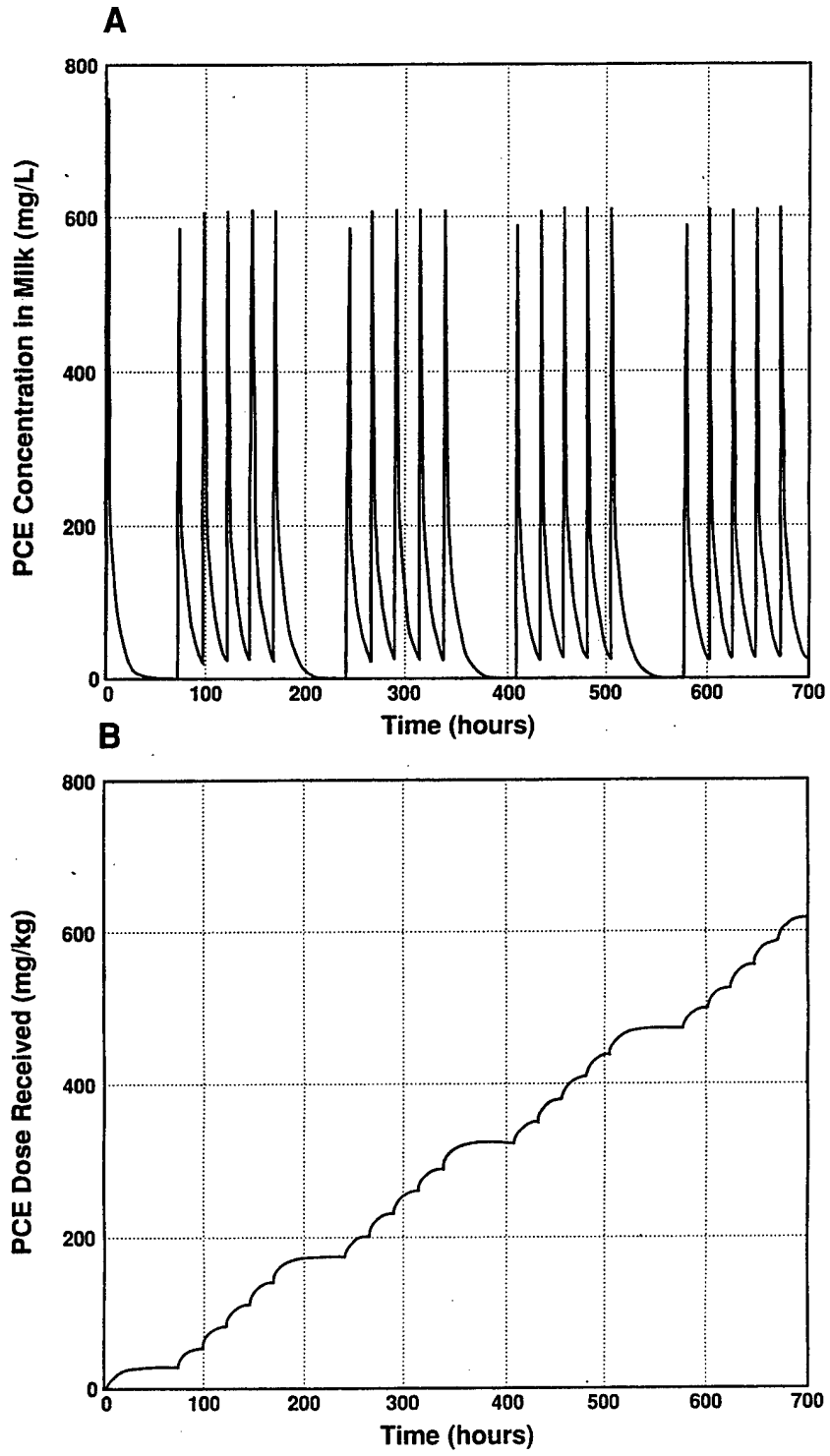
Using these parameters, with the milk compartment turned off, and adopting the exposure scenario described by ACGIH (19), the computer simulations of PCE concentrations in human blood (Figure 43a) and exhaled air (Figure 43b) were run and then compared to the published BEI values. The model slightly underpredicted both blood and exhaled air PCE concentrations for human subjects, prior to the last shift of the workweek (Figures 43a,b). Much better fit of the computer-simulated time-course was obtained with the data reported by Fernandez *et al.* (22) for exhaled air of human subjects exposed to 100 ppm of PCE for 1 h (Figure 44a) and 8 h (Figure 44b). Similarly, the model accurately predicted PCE concentrations in exhaled air of human subjects exposed to 194 ppm of PCE for 90 min and 3 h

(Figure 45), as reported by Stewart *et al.* (7). Figure 46 shows computer simulation of the rate of PCE exhalation (RAX) run *versus* data reported by Bolanowska and Golacka (23) for two selected human subjects, a slim man and an obese woman (Bolanowska, personal communication). The model predictions of PCE exhaled breath clearance rates for both subjects were in general agreement with values measured by Bolanowska and Golacka (23), with modest overprediction of experimental data after the first measured time point. However, the model predictions fit better to the experimental data from the slim man than from the obese woman (Figure 46).

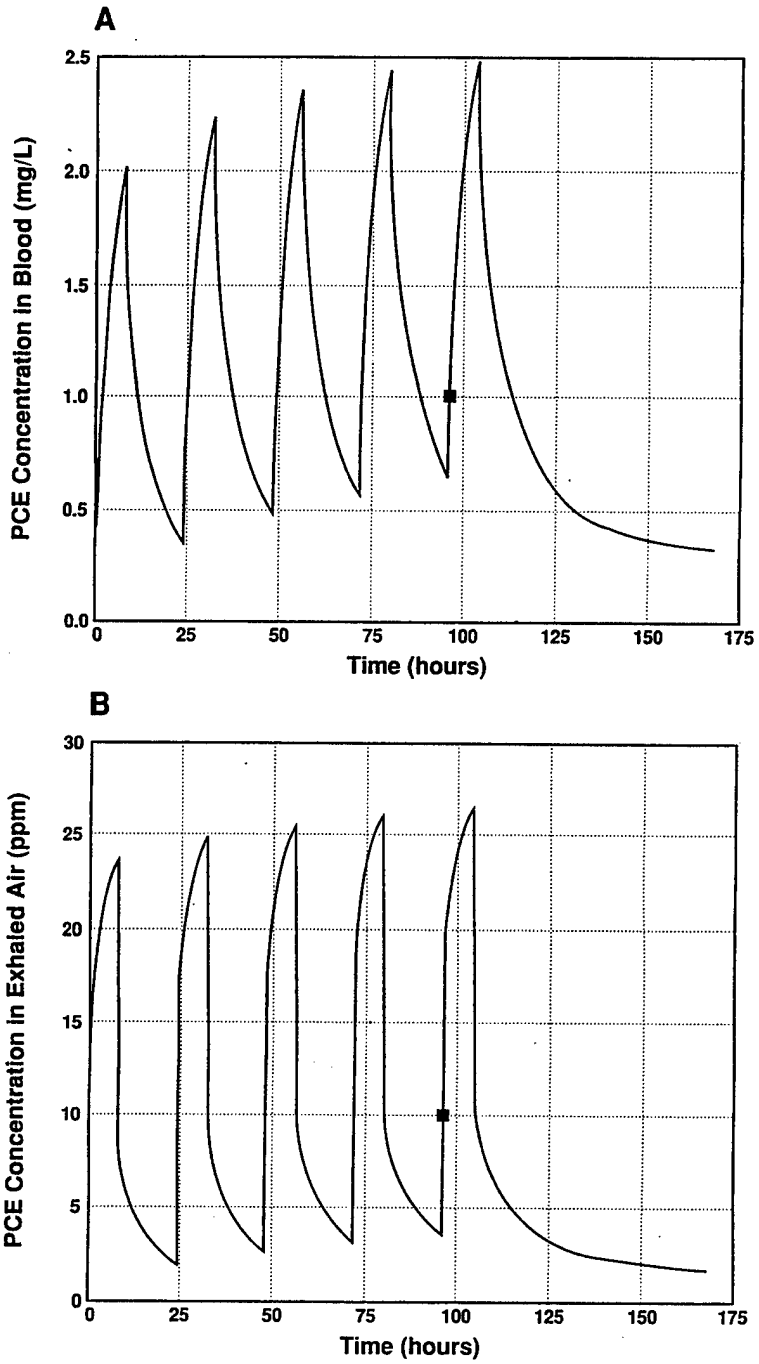
Computer Simulations and Predictions of PCE Distribution in the Mother and Her Nursing Infant

We have attempted also to simulate the only documented case of the lactational transfer of PCE from mother to infant, described by Bagnell and Ellenberger (5). Although the PCE concentration in inhaled air was not measured, the reported incidents of dizziness after exposure of mother to PCE without symptoms of general anesthesia (Bagnell, personal communication) suggested the PCE air concentration within the range of several hundred ppm. The best approximation of the computer-simulated values to PCE concentrations determined in blood and breast milk by Bagnell and Ellenberger (5) was achieved when the exposure concentration in inhaled air was assumed to be 600 ppm (Figures 47a,b). This concentration, exceeding more than 10 times the air TLV-TWA level recommended by ACGIH (19) for PCE, could result in the infant blood concentration of not more than 0.035 mg/L within one month of exposure to PCE *via* mother's milk (Figure 48). This concentration is more than one order of magnitude lower than the no-effect threshold assumed for adults by ACGIH (19). It is unknown if this concentration may cause any health effect in the infant.

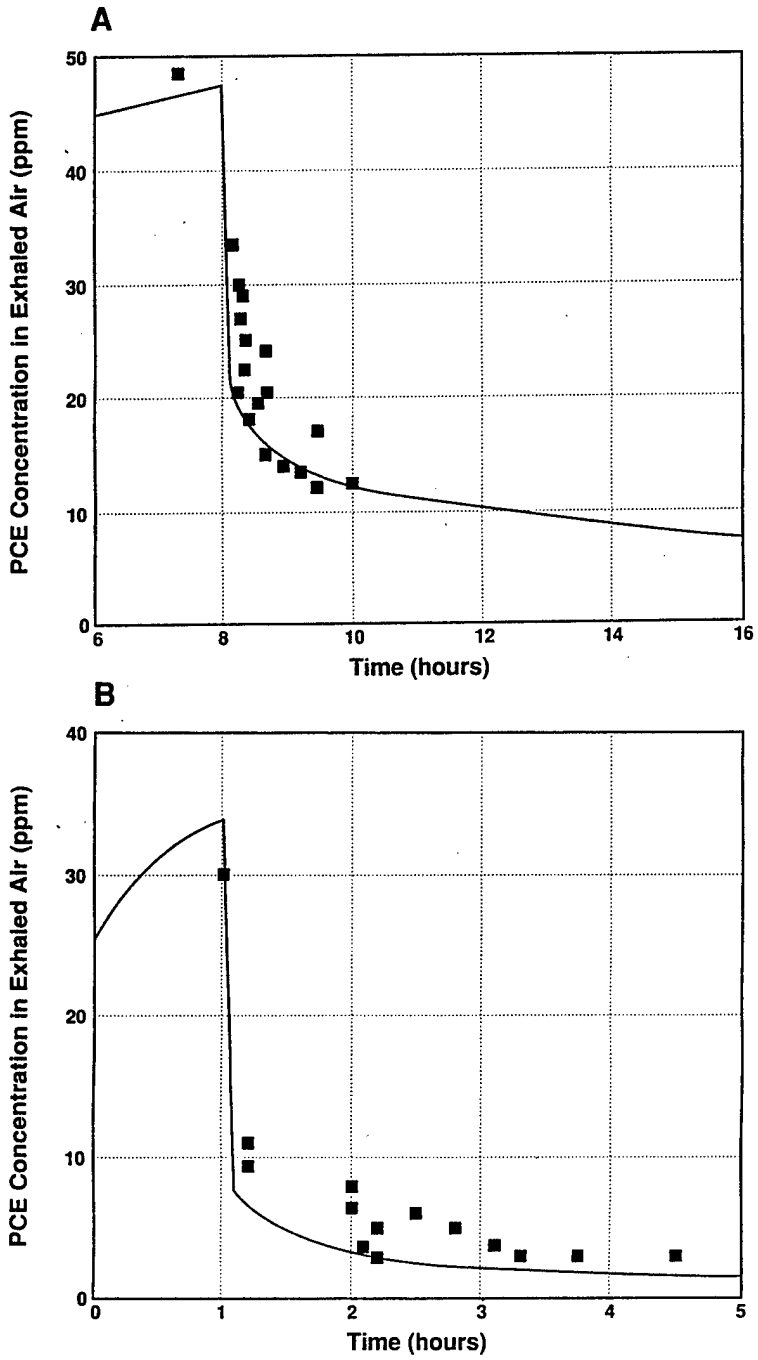
From these several predictions of PCE distribution and its kinetic behavior in exhaled air, blood and milk of exposed human subjects, and especially from the comparison of computer simulations with the available human data from literature, it is concluded that the PBPK model successfully described the concentrations of PCE in both lactating rats and humans. It seems that the validated PBPK model may be used, consequently, to predict the absorbed doses of PCE by nursing infants from the concentration in the mother's breathing zone. Although this approach will require monitoring of concentrations of PCE and other volatile chemicals in the air, it would aid the future attempts of risk assessment for infants.



Figures 42a,b. Computer Simulation of Time-Dependent Concentrations of PCE in Rat's Milk (A) and Cumulative Uptake of PCE Received by 8 Pups With Milk From the Dams (B). The computer simulation was run assuming 2-h exposure of dams to 600 ppm of PCE, five times per week (Monday through Friday, beginning on Friday), during 1 month.



Figures 43a,b. Computer Simulation of Time-Dependent Concentrations of PCE in Blood (A) and Exhaled Air (B) of Human Subjects Exposed to 50 ppm of PCE (TLV-TWA), According to Scenario Reported by ACGIH (19); Five Times per Week for 8 h, Monday Through Friday, Beginning on Monday). Small rectangles show biological exposure indices (BEI) measured in blood (A) and exhaled air (B). Data according to (19).



Figures 44a,b. Computer Simulation of Time-Dependent Concentrations of PCE in Exhaled Air of Human Subjects Exposed to 100 ppm of PCE for 8 h (A) and 1 h (B), According to the Scenario Reported by Fernandez *et al.* (22). Exposure starts at time = 0. Small rectangles show data measured in exhaled air according to (22).

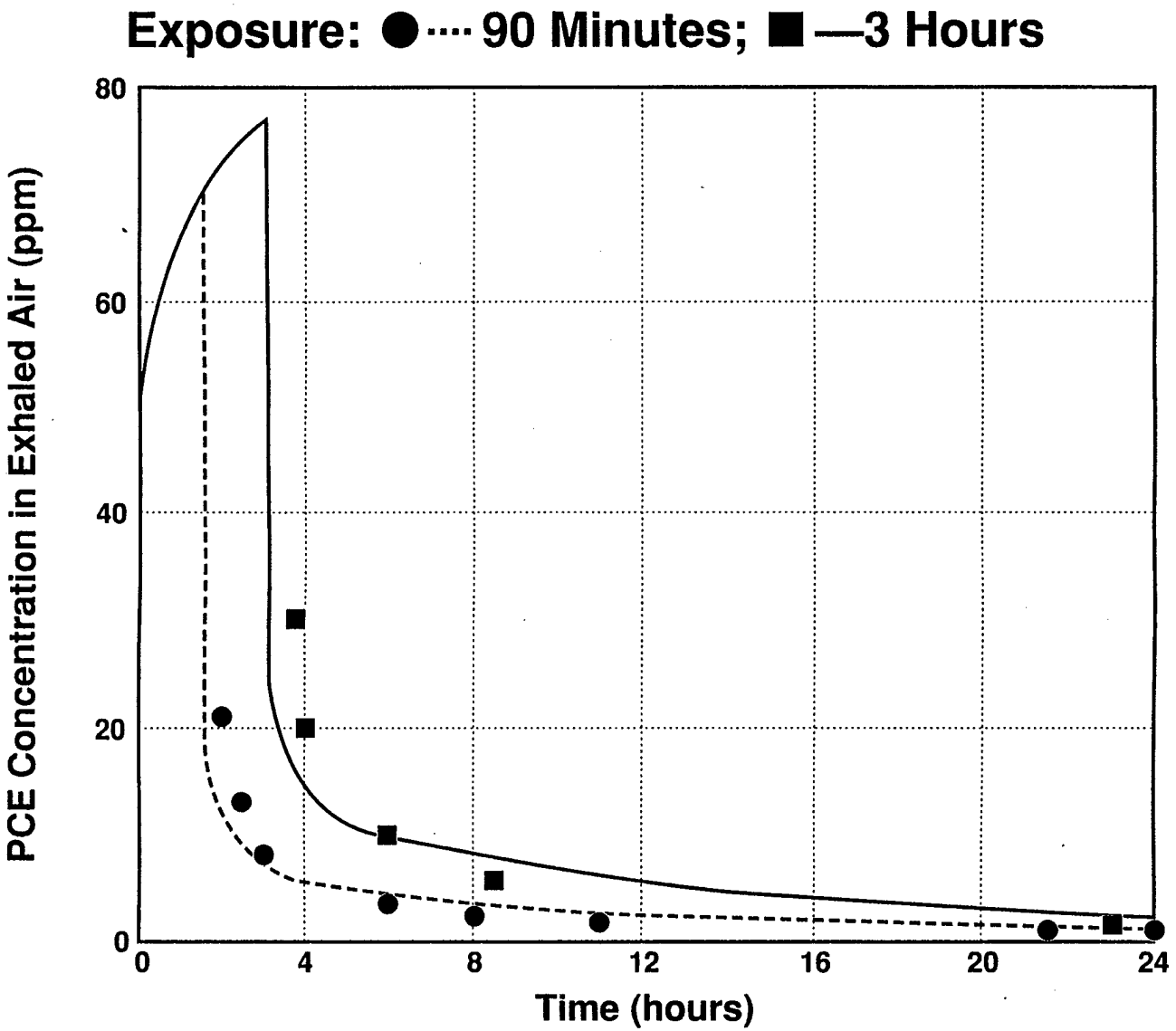


Figure 45. Computer Simulation of Time-Dependent Concentrations of PCE in Exhaled Air of Human Subjects Exposed to 194 ppm of PCE for 1.5 h (●) and 3 h (■), According to the Scenario Reported by Stewart *et al.* (7). Small Rectangles and circles show data measured in exhaled air according to (7).

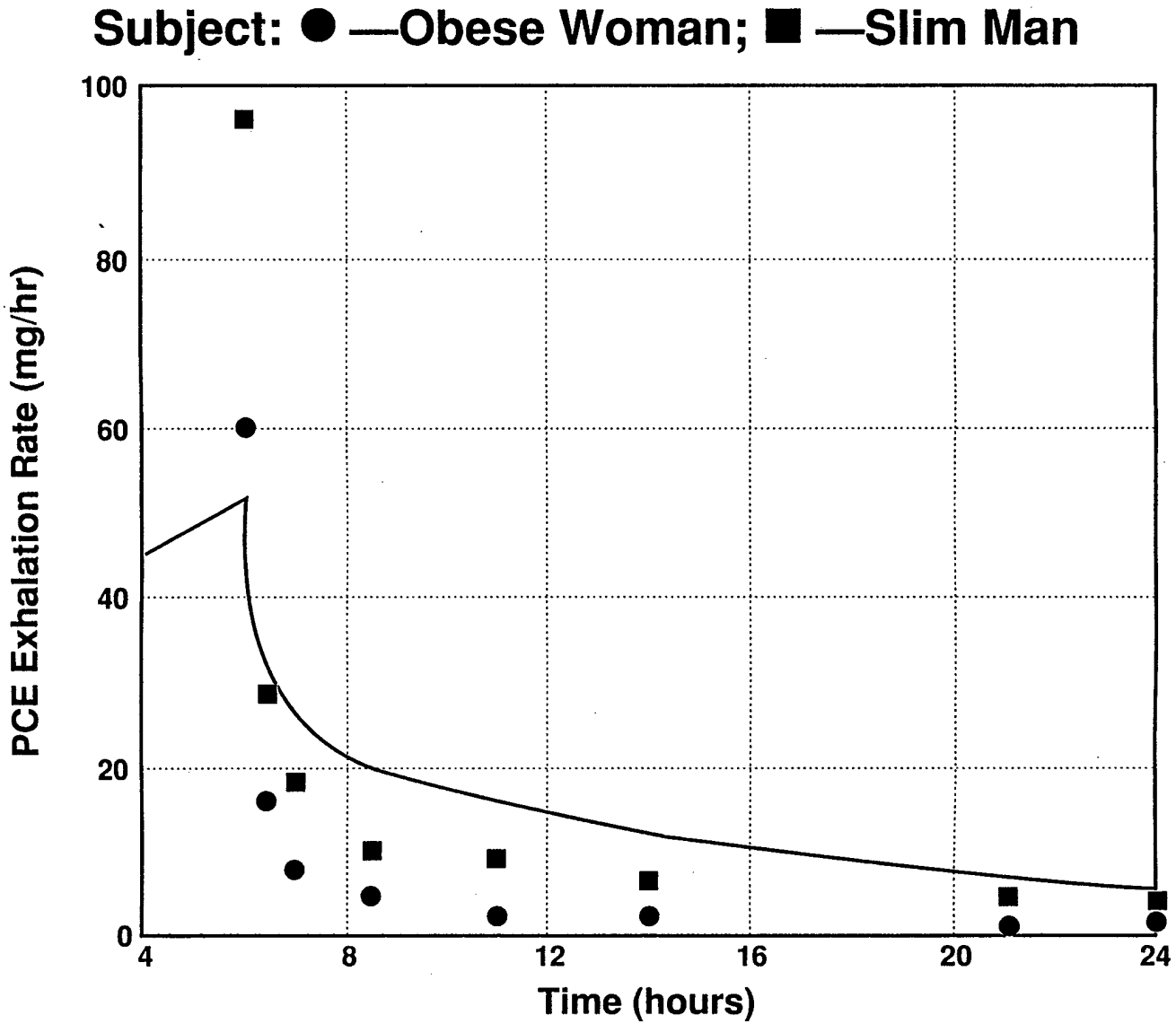
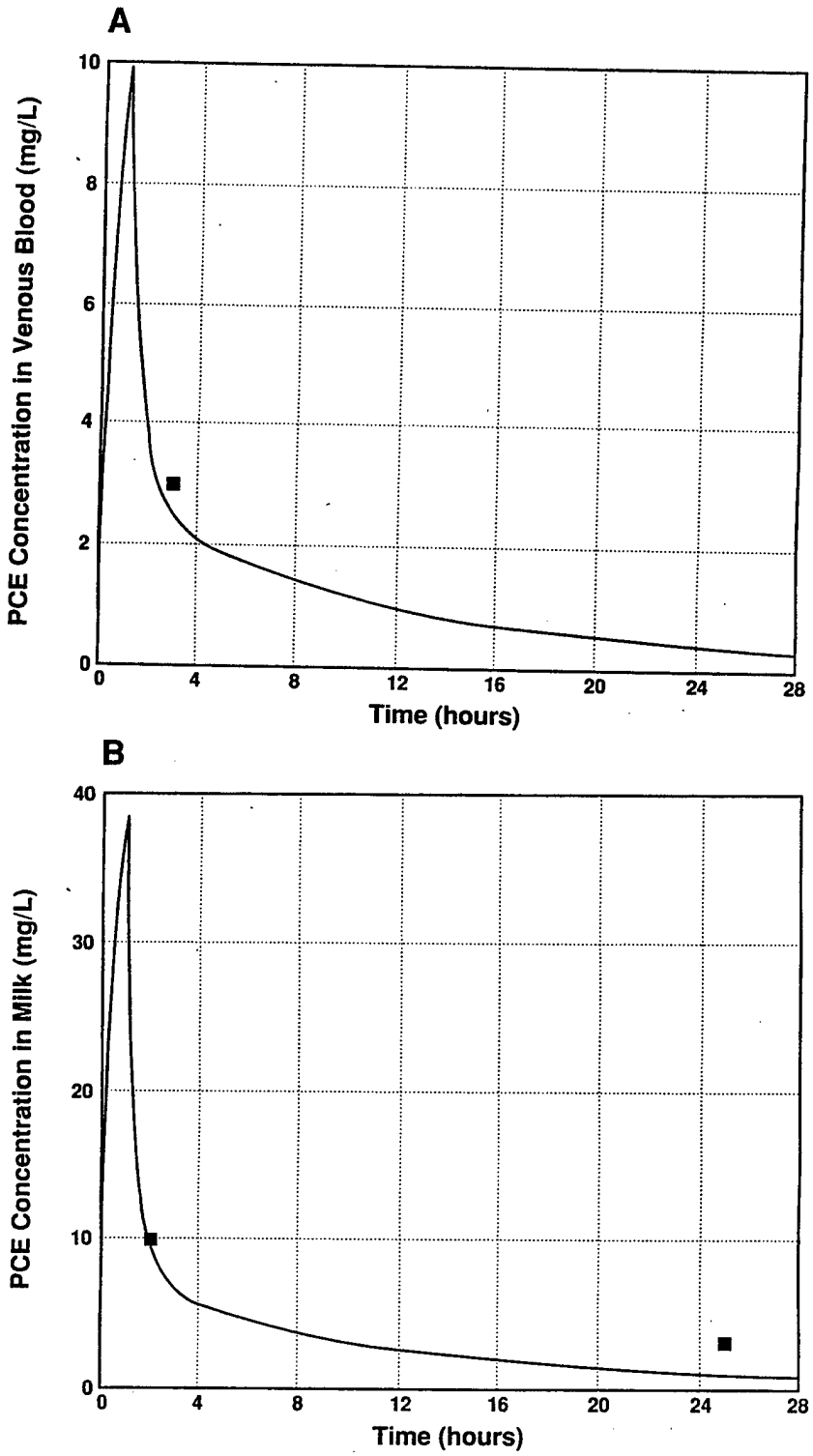


Figure 46. Computer Simulation of Time-Dependent Rates of PCE Exhalation by Obese Woman (●) and Slim Man (■), Exposed to 55 ppm of PCE for 6 h, According to the Scenario Reported by Bolanowska and Golacka (23). Small rectangles and circles show data measured in exhaled air according to (23).



Figures 47a,b. Computer Simulation of Time-Dependent Concentrations of PCE in Blood (A) and Milk (B) of Lactating Mother Assumed to be Exposed to 600 ppm of PCE for 1 h, According to the Scenario Reported by Bagnell and Ellenberger (5). Small rectangles show data measured in blood or milk of human subject according to (5).

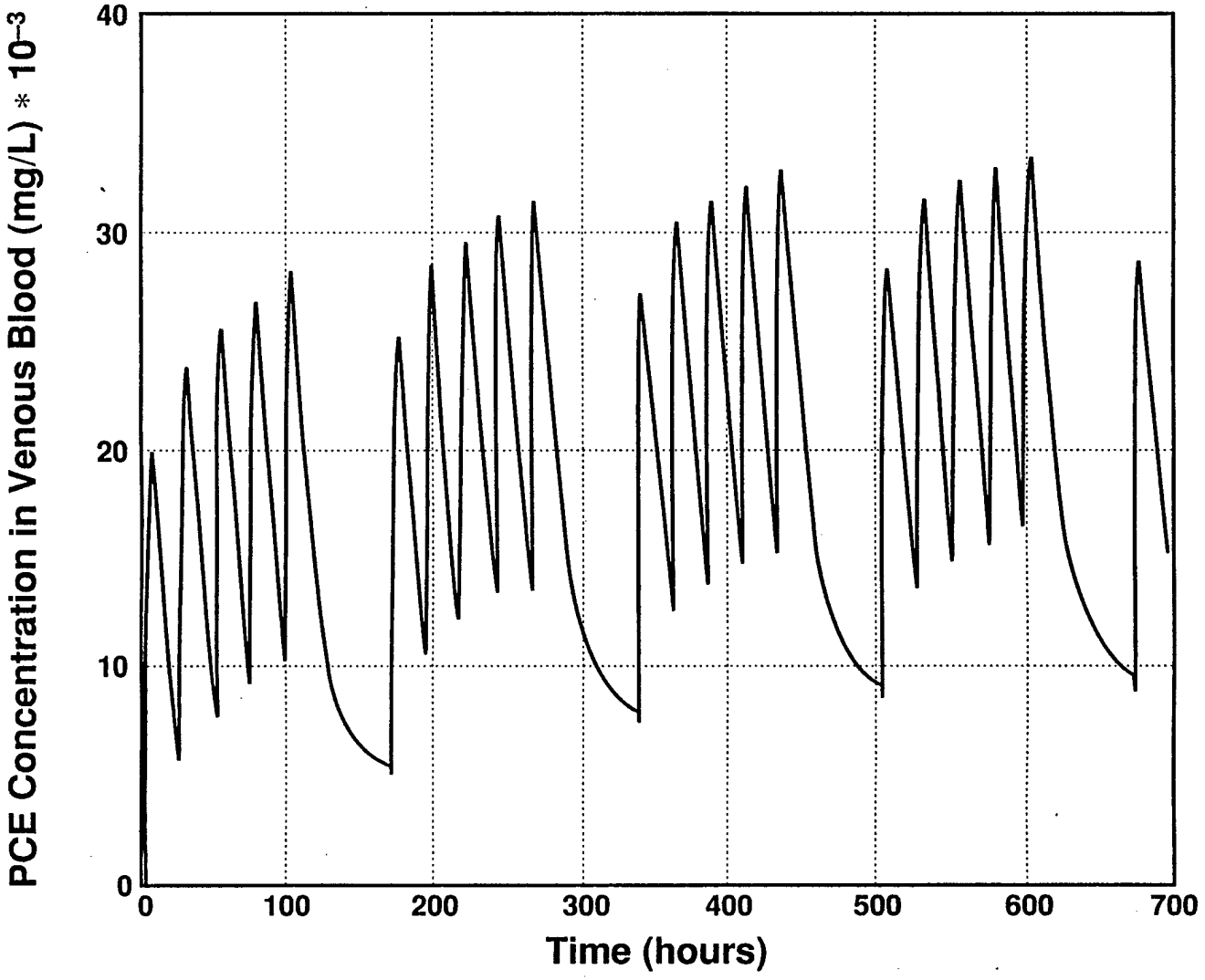


Figure 48. Computer Simulation of Time-Dependent Concentrations of PCE in Infant's Venous Blood. The computer simulation was run assuming 1-h exposure of lactating mother to 600 ppm of PCE, five times per week (Monday through Friday, beginning on Monday) during 1 month, according to the scenario reported by Bagnell and Ellenberger (5).

From these several predictions of PCE distribution and its kinetic behavior in exhaled air, blood and milk of exposed human subjects, and espectially from the comparison of computer simulations with the available human data from literature, it is concluded that the PBPK model successfully described the concentrations of PCE in both lactating rats and humans. It seems that the validated PBPK model may be used, consequently, to predict the absorbed doses of PCE by nursing infants from the concentration in the moter's breathing zone. Although this approach will require monitoring of concentrations of PCE and other volatile chemicals in the air, it would aid the future attempts of risk assessment fr\or infants.

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REFERENCES

1. M.V.Cone, M.F.Baldauf, D.M.Opresko, and M.S.Uziel, *Chemicals Identified in Human Breast Milk, a Literature Search* (EPA 560/5-83-009 Report. U.S. Dept. of Commerce Natl. Technical Inform. Service, Washington, DC, 1983).

2. D.Giroux, G.Lapointe, and M.Baril, "Toxicological Index and the Presence in the Workplace of Chemical Hazards for Workers who Breast-feed Infants," *Am. Ind. Hyg. Assoc. J.* **53**, 471-474 (1992).

3. A.A.Jensen and S.A.Slorach, "Chemical Contaminants in Human Milk" (CRC Press Inc., Boca Raton, FL, 1991).

4. Committee on Drugs, American Academy of Pediatrics, "Transfer of Drugs and Other Chemicals into Human Milk," *Pediatrics* **84**, 924-936 (1989).

5. P.C.Bagnell and H.A.Ellenberger, "Obstructive Jaundice Due to a Chlorinated Hydrocarbon in Breast Milk," *Can. Med. Assoc. J.* **117**, 1047-1048 (1977).

6. R.D.Stewart, "Acute Tetrachloroethylene Intoxication," *J. Am. Med. Assoc.* **208**, 1490-1492 (1969).

7. R.D.Stewart, E.D.Baretta, H.C.Dodd, and T.Torkelson, "Experimental Human Exposure to Tetrachloroethylene," *Arch. Environ. Health* **20**, 224-229 (1970).

8. R.D.Stewart, D.S.Erley, A.W.Schaffer, and H.H.Gay, "Accidental Vapor Exposure to Anesthetic Concentrations of a Solvent Containing Tetrachloroethylene," *Ind. Med. Surg.* **30**, 327-330 (1961).

9. R.D.Stewart, H.Gay, D.Erley, C.Hake, and A.Schaffer, "Human Exposure to Tetrachloroethylene Vapor," *Arch. Environ. Health* **2**, 516-522 (1961).

10. R.C.Ward, C.C.Travis, D.M.Hetrick, M.E.Andersen, and M.L.Gargas, "Pharmacokinetics of Tetrachloroethylene," *Toxicol. Appl. Pharmacol.* **93**, 108-117 (1988).

11. J.S.Schreiber, "Summary of an Exposure and Risk Assessment Regarding the Presence of Tetrachloroethylene in Human Breast Milk," *J. Exposure Anal. Environ. Epidem.* **2**, Suppl. 2, 15-26 (1992).

12. M.L.Gargas, M.E.Andersen, and H.J.Clewell III, "A Physiologically Based Simulation Approach for Determining Metabolic Constants from Gas Uptake Data," *Toxicol. Appl. Pharmacol.* **86**, 341-352 (1986).

13. M.L.Gargas, R.J.Burgess, D.E.Voisard, G.H.Cason, and M.E.Andersen, "Partition Coefficients of Low-Molecular Weight Volatile Chemicals in Various Liquids and Tissues," *Toxicol. Appl. Pharmacol.* **98**, 87-99 (1989).

14. J.C.Ramsey and M.E.Andersen, "A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Rats and Humans," *Toxicol. Appl. Pharmacol.* **73**, 159-175 (1984).

15. M.L.Shelley, M.E.Andersen, and J.W.Fisher, "An Inhalation Distribution Model for the Lactating Mother and Nursing Child," *Toxicol. Lett.* **43**, 23-29 (1988).

16. J.W.Fisher, T.A.Whittaker, D.H.Taylor, H.J.Clewell III, and M.E.Andersen, "Physiologically Based Pharmacokinetic Modeling of the Lactating Rat and Nursing Pup: A Multiroute Exposure Model for Trichloroethylene and Its Metabolite, Trichloroacetic Acid," *Toxicol. Appl. Pharmacol.* **102**, 497-513 (1990).

17. J.Z.Byczkowski, E.R.Kinkead, R.J.Greene, L.A.Bankston, and J.W.Fisher, "Physiologically-Based Modeling of the Lactational Transfer of Tetrachloroethylene," *The Toxicologist* **13**, 354 (1993).

18. M.L.Shelley, M.E.Anderson, and J.W.Fisher, "A Risk Assessment Approach for Nursing Infants Exposed to Volatile Organics through the Mother's Occupational Inhalation Exposure," *Appl. Ind. Hyg.* **4**, 21-26 (1989).

19. The American Conference of Governmental Industrial Hygienists, "Threshold Limit Values for Chemical and Physical Agents and Biological Exposure Indices," (ACGIH, Cincinnati, OH, 1992).

20. J.W.Fisher, J.M.Gearhart, R.J.Greene, L.A.Bankston, C.Bryant, and S.J.Fortunato, "Estimating the Lactation Transfer of Volatile Chemicals in Women Using a Physiological Model," *The Toxicologist* **13**, 356 (1993).

21. International Commission on Radiological Protection, "No. 23 Report on Reference Man," in W.S.Snyder, M.J.Cook, L.R.Karhausen, G.P.Howells, and I.H.Tipton (eds.), (Pergamon Press, Elmsford, NY, 1984).

22. J.Fernandez, E.Guberan, and J.Caperos, "Experimental Human Exposures to Tetrachloroethylene Vapor and Elimination in Breath After Inhalation," *Am. Ind. Hyg. Assoc. J.* **37**, 143-150 (1976).

23. W.Bolanowska and J.Golacka, "Inhalation and Excretion of Tetrachloroethylene in Men in Experimental Conditions," *Medycyna Pracy* **23**, 109-119 (1972).

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SESSION VI
RISK COMMUNICATION

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Improving Risk Communications

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World Resources Institute
Washington, DC

Let me begin with an anecdote about communicating with the public in general. In the winter of 1947 to 1948, a coalition of civic organizations in Cincinnati determined to raise public knowledge about the newly established United Nations, and launched an intensive six-month public information campaign. One radio station broadcast 150 spots. Sixty thousand pieces of literature were distributed. The Parent Teacher Association organized presentations to 13,000 people, and a speakers bureau made presentations to 2,800 clubs. To evaluate the campaign, a survey was made before it began, and the same individuals were again surveyed after the campaign had run for six months. It was a simple six-item test. Before the program, 15% of those surveyed got five or six of the questions right. After six months of the intensive public information campaign, 15% of the respondents got five or six of the questions right.

This illustrates a basic principle in communications: most messages reach people who are already informed. Few people pay attention, except when they have a reason. This applies especially to risk communications. Most people ignore or quickly forget risk messages. But when they believe something impinges on their lives, or those of their family, they become information seekers. In that situation, people from all socioeconomic classes become information seekers. And they use it effectively, as well.

There is a second aspect of risk communication that I would like to highlight. It draws upon our experience with smoking. When the Surgeon General issued the first report on smoking in 1964, there was not widespread opposition to smoking in our society. But 20 years of reading the same warnings in newspapers that are heard on television and radio, of having the family doctor urge quitting, of having the kids come home from school with the same message, and finally of hearing complaints from one's colleagues, we can observe significant reductions in the numbers of people smoking.

Risk communications aimed at changing people's behavior takes time. It takes many messages from many different sources. In essence, it means that risk communications cannot be thought of as the sending of a single message which stimulates some change in behavior. Thus, it is almost impossible to

observe simple cause-and-effect relationships regarding risk communications. Evaluations of risk communications require a more sophisticated understanding of the processes by which people receive and process risk information.

In 1989, the Committee on Risk Perception and Communication of the National Research Council issued *Improving Risk Communication*. It focused on the context within which people receive information and how that context influences the way they process risk messages. It also looked mostly at human health effects of exposure to environmental chemicals, although it did examine other topics like exposure to ionizing radiation and bridges falling down. The committee felt its findings generalized to most risk communications situations, and those findings are the basis for my comments.

The committee said that risk communication is successful to the extent that it raises the level of understanding of relevant issues for those involved, and satisfies them that they are adequately informed within the limits of available information.

That is a challenging goal. Not only must risk communication be clear and understandable, but people must be satisfied with the information they are receiving. In other words, solving risk communications problems usually requires changing the procedures with which one interacts with the target audience as much as it requires changing what is said.

The committee identified several common misconceptions that interfere with good risk communications. I want to present those briefly.

The first misconception, and the most important, is that there is no simple solution. There is no single overriding problem, and thus no simple way of making risk communication easy. It requires a lot of effort, and a lot of time.

Next are two unrealistic expectations. The first of these is that good risk communications always reduced conflict and smoothed management of risk issues. However, many conflicts are based in people's different preferences regarding the outcomes rather than disagreement about the facts of the matter. If risk communications helps them better understand the issues and those hinge on preferences regarding outcomes, they may become more convinced of the need to defend their own position. Even though it

may make risk management more difficult in a given instance, the committee felt that our democratic system makes good risk communication an imperative.

The second unrealistic expectation is that risk comparisons can establish acceptable levels of risk. This is most easily illustrated by referring to risks with which we are all familiar. The annual risk of death in this country due to fire or electrocution in the home is about one-fortieth that of death in an automobile accident. But this does not mean that a homeowner should ignore fire or electrical hazards. Appropriate action should be taken to reduce risk or each type even though the risk is greater in one category than in another. Risk comparisons can help people understand the unfamiliar magnitudes encountered in looking at risks. But simple comparing risk levels is never adequate to establish an acceptable level of risk in a particular instance.

Then there are three mistaken beliefs about risk communications. First is that scientific information resolves all important risk issues. I do not have to elaborate on this--you have been hearing about issues in risk assessment throughout this conference. Second is that scientists always agree about the meaning of the available scientific evidence. The conference has also described many of the alternative ways of treating key components of risk assessment. The third, however, has not been treated by other sessions in this meeting. It is that the values, preferences, and information needs of people can be easily identified. Unfortunately, this is not so. Despite the fact that individuals often stand on a platform and talk about "the people" and their "needs and wishes," the attitudes and beliefs of people in a particular situation are extremely difficult to determine. They are hard to specify in a scientific way, so that the findings can be replicated. It takes hard work, and risk communication efforts can be misplaced and ineffective if that hard work is not done.

Finally, there are two false stereotypes. The first is that journalists and the media are a significant, independent cause of risk communications problems. To be sure, risk communicators often have problems with journalists. Journalists often present information in quite different ways than we would. That should be no surprise – they are not writing scientific articles. They have to attract people's attention (remember the good citizens of Cincinnati), and to do so, they need dramatic tension. Part of what we expect from the news media is that they ferret out all the relevant positions in instances of political or social conflict. We also want them to illuminate even those that are uncommon or unpopular. When they do so, regarding scientific issues or conflicts about risk issues, however, we are

less understanding. The committee felt that the treatment of scientific and risk issues in the print media has improved greatly over the last decade. It also admonished scientists and risk assessors to learn more about how journalists do their work and the way the news sector functions.

The other false stereotype is that people always want simple, clear-cut answers. Think about medical problems. It used to be that medical doctors were expected to make decisions about their patients. Now, however, many patients want full explanations and data about success rates of procedures. Often, second opinions are sought as a matter of course. In some instances, people do want simple directions about what to do. But, often they prefer to understand the issues and make up their own mind.

This briefly summarizes the common misconceptions described in *Improving Risk Communications*. If you are interested in going into these issues in more detail, the book contains an excellent summary of these ideas, as well as the committee's findings and recommendations regarding management of the process of risk communications and the content of risk messages.

Role for Risk Communication in Closing Military Waste Sites

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ABSTRACT

Lessons learned from environmental and occupational hazard risk management practices over the past 30 years have led the Department of Defense to explore alternative risk management approaches. Policies for cleanup of environmentally hazardous waste sites are undergoing examination and are being reframed. A Demonstration Risk Communication Program is described that incorporates principles that integrate risk-based scientific information as well as community values, perceptions, and needs in a democratic process that includes the public as an active participant from the earliest stages. A strong scientific foundation for assessment and characterization of risk is viewed as necessary but not sufficient; the public's values must be actively integrated into the negotiated criteria. The Demonstration Program uses a model to prepare the participants and to guide them through the process. A five-step process is presented: (1) create risk communications process action team including at least one member of the specific site audience; (2) professionally train participants on team dynamics including interpersonal communication skills; (3) train risk communicators to deliver a cogent presentation of the message to secure a decision acceptable to both the government and the public; (4) identify existing biases, perceptions, and values held by all participants; and (5) develop risk message incorporating science and values. The process action team approach assumes the participants enter into the effort with the goal of improved environment and safeguarded public health. The team approach avoids confrontational or adversarial interactions and focuses on a dialogue from which a negotiated team response develops. Central to the program is the recognition that communication is only effective when the dialogue is two-way.

INTRODUCTION

The federal government has placed significant emphasis on the cleanup of environmentally hazardous waste sites. This emphasis is increasing as installations are identified for closure and as the commitment to complete cleanup in this decade becomes one of the principal management goals (1). Reflecting the current views on good practice, the Department of Defense (DoD) adopted the separately

funded Defense Installation Restoration Program. In a systematic approach modeled after the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as Superfund), waste sites are identified and characterized, cleanup levels are defined, and restoration programs are implemented. The various governmental and regulatory bodies interact under a set of federal facilities agreements. Typically, preferred action consequences are compared to consequences of alternatives or no action, the criteria for site cleanup are negotiated, and then submitted to the agency for a Record of Decision (ROD). Historically, the public usually only had input into the decision making towards the end of the process. This practice is undergoing transition toward a more democratic process that includes the public earlier and gives greater weight to perceptions and values. An Air Force Demonstration Risk Communication Program in the format of a process action team (PAT) addresses the need to incorporate the public into the cleanup management process.

Communication of risk of hazardous environment to the public is required by statutes, regulations, and court decisions. Enhanced risk communication rules are being required by Occupational Safety and Health Administration, the Clean Air Act Amendments of 1990, the Resource Conservation and Recovery Act of 1986, the National Environmental Policy Act of 1969 (NEPA), and CERCLA, as amended by the Superfund Amendment Reauthorization Act (SARA) of 1986, and as specifically outlined in SARA Title III (Federal Emergency Response and Community Right To Know). Risk communication is a critical component of the Environmental Impact Assessment (EIA) process required under NEPA.

Attaining these mandates has proven to be a difficult task. Budgets for cleanup for some of the federal agencies, even though extremely large, will not yield closed sites for 10 to 100 years (1,2). Cost effectiveness is a powerful driver in any environmental cleanup action. Recent review of the successes and failures of environmental practices cautions that society will "face heavy costs... if finite resources are expended on low priority problems at the expense of high priority risks" (3). The recently drafted Environmental Risk Reduction Act (S.110, originally Bill S.2132) calls for the Environmental Protection Agency (EPA) to identify a prioritized list of risks, evaluate the public awareness of each risk, determine alternative options for reducing risks with estimates of cost and time required, and to identify uncertainty associated with the assessment process.

Two significant initiatives are occurring within the scientific community, which promise to facilitate progress in site closure. First, we are slowly evolving towards use of redefined risk-based

health effects criteria in the assessment and prioritization of environmental protection and occupational safety and health issues. To more thoroughly evaluate various approaches, the Congressional Office of Technology Assessment currently is assembling data on the various agencies' efforts in risk assessment research. Second, the agencies are developing an awareness of how little impact our knowledge of risk-based criteria has on the public. The Department of Energy (DOE) has initiated a senior-level Steering Group (4) of representatives from their National Laboratories with the specific objective of improving public understanding of the risks existing at DOE sites. In their report on lessons learned, they identified three areas as requiring emphasis: public understanding of the scientific underpinnings of risk-based standards, prioritization based on risk-based criteria, and use of cost-benefit analysis in standards setting. This approach places emphasis on development and adherence to standards that can provide credibility for risk-management practices and risk communication.

Significant scientific controversy continues over whether the practices employed during the last decade in estimating risk are yielding clear national objectives for environmental restoration (2, 5-14). Economic pressures on strategy selection (15) have given rise to controversial concepts of risk management, for example that of risk-risk reduction (16). Review of alternative cleanup policies (2) suggests that we may need to redefine our approach to bring the human health and environmental benefits more in-line with the very large cleanup investments. The public must be involved in this dialogue, particularly at individual sites.

Environmental Protection Agency policy recently stated that...."solid scientifically based risk assessment is critically important to our ability to set risk-based priorities" (17). However, scientific consensus on health risk is only one of the variables currently involved (14). Social and political values held by all participants in the risk process are becoming recognized as important variables. This is a recent development in the evolution of risk assessment and risk communication. The 1991 Regulatory Program of the United States Government stated that risk assessments under federal oversight should be based on science only (16), continuing an EPA management policy set a decade earlier that "risk assessment must be based on scientific evidence and nothing else." (18). The EPA Science Advirsory Board (SAB) recently concluded that "there has been little correlation between the relative resources dedicated to different environmental problems and the relative risks posed by those problems," (3). In fact, the EPA report, *Unfinished Business* (19), concluded that the only factor that correlated with EPA programmatic priorities of budget and staff allocations was apparent public perception of risk. Thus,

public perception, rather than scientific understanding, appears to drive Congressional allocation of resources to the EPA.

The EPA's Comparative Risk Assessment (CRA) process (3) proposes that environmental priorities be based on expert scientific opinion regarding the greatest and/or most cost-effective opportunities for reducing risks. However, it has been shown that the public is not the only participant that is influenced by values and experiences. Scientists' interpretations of the facts can be influenced by personal values and experiences (20). This influence appears especially when the data are unclear and when social and political stakes are high (21, 22). The PAT should recognize that data that are less than complete, at threshold values, and thus not especially convincing, will present the greatest challenge to communication.

The EPA strategy to guide risk reduction efforts has evolved during the last two years from a "hard" version of the CRA process to a "softer" version that integrates science, values, and democracy (23). "Hard" CRA refers to experts calculating aggregate risk to populations and cost of reducing risk and then setting priorities by allocating resources to interventions that have the lowest cost per unit of risk reduction. In contrast, in the "soft" version of CRA, experts and the public would negotiate in an attempt to reach consensus on risk rankings that reflect the magnitude of possible hazard reductions and other social and political considerations that affect perception of risks. Risk characterization is more than the single dimension of hazard assessment; it directly involves public values focusing on the outrage (24,25) perceived by the general public. Achieving the goal of site closure to protect the public health requires meeting the public's (and often the scientists' and regulators') value sets. To fully develop the "soft" version, the public must be informed and scientific data must be decoded and expressed in language that the public/nonscientists can evaluate. Only then can true negotiation proceed in setting risk communication and risk management strategies. The Demonstration Program embraces the "soft," more democratic, version as we believe attention to public perceptions and concerns is critical to success, yet it incorporates a "hard" central foundation of sound science.

Any risk communication PAT must understand that differing degrees of evidence are judged as cause for action by individuals following varied policy agendas. For example, although scientists tend to demand solid evidence before acceptance, public health officials adopt a standard of taking action on the basis of suggestive but still inconclusive evidence (26). As a result, public health policy is often

promulgated based upon social and political considerations and not solely upon science. The EPA SAB noted (3) that "some view risk reduction in the face of incomplete or uncertain risk assessment as a kind of insurance premium, since the risks of postponing action can be greater than the risks entailed in taking inefficient or unnecessary action." The SAB deemed this appropriate stating that "because they experience risks first hand, the public should have a substantial voice in establishing risk-reduction priorities" (3). This Demonstration Program includes the public throughout the process.

APPROACH

The risk communication PAT should be formed early, be professionally trained on team dynamics including interpersonal communication skills, and interact routinely and concurrently with discovery steps in the risk assessment process until a ROD is signed. Previous experience with PATs formed under Total Quality Management guidance has shown that preliminary training results in greater team success. The team will be responsible for preparing informational materials and fostering dialogue between the government and the public including town meetings, official scoping meetings, and public hearings. The team will have responsibility for interagency communication required during the approval process.

Models for the communication process identify the elements and the transfer functions that impact the effectiveness of communications (25, 27-34). Most models of communication presume a single communications event is occurring. Figure 49 represents the classical models for risk assessment (5) and instantaneous communication (35). For our demonstration, we develop the risk communications structure with the expectation that at least four separate, but interacting, scenarios may occur simultaneously. First, health-based risk assessment information must be conveyed to the public. Risk communicators have been criticized for incorrectly assuming the public is naive about environmental and health issues (28,36). The interested public can be expected to include members representing the full spectrum of risk awareness, subject knowledge, acceptance/avoidance, and bias. Second, the risk must be conveyed to the environmental activists and government/industry special interests which are often at opposite poles of the spectrum. These participants have distinct biases that can impact communication. Third, the risk assessment, the risk management plan, and current public perceptions must be conveyed to the media in a concise and unambiguous style that can be easily formatted to the particular media channel. Lastly, the risk must be communicated to the scientific community and the regulators/lawyers/public policy officials who have objectives of protecting the public but also frequently strive to maintain status quo. Each of the scenarios involves audiences with varying levels of existing expertise, varying levels of

commitment and outrage, and varying objectives. Risk communication strategies should not be directed at winning over those whose objectives are planned attempts to obfuscate the process (37) but instead should be directed towards those who have legitimate concerns. The National Research Council's suggestions (38) that (a) the process of communication, not just the message, should be important, and (b) communication should be two-way are central to the approach. Figure 50 represents the situation when multiple simultaneous communications occur where participants each have their own encoders and decoders actively impacting information exchange. At any instant, communication is in one direction. The role of sender and receiver switch as the direction of information flow switches instant to instant.

Meaningful risk communication is a component of effective risk management (39). Risk communication becomes integral to environmental performance and not just an arm of Public Affairs. Amplification of risk communication has been shown to enhance an organization's ability to reduce risks (39). An open, two-way dialogue facilitating information flow between external as well as internal sources and those responsible for risk management is necessary to establish credibility and to maintain trust. Thus, risk communication may be viewed as integral to environmental performance rather than merely a mouthpiece for it or, at worse, a tool to make the public see and accept the issue from management's view point.

This procedural approach (40) to risk communication tailors messages to the concerns of the particular audiences. The public is empowered to influence risk management efforts through participation in the Risk Communication PAT, advisory groups, and at public meetings. Citizen input is encouraged and efforts are taken to inform the population about the risk assessment and risk management processes. Citizen criteria and citizen-initiated management suggestions are considered especially valid and are taken into account. The model focuses on issues (e.g., hazardous waste), venues (e.g., public meeting or advisory group), and concepts demonstrated to be effective tools for PATs (e.g., trust, respect, concern). The process stresses understanding people's concerns and the constraints that they perceive they must operate under.

CLASSICAL MODELS

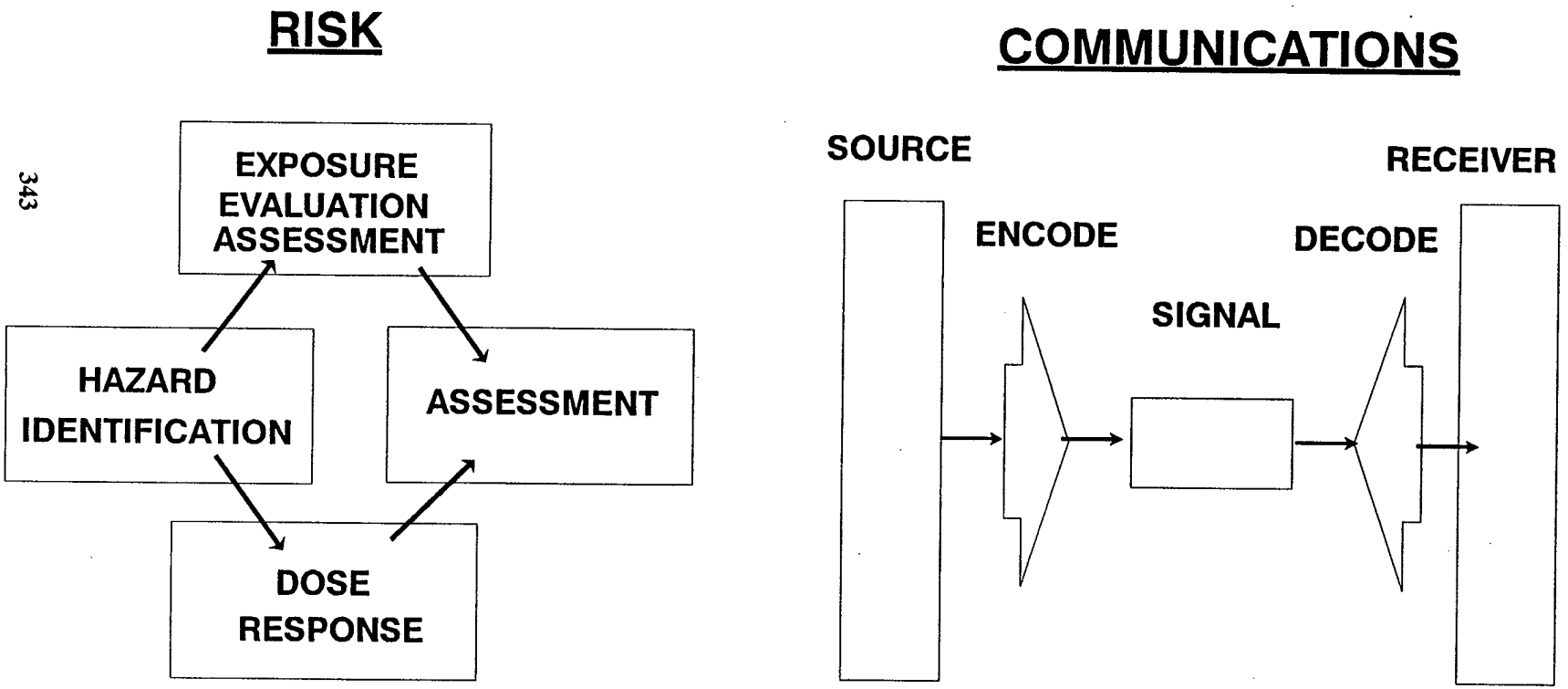


Figure 49. Concurrent Processes for Risk Analysis and Simple Communication Model.

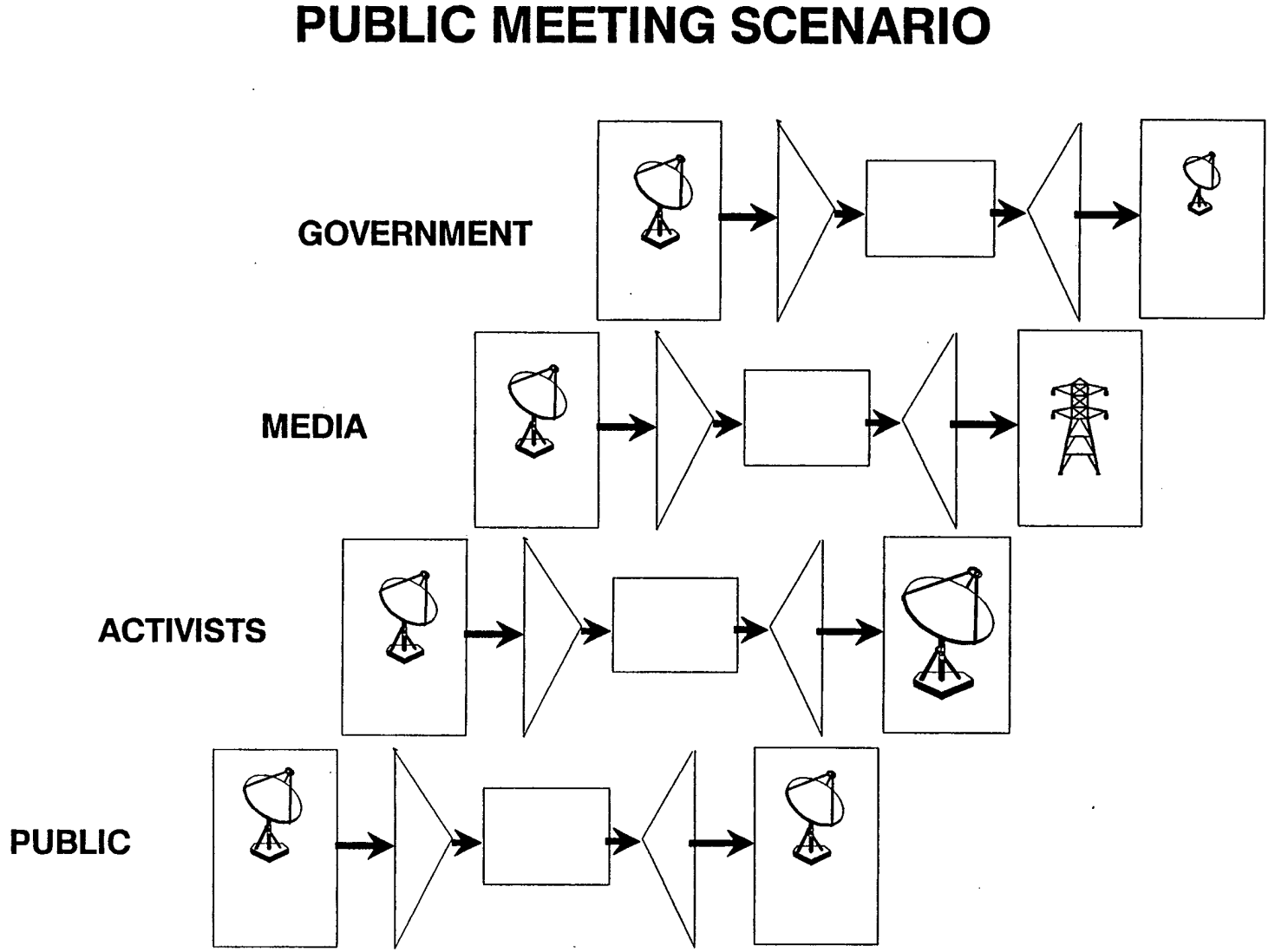


Figure 50. In Concurrent, Parallel Communications the Message is Decoded Differently Depending Upon the Receiver's Perspective.

With careful design and evaluation, it is possible to develop balanced materials that provide the public with the information that they may need to make informed decisions about the risk that they face. The design must include communication of choices people have. It must assess their present cognitive set about the risk, their beliefs and values, and what information they have and need (36). This education step must occur early and possibly reoccur as participants change to facilitate real discussion of risk. The "Mental Models" approach (36) suggests that understanding the "intuitive thought processes" the public uses will facilitate communication of information that is needed for making informed judgements. This approach attends to individual costs including those incurred by thinking about hazards. "Prudent Avoidance" (41) springs from this emphasis on encouraging private management of the hazard when possible and when individual costs are acceptable.

Sustained risk communication involving citizen-participatory advisory groups substantially improves negotiations toward a ROD. Several public meetings occur during the process and the risk communication PAT must be prepared with a well-developed message. The risk communication team must receive training in interacting with the public and the media, as well as training in meeting the challenges of activist techniques, and sensitization to the concerns of the specific community involved (25,28,34,37). With the complex communications scenario that develops, a significant amount of structure will be required to accommodate the existing diverse set of personal objectives.

In order for any of the attendees at the public scoping meetings or the public hearings to express their objectives, a discipline for the process needs to be established. A "model format" will be presented and negotiated with the attendees in order that all the objectives be recognized. It is structured for the necessary condition of including sound science but recognizes science in itself is not sufficient (14). The intent of the discipline is to focus the dialogue on the explicit issues and reduce the confusion over hazard, exposure, scientific uncertainty, and the acceptability of those factors with respect to public values. Discipline in the agenda assures each participant an opportunity to air issues and resolve conflicts.

To establish and maintain discipline, a respected but neutral party must act as a moderator. In public hearing scenarios, a Trial Judge or similar official is typically required by statute. The moderator, as part of the introduction, negotiates a "contract" with the audience for rules of order and the use of the model format as part of the conduct of the meeting. Because it is expected that the attendees may be

suspicious of a government assessment, all the tools to establish credibility and achieve transference must be employed prior to the development of the hazard characterization phase (25,29,35). Care must be taken to ensure that the audience is "calibrated" for the concepts of comparative risks to be addressed. Communication must address both the relative levels of previously accepted risk and must also explicitly include the issues of equity; whether the risk is voluntary, catastrophic, novel, or embodies any of the 20 to 30 factors that have been identified to impact the perception and acceptability of risks (24). This "calibration" needs to be tailored to each specific risk situation. The lessons learned by the DOE Steering Group (4) support the concept of added attention to understanding the objective and processes of the attendees along with the need for discipline in the communications process.

DISCUSSION

Establishing priorities for environmental risk management continues to be an inexact process. Not only are we dealing with concepts and probabilities that we find difficult to grasp in total, but we are dealing with personal values that vary widely across the population. Journal policies not to publish and pressures on scientists not to pursue "no effect" studies continues to artificially skew the literature toward positive findings (14). Additionally, our policy makers in their attempt to deal with uncertainty are pursuing questionable practices such as incorporating concepts like "prudent avoidance" into risk management decisions. With these pressures to distort risk and with the difficulty we have in perceiving (28) the extent of risk, if we are to arrive at a sound decision for the extent and priority of risk management actions, discipline must be involved in the dialogue. Through the use of a structured PAT model format, the tendency to propagate conservative assumptions and distort margins of safety may be partially controlled. It also keeps the focus on protecting the public health in an open and full disclosure process. As stewards of our natural environment and of the taxpayers' trust, our goal must be to establish health-based criteria that reduce our population risks to levels that are agreed upon in a negotiated open forum. It ensures that scientific assessments and public values and perceptions are taken into account. The described demonstration's approach at characterizing the risk, empowering the community through communication, and searching for effective risk management through a priority process reflecting public concern and finite resources promises to aid the DoD in its risk reduction management goal.

CONCLUSION

The risk communication PAT approach needs to be tailored to each situation. It provides an organized procedure for incorporating the public into the process to achieve protection of the public health while providing stewardship of the taxpayers resources. The approach describes concurrent processes that must occur over a significant period of time leading to a sound scientific and value-based decision. It contains the necessary and sufficient conditions of both scientific and social processes to reach a sound cost-effective value and risk-based decision.

REFERENCES

1. G. Vest, "The Environmental Umbrella," Presentation to Commander's Environmental Leadership Course (Wright-Patterson AFB, OH, March 31, 1993).

2. P. Abelson, "Remediation of Hazardous Waste Sites," *Science* **255(2)**, 901 (1992).

3. U.S. Environmental Protection Agency, Science Advisory Board: Relative Risk Reduction Strategies Committee, "Reducing Risks: Setting Priorities and Strategies for Environmental Protection" SAB-EC-90-021, (1990).

4. Department of Energy, Office of Environmental Safety and Health, "Risk-Based Standards – Lesson Learned," (Sandia National Laboratories, Albuquerque, NM, 1992).

5. National Academy of Sciences, Committee on the Institutional Means for Assessment of Risks to Public Health, "Risk Assessment in the Federal Government: Managing the Process" (National Academy of Sciences, National Academy Press, Washington, DC, 1983).

6. U. S. Environmental Protection Agency, *Risk Assessment Guidance in Superfund Volume 1: Human Health Evaluation Manual, Part A,* EPA 540/1-89-002, (1989).

7. J. Doull, "Toxicology and Exposure Limits," *Appl. Occup. Environ. Hyg.* **7(9)**, 583-585 (1992).

8. D. Stipp, "How Sand on a Beach Came to Be Defined as a Human Carcinogen: Tests Using Common Silica Spark a Scientific Clash Over Safety, Procedures" (*The Wall Street Journal*, **22**, March 1993).

9. B. Ames, L. Gold, and M. Profit, "Dietary Pesticides (99.99% All Natural)," Medical Sciences Proceedings, *Natl. Acad. Sci.*, U.S.A. **87** (1990).

10. B. Ames, L. Gold, M. Profit, "Natures Chemicals and Synthetics Chemicals: Comparative Toxicology," Medical Sciences Proceedings, *Natl. Acad. Sci.*, U.S.A. **87** (1990).

11. D. Paustenbach, "Health Risk Assessment and the Practice of Industrial Hygiene," *Am. Ind. Hyg. Assoc.* **51(7)**, 339-351 (1990).

12. F. Johansen, "Risk Assessment of Carcinogenic and Noncarcinogenic Chemicals," *Cri. Rev. Toxicol.* **20(5)**, 341-367 (1990).

13. E. Efron, *The Apocalyptic: How Environmental Politics Controls What We Know About Cancer* (Simon and Schuster, New York, NY, 1984).

14. B.J. Klauenberg, "Does Public Health Policy Require Scientific Consensus?" (*HPS Newsletter*, October 1991).

15. D.J. Paustenbach, "Jousting with Environmental Windmills," *Risk Anal.* **13**, 13-15 (1993).

16. Office of Management and Budget, Regulatory Program of the United States Government (April 1, 1990-March 31, 1991).

17. F.H. Habicht II, EPA Assessment Program, Featured Speaker, Society for Risk Analysis Annual Meeting (1991).

18. W. Ruckelshaus, *Science, Risk and Public Policy, Vital Speeches of the Day*, Vol. 49, No. 20, pp. 612-615 (August 1, 1983).

19. U. S. Environmental Protection Agency, Office of Policy Analysis, *Unfinished Business: A Comprehensive Assessment of Environmental Problems*, Vol. 1, Overview Report, (U.S. Government Printing Office, Washington, DC, 1987).

20. G.L. Carlo, N.L. Lee, K.G. Sund, and S.D. Pettygrove, "The Interplay of Science, Values, and Experiences Among Scientists Asked to Evaluate the Hazards of Dioxin, Radon, and Environmental Tobacco Smoke," *Risk Anal.* **12**, 37-43 (1992).

21. A. Whittemore, "Facts and Values in Risk Analysis for Environmental Toxicants," *Risk Anal.* **3**, 23-33 (1983).

22. D. Robins and R. Johnson, "The Role of Cognitive and Occupational Differentiation in Scientific Controversies," *Soc. Stud. Sci.* **6**, 349-368 (1976).

23. F.H. Habicht II, National Environmental Priorities: The EPA Risk-Based Paradigm and its Alternative Conference, held in Annapolis, MD, Nov 16-17, 1992 (Organized by A. Finkel, Center for Risk Management, Resources for the Future. Reported in *RISK Newsletter*, **13**-1 (1993).

24. P.M Sandman, "Risk Communication: Facing Public Outrage," *EPA J.*, pp. 21-22 (November 1987).

25. V. Covello, P. Sandman, and P. Solvic, *Risk Communication, Risk Statistics and Risk Comparisons: A Manual for Plant Managers* (Chemical Manufacturers Association, Washington, DC, 1988).

26. G. Morgan, "Expose' Treatment Conforms Understanding of a Serious Public Health Issue," *Scient. Am.* (April 1990).

27. C. Chess, B. Hance, and P. Sandmond, "Improving Dialogue with Communities: A Short Guide for Government Risk Communication," New Jersey Department of Environmental Protection (1988).

28. H. Otway and B. Wynne, "Risk Communication: Paradigm and Paradox," *Risk Anal.* **9(2)**, (1989).

29. F. Johnson and A. Fisher, "Conventional Wisdom on Risk Communication and Evidence from a Field Experiment," *Risk Anal.* **9(2)**, (1989).

30. R. Schrum, "Challenges in Crisis Management," *Contingency J.* (July-September 1990).

31. C. Howard, "Managing Media Relation for Environmental Issues," *Public Relations Quarterly* (Summmer, 1988).

32. Fact Sheet: Community Relations in the Environmental Restoration Program, Office of Environmental Restoration and Waste Management, Oak Ridge Operations, Oct 1990.

33. V. Covello, D. McCallum and M. Pavlova, "Principles and Guidelines for Improving Risk Communication, Effective Risk Communication: The Role and Responsibility of Government and Nongovernment Organizations," (Plenum Press, New York, NY, 1989).

34. V. Covello and F. Allen, "Seven Cardinal Rules of Risk Communications," (Environmental Protection Agency, Washington, DC).

35. S. Certo, *Principles of Modern Management* (Wm. C. Brown Co., Dubuque, IA, 1980).

36. M.G. Morgan, B.B. Fischoff, L. Lave, and C.J. Atman, "Communicating Risk to the Public: First, Learn What People Know and Believe," *Environ. Sci. Technol.* **26**, 2048-2056 (1992).

37. I. Kornfeld, W. Subra, and W. Collette, "How to Win in Public Hearings," (Citizen's Clearinghouse for Hazardous Waste, Inc., Falls Church, VA, 1990).

38. National Research Council, *Improving Risk Communication* (National Academy Press, Washington, DC, 1989).

39. C. Chess, A. Saville, M. Tamuz, and M. Greenberg, "The Organizational Links Between Communication and Risk Management: The Case of Seaborne Chemicals Inc.," *Risk Anal.* **12**, 431-438 (1982).

40. B.B. Johnson, "The "Mental Model" Meets the "Planning Process": Wrestling with Risk Communication Research and Practice," *Risk Anal.* **13**, 5-8 (1993).

41. I. Nair, M.G. Morgan, and H.K. Florig, "Biological Effects of Power Frequency Electric and Magnetic Fields," Background paper prepared for the Congress of the United States, Office of Technology Assessment, OTA-BP-E-53 (May 1989).

Risk Communication in Environmental Restoration Programs

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ABSTRACT

The author advocates adoption of a convergence model in place of the traditional source-receiver model of communication for communicating with members of the public who have a stake in remediation of a nearby site. The source-receiver model conceives of communication as the transmission of a message from a risk management agency (sender) to a target audience of the public (receivers). The underlying theme is that the sender intends to change the perception of the receiver of either the issue or the sender of information. The author draws on her experience at a Department of Energy (DoE) site undergoing remediation to illustrate why the convergence model is more appropriate in the context of cleanup. This alternative model focuses on the Latin derivation of communication as sharing or making common to many, (i.e., as involving a relationship between participants who engage in a process of communication). The focus appears to be consistent with recently issued DoE policy that calls for involving the public in identifying issues and problems and in formulating and evaluating decision alternatives in cleanup. By emphasizing context, process, and participants, as opposed to senders and receivers, the model identifies key issues to address in facilitating consensus concerning the risks of cleanup. Similarities between the institutional context of DoE and Department of Defense (DoD) suggest that a convergence model may also prove to be an appropriate conceptual foundation for risk communication at contaminated DoD sites.

INTRODUCTION[1]

At a national conference on risk communication held in 1986, William Ruckelshaus (1), former Administrator of the Environmental Protection Agency (EPA) emphasized:

> My point is not to say whether a sharing of power to make risk management decisions is right or wrong; it is simply to state that it is a fact of life in the United States. We have decided in an unprecedented way, that the decision-making responsibility involving risk issues must be shared with the American people . . . . So the question before us is

not whether there is going to be any sharing, whether we will have participatory democracy with regard to the management of risk, but how.

This paper discusses risk communication in the context of sharing in risk decisions with members of the public who have a stake in remediation of a nearby contaminated site. First, the paper summarizes and provides the author's personal perspective on developments occurring under the Environmental Restoration and Waste Management (EM) program of the Department of Energy (DoE). Subsequently, it outlines two models of communication – the linear model and the convergence model. The focus of the discussion is on the implications of each model for practice: which model provides the more appropriate basis for risk communication in the context of sharing in the management of risk? I argue that lack of clarity concerning the purpose of risk communication in different contexts and for different types of policy is contributing to continued confusion over how to structure the sharing process.

EXPERIENCE FROM THE FIELD

Creighton (2) has recently pointed to changes that have occurred over the past few decades in public expectations concerning agency decision making. He argues that, in the post World War II era, expectations were limited to information, which was provided at the agency's discretion. Expectations shifted to procedural public involvement in the 1960s and 1970s, while the 1980s witnessed increasing calls for "meaningful" participation in agency decisions (i.e., a role in defining the problem and in determining the range of alternatives considered and the criteria used to evaluate those alternatives). Creighton has identified collaborative decision making as the expectation of the 1990s.

Changed expectations, in combination with pervasive public distrust of government institutions (3), have had particular impact on the DoE, where decision making was formerly cloaked in the secrecy afforded by a national security mission. In the post-cold war era, the agency is now faced with the need to remediate over 9,000 sites in full compliance with a range of environmental regulations. The Decide-Announce-Defend approach of former years perforce is giving way to attempts to develop a new culture of openness and involvement of the various publics (stakeholders)[1] in agency decision making. Culture changes begun under the Bush Administration are receiving increased emphasis by the Clinton Administration through appointment of service personnel who are strongly supportive of public participation.

As with any culture change, the transition will not occur overnight. The agency is likely to encounter disagreements, lack of understanding, and confusion over the extent and implications of this new approach to decision making. Individual staff may be expected to vary in their commitment to public participation; preferred approaches are likely to span the continuum from maintaining public control to public persuasion (i.e., providing information "with the intent of putting DoE in the best possible light"), and ultimately to providing for public involvement in formulating and reaching decisions (4).

Meanwhile, public participation activities are increasing at the many sites in the DoE complex. Within the past year, a newly established office of policy and program information in the EM program has provided for increased coordination among the sites and between headquarters and field offices. At the national level, two advisory groups meet and interact on a regular basis with program staff: the State and Tribal Government Working Group and the EM Advisory Committee for the Programmatic Environmental Impact Statement. Additionally, the Department has instituted training in public participation for managers across the DoE complex.

These activities will need strong management support if they are to address what I consider to be the most critical need in meeting public expectations of the 1990s and in increasing public trust in the decision-making process. In my view, this is the need to integrate public participation activities into the technical decision-making process specified under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Significantly, although the CERCLA regulations provide for a more formal program in public participation than other environmental statutes do, DoE (or the applicable regulated entity) is not required to engage the public in early discussion of plans and alternatives, including discussion of risks. All that is required is availability in a nearby information repository of site information and notification of the availability of Technical Assistance Grants, and formal public comment on the agency's preferred cleanup alternative. The regulations therefore do little to facilitate a change in DoE's normal way of doing business (i.e. of making decisions, frequently classified as "purely technical," without benefit of early public input). Too often, public participation becomes an add-on to the real action: public participation activities consist of informing the public and/or seeking formal comments on decisions that, essentially, have already been reached. Such an approach is unlikely to meet public expectations in the 1990s or to engender public trust.

The lack of integration between technical and public participation activities is especially marked in addressing and communicating about risk, which frequently symbolizes and serves as the lightning rod for disagreement between technical and lay communities on cleanup issues. An increasingly formalized approach to risk assessment underlies the entire Remedial Investigation/Feasibility Study (RI/FS) decision-making process, culminating in selection of a preferred cleanup alternative. Here, the lack of CERCLA requirements for early public input is reinforced by EPA policy which has consistently attempted to make a clear distinction between risk assessment and risk management.[2] The theoretical premise underlying this policy assumes that risk is a completely objective, neutral entity. The unfortunate practical result of the policy is that it contributes to continuation of a technocratic, rather than a participatory approach to decision making. This approach views risk communication as a process of providing technical risk information **to** the public – in effect, attempting to persuade the public to accept as the basis for action the technical risk assessments from which they are excluded. Fiorino (5) summarizes the current situation as follows:

> The risk community has focused its attention on the technical and economic aspects of policy making. Yet the challenge to effective risk management may not be so much the technical or economic as political. By political, I mean the ways people view their relationship to institutions making collective decisions about environmental risk and their capacities for influencing those decisions. Yet the literature on environmental risk – whether it assumes the label of risk assessment, management, or communication – often ignores this aspect of risk problems and their solutions. . . . We refine, polish, and perfect our formal models for determining acceptable levels of risk, despite evidence that the assumptions and methods bear little relationship to the lay public's conception of the problems. We test techniques for communicating risk information to the public, but conduct almost no research on mechanisms for the lay public to communicate with government officials and technical experts.

The notion that risk is a completely objective, neutral entity has been challenged by writers who adhere to a social constructivist viewpoint (6-13). This viewpoint conceives of risk as a socially constructed attribute rather than a physical entity that exists independently of the humans who assess and experience its effects. Authors who adhere to this viewpoint emphasize the inherent uncertainties in risk assessment and the element of judgment that underlies both technical and lay assessments of risk.

Accordingly, they advocate negotiation on value differences as the appropriate approach to programs and policies involving risk issues.

Several events that have occurred within the past few months may, in combination, provide greater support for early stakeholder input and foster collaborative decision making among DoE and its various stakeholders. First, EM public participation policy, which was issued in the fall of 1992, commits the program to provide opportunities for public participation in program planning, design, and implementation. The policy specifically promises to provide "opportunities for the public to assist DoE in identifying EM-related issues and problems and in formulating and evaluating decision alternatives." Second, the interim report of the Federal Facilities Environmental Restoration Dialogue Committee includes a recommendation that will impact all Federal agencies involved in remediation.[3] The Committee recommends establishment of site-specific advisory boards composed of a representative cross-section of stakeholders and including senior staff of the agency subject to regulation. Third, the Defense Reauthorization Act for Fiscal Year 1993 requires DoE to submit a report to Congress on the effectiveness of existing advisory groups, the desirability of establishing new or replacement advisory groups, and methods for improving public participation in EM activities. The DoE is already in the process of forming advisory groups of stakeholders at five large sites within the DoE complex.

Establishment of site-specific advisory boards may prove to be a critical development. Over time, such boards could provide for genuine dialogue, setting the stage for negotiation on contentious issues surrounding cleanup. Confusion in the social and communication sciences, however, has hindered development of a sound theoretical foundation for risk communication in such settings. The lack of conceptual clarity is particularly regrettable, given DoE's need for guidance in structuring participation during an era of fundamental culture change.

TWO MODELS OF COMMUNICATION

Everett Rogers, a leading scholar in the field of communication, has distinguished between two models of communication: the linear model and the convergence model. The practical implications for risk communication in the context of remediation are demonstrated by an examination of the graphical illustrations of the models provided by Rogers and Kinkaid (14).

The Linear Model portrays communication as the transmission of a message, via a channel (media), from a source (sender) to a receiver. Communication, here, is conceptualized as a one-way process, albeit with feedback loops that allow for a reaction, in which information is provided to someone. As emphasized by Rogers, information is viewed as context-free, to be "carried from a source to a receiver the way a bucket carries water" (15). The focus of the linear model is the effect of communication on the receiver – essentially the goal is persuasion; typically, as suggested recently by Renn (16), a risk management agency is viewed as the communicator and groups of the public are the audiences. Important issues for research and practice include how to facilitate attitude change and how to promote consistency between attitudes and behavior in the intended receiver. Training and assistance for technical personnel is likely to focus on ways to ensure that the technical message gets across.

Renn has observed that the linear model continues to be the dominant model underlying much of the current discussion of risk communication. Covello, von Winterfeldt, and Slovic (17), for example, emphasize problems with the source, message, channel, and receiver that hinder the recipient's ability to receive (and act on) the message. This emphasis, implicitly incorporating the "blame the receiver" orientation of the linear model which has been criticized by Rogers, reinforces the viewpoint that the problem lies in lack of public understanding of the so-called "real" risks. More explicitly, Renn (18) asserts that "most authors" view the general purpose of risk communication as "aim[ing] at changing behavioral response."

In their discussion, however, these authors distinguish different types – and associated purposes – of risk communication. Covello, Von Winterfeldt, and Slovic provide a typology of four risk communication objectives:

- Information and education
- Behavior change and protective action
- Disaster warnings and emergency information
- Joint problem solving and conflict resolution

Renn similarly distinguishes three specific purposes of risk communication:

- To make sure that all receivers of the message are able and capable of understanding and decoding the meaning of messages sent to them

- To persuade the receivers of the message to change their attitudes or their behavior with respect to a specific cause or class of risk
- To provide the conditions for a rational discourse on risk issues so that all parties can take part in an effective and democratic conflict-resolution process

The authors' recognition that risk communication may be employed for different purposes, however, is not accompanied by recognition that the linear model provides an inadequate foundation for all of these purposes. It is questionable whether engaging in risk communication to "change [the public's] behavioral response" is consistent with Ruckelshaus's goal of "sharing. . . [and] participatory democracy with regard to the management of risks" or with DoE's new, participatory approach to decision making. Indeed, the social engineering orientation of the linear model is likely to prove counterproductive: risk bearers such as citizens affected by contamination and remediation of a nearby site are more likely to demand involvement in the decisions that affect them and to resent implications that their attitudes are in need of change.

The linear model may initially appear to provide an appropriate conceptual foundation for programs such as health campaigns that seek to persuade targeted groups of the need for changes in behavior. In this context, the database is firmly established and programs that seek to change behavior seem justified: an accumulation of data indicates that changing public behavior (in relation to smoking, diet, etc.) would result in improved health and longevity. Even here, however, the model fails to address the element of interpretation that enters into all human communication. The need for a different model on which to base policy is more pronounced in the context of remedial programs, where needed data may be lacking and uncertainty is a key aspect of the decision problem. Moreover, implementing an effective cleanup remedy that is acceptable to stakeholders calls for joint problem solving and conflict resolution rather than persuasion.

The need for a different model is highlighted by contradictions discernable in the National Research Council 1989 publication, *Improving Risk Communication*. The authors emphasize that risk communication is a two-way, interactive process – a "form of democratic dialogue," rather than a persuasive attempt to "get one's message across" (19). However, continued reliance on the terminology of the linear model for risk communication undermines their attempt to provide an interactional orientation. The implicitly persuasive orientation of the linear model provides a foundation that is in

fundamental contradiction to an explicit emphasis on communication as a mutual process. The authors acknowledge the lack of "complete knowledge" that forms the basis for risk assessment, the difficulty of separating the underlying values of the risk assessor, and the factors that affect human, including scientific, judgment. However, they fail to address this key issue of the inherent uncertainty and value-embedded nature of risk assessment by implicitly adhering to a concept of risk as a physical attribute of hazardous technologies rather than a social construction that is affected by the social and cultural context. While advocating democratic dialogue on risk, the authors' frequent use of the term "risk messages" endorses a view of communication as the transmission of context-free information. This emphasis on what is transmitted, rather than interpretation of the transmission and the transaction between groups who adhere to different values, reinforces the orientation toward communication as persuasion. Consequently, the authors fail to provide an adequate basis for structuring risk communication as a more participatory process.

The Convergence Model, in contrast, draws on the Latin derivation of communication: sharing, making common to many, or giving to another as a partaker. As Rogers and Rogers have emphasized, "Sharing implies a relationship . . . that two or more people do something together, not that one individual does something to another . . . . [it] is not simply a matter of action and reaction – it is a transactional exchange between two or more individuals" (20).

The convergence model shows communication as an iterative, long-term process in which participants are mutual communicators rather than senders and receivers. The model highlights the role of interpretation and the creation and sharing of information. As indicated by the phrase "and then," participants bring to the process their own cultural frame of reference – their different experiences, values, and organizational background. The written or verbal symbols, which are the physical aspects of communication, may have a different meaning to each. In the communication process, participants share and create information, either diverging or converging on a common meaning or understanding. This sharing, transactional process is inherently a dialogue which takes place over a period of time. It is important to note that convergence on meaning does not necessarily mean agreement and the elimination of conflict.

Adopting a convergence model shifts the linear model focus on the effect of the transmission on a recipient to a focus on the mutual nature of the communication process. The model also shifts the focus

from differences between expert and laypersons to differences in expertise among all stakeholders, indicating different contributions that various parties provide in the communication process. As a result of this shift in focus, the model is better able to address the political challenge to risk management identified by Fiorino. Issues for research and practice include identification of these different sources of expertise, differences between frames of reference that hinder communication, and how and when to include risk bearers in the decision-making process. Training and assistance for technical managers is more likely to focus on how to integrate public participation within overall planning (21), so that communication with the various stakeholders occurs on a continuous, iterative basis.

SELECTING AN APPROPRIATE MODEL

The convergence model provides a more appropriate foundation than the linear model for risk communication in the context of dialogue among stakeholders concerning site remediation. Key, relevant features of the model are: (1) the focus on risk communication as a transaction process; (2) justification for early stakeholder input; (3) the long-term, interactional nature of the process; and (4) consistency with relevant theoretical developments in the social sciences.

Risk Communication as a Transaction Process. The literature on public perception of risk has illuminated ways in which laypersons' assessments of risk differ from those of the technical risk assessor. There has been less widespread recognition, however, that technical judgments also represent perceptions.[4] In particular, there has been less attention paid to the implications for policy of cultural differences among stakeholders in terms of perspectives and in acquiring and using knowledge (23). The convergence model points to the cultural filters that all participants bring to the communication process and highlights the need to take public concerns and knowledge seriously. It emphasizes the importance of communication as a transaction between participants with different frames of reference. The issues to address are not the effect of communication on the receiver and on how technical experts can convey their message more effectively (i.e., convince the public, as in the linear model). Rather, the issues are the barriers to communication on the part of all participants and the best way to structure a constructive dialogue among them.

Need for Early Stakeholder Input. By highlighting cultural differences between participants and the context in which communication occurs, the convergence model indicates the importance of communicating with rather than to stakeholders in the early stages of the risk assessment process. The

level of acceptable risk to a community may depend on a variety of factors, including intended use of the site after remediation and the human health and environmental issues of most concern to those involved. Value-laden factors like these, which underlie many of the decisions that guide the conduct of risk assessment, confirm the fundamental impossibility of neatly distinguishing between the facts of risk assessment and the values of risk management. Establishing an early mechanism for communication will facilitate identification of value differences between risk assessors and other stakeholders, bringing risk bearers into the process to provide early guidance on their values. This guidance is required throughout, not simply after the conduct of the risk assessment.

The Ongoing Nature of Communication. In the convergence model, communication is an iterative, long-term process rather than a single act of transmission. The model underscores the interactional nature of constructing common meanings. It also points to the importance of building a relationship as the basis on which a civilized debate can proceed.

Consistency with Theoretical Developments in the Social Sciences. Perhaps most significantly, the convergence model is consistent with several related trends in social thought that, in combination, provide a sound basis for designing policy on issues involving risk. First, the model provides a conceptual basis for communication as dialogue, consistent with the insights of Ravetz (24). Ravetz explicitly addresses the value-embedded nature of risk assessment in recommending a policy approach that is directly applicable to remediation policies and programs. He differentiates between types of policy problems according to location on two dimensions: a factual and a valuative dimension. A traditional, technical approach is suited to problems where a substantial body of data exist and value disputes among stakeholders are lacking. However, where the database is not well established and value disputes arise, Ravetz recommends dialogue among stakeholders as a more appropriate approach to policy resolution.

Second, the convergence model is consistent with knowledge utilization theory, which highlights the role of frames of reference in interpreting new information and constructing meanings. Differences in frames of reference, which serve as "the unreflected basis for structuring inquiry," are associated with different ways of assessing the validity, relevance, and cogency of new information (25). Accordingly, knowledge is transacted among rather than transmitted to groups with different reference frames. Risk communication, essentially involves this process of negotiating shared meanings (though not necessarily shared agreement) over time.[5]

Third, the convergence model supports an approach to policy that recognizes the limits of analysis as the basis for consensual social action. Habermas (26) has been a leading contributor to the argument that stakeholders use different forms of rationality in evaluating proposed social actions. Forms of rationality include subjective and social dimensions in addition to the objective, analytic dimension provided by technical risk assessment. Issues such as trust and legitimacy of the decision process, which have been frequently discussed in the literature, are key components of the subjective and social forms of rationality, respectively. The value of the convergence model is that the emphasis on establishing dialogue over a long period of time explicitly provides for the development of consensus based on all forms of rationality.

CONCLUSION

The convergence model provides a conceptual foundation consistent both with theory and with the trend toward collaborative problem solving that is evident in the field. A key feature that demonstrates this consistency is the emphasis on communication as a transactional exchange between two or more individuals that involves developing common meanings and implies building a relationship. Communicating with rather than to the public is more likely to meet public expectations in the 1990s.

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FOOTNOTES

1. The terms *public* and *stakeholder* are used interchangeably throughout to include the range of individuals and groups that are interested in or potentially affected by a policy or project (e.g., regulators and other federal, state, tribal, or local agencies; civic groups; national and regional interest groups; and the general public).

2. These distinctions, endorsed by the U.S. Environmental Protection Agency (EPA) in 1984 (*Risk Assessment and Management: Framework for Decision Making),* were relied on to develop risk assessment guidelines (51 *Federal Register,* 33992-34054, September 24, 1986). The most recent statement confirming EPA's position is the Habicht memo, *Guidance on Risk Characterization for Risk Managers and Risk Assessors,* February 26, 1992.

3. The committee, which was established by EPA, is composed of 40 representatives of federal, tribal, and state governments and associations, and local and national environmental, community, and labor organizations. The goal of the committee is to develop policy recommendations aimed at improving the decision-making process to ensure that cleanup decisions reflect the priorities and concerns of all stakeholders.

4. As noted by Fischhoff, Slovic, and Lichtenstein: "Although there are actual risks, nobody knows what they are. All that anyone does know about risks can be classified as perceptions" (22).

5. Differences in frames of reference for structuring inquiry, assessing knowledge, and constructing meanings can be linked more broadly to cultural theory, as discusssed by Rayner (27). Rayner, who adopts a social accountability view of cultural theory that conceptualizes culture as "the framework by which we justify our actions to others and call them to account to us for theirs," has identified three ideal types of organizational cultures. These cultures – market, hierarchical, and egalitarian/collectivist – frame the way in which decisions concerning technology and the environment are made.

REFERENCES

1. W.J. Ruckelshaus, "Overview of the Problem. Communicating about Risk," in J. C. Davies, V.T. Covello, and F.W. Allen (eds.), *Risk Communication: Proceedings of the National Conference on Risk Communication*, held in Washington, DC, January 1986 (Conservation Foundation, Washington, DC, 1986), p. 5.

2. J.L. Creighton, Welcoming Speech, *International Association of Public Participation Practitioners Conference,* held in Portland, OR (September 1992).

3. Draft Final Report, *Secretary of Energy Advisory Board on Radioactive Waste Management,* (U.S. Department of Energy, Washington, DC, 1992).

4. D. Beck, "The Changing Nature of DoE's Relationship with the Public," (U.S. Department of Energy, Washington, DC, 1993), p. 1.

5. D. J. Fiorino, "Environmental Risk and Democratic Process: A Critical Review," *Columbia J. Environ. Law* **14**, 501-547 (1989), p. 503.

6. J. R. Ravetz, "Usable Knowledge, Usable Ignorance: Incomplete Science with Policy Implications," *Knowledge* **9(1)**, 87-116 (1987).

7. M. Douglas and A. Wildavsky, *Risk and Culture* (University of California Press, Berkeley, CA, 1982).

8. S. Rayner, "Risk and Relativism in Science for Policy," in B.B. Johnson and V.T. Covello (eds.), *The Social and Cultural Construction of Risk: Essays on Risk Selection and Perception* (Reidel Publishing Company, Boston, MA, 1987).

9. S. Krimsky and A. Plough, *Environmental Hazards: Communicating Risks as a Social Process* (Auburn House, Dover, MA, 1988).

10. S. Jasanoff, "Cultural Aspects of Risk Assessment in Britain and the United States," in B.B. Johnson and V.T. Covello (eds.), *The Social and Cultural Construction of Risk: Essays on Risk Selection and Perception* (Reidel Publishing Company, Boston, MA, 1987).

11. H.J. Otway and K. Thomas, "Reflections on Risk Perception and Policy," *Risk Anal.* **2**, 69-82 (1982).

12. J.A. Bradbury, "The Policy Implications of Differing Concepts of Risk," *Sci. Technol. Human Values* **14(4)**, 380-399 (1989).

13. Center for Technology, Policy and Industrial Development, *Monitoring the Community for Exposure and Disease: Scientific, Legal, and Ethical Implications* (Massachusetts Institute of Technology, Cambridge, MA, 1991).

14. E.M. Rogers and D.L. Kinkaid, *Communication Networks* (Free Press, New York, NY, 1981), pp. 33 and 65.

15. E.M. Rogers, *Communication Technology* (Free Press, New York, NY, 1986), p. 197.

16. O. Renn, "Risk Communication: Toward a Rational Discourse with the Public," *J. Haz. Mater.* **20**, 465-519 (1992), p. 467.

17. V.T. Covello, D. von Winterfeldt, and P. Slovic, "Communicating Scientific Information about Health and Environmental Risks," in J. C. Davies, V. T. Covello, and F.W. Allen (eds.), *Risk Communication: Proceedings of the National Conference on Risk Communication*, held in Washington, DC, January 1986 (Conservation Foundation, Washington, DC, 1987).

18. Renn, loc. cit.

19. National Research Council, Committee on Risk Perception and Communication, *Improving Risk Communication* (National Academy Press, Washington, DC, 1989), pp.20-21.

20. E.M. Rogers and R. Agawarla-Rogers, *Communication in Organizations* (Free Press, New York, NY, 1976), p. 18.

21. J.E. Beck and S.A. Davidson, "Empowerment Through Public Involvement Functional Interactive Planning," (Pacific Northwest Laboratories, Richland, WA, 1993).

22. B. Fischoff, S. Lichtenstein, and P. Slovic, "The Public vs. the 'Experts," in V. T. Covello, W.G. Flamm, J.V. Rodricks, and R.G Tardiff (eds.), *The Analysis of Actual vs. Perceived Risks* (Plenum Press, New York, NY, 1983), pp. 236-237.

23. Bradbury, op. cit.

24. Ravetz, op. cit.

25. B. Holzner and E. Fisher, "Knowledge in Use," *Knowledge* **1(2)**, 219-43 (1979), p. 231.

26. J. Habermas, *The Theory of Communicative Action, Vol. 1: Reason and the Rationalization of Society* (translated by Thomas McCarthy), (Beacon Press, Boston, MA, 1981).

27. S. Rayner, "A Cultural Perspective on the Structure and Implementation of Global Environmental Agreements," *Evaluation and Review* **15(1)**, 75-102 (1991), p. 84.

Why Rules for Risk Communication Are Not Enough: A Problem-Solving Approach to Risk Communication

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ABSTRACT

Current work on risk communication offers practitioners helpful guidelines and rules such as "speak clearly" and "speak with compassion." Although important, these guidelines have limitations. They offer practitioners and scholars little aid in determining why a communication encounter failed. Also, they do not help practitioners anticipate and overcome likely difficulties in future risk situations, nor can they help locate information about how to reduce these difficulties.

To overcome the limitations of rule-based approaches to risk communication, this paper describes a diagnostic or problem-solving approach. This approach maintains that instead of rules, people need bases for anticipating likely obstacles to effective communication and selecting approaches that reduce these difficulties. Research on building trust, increasing awareness, deepening comprehension, gaining agreement, and motivating action is available in fields such as communication, educational psychology, science education, marketing, counseling, negotiation, and disaster response. This paper describes a framework that assists scholars and practitioners in (a) identifying communication goals, (b) determining principal obstacles to those goals, and (c) selecting research-based methods for overcoming or minimizing these difficulties and achieving communication objectives.

INTRODUCTION

The date is June 15, 1990. You live in a home bordering Wright-Patterson Air Force Base, and you are reading the Dayton *Daily News*. Seeing a story headlined "Toxic Waste in Well Water Near Base," you read, "Dayton's environmental officials have found extremely high levels of a toxic solvent in a groundwater monitoring well drilled at the Wright-Patterson Air Force Base property line." The story continues:

> "Trichloroethylene [TCE] concentrations of 876 parts per billion were found in one of five monitoring wells drilled by the city in an area just west of the base." According

to Douglas "Dusty" Hall, Dayton's environmental protection manager, "Those are the highest numbers of TCE levels in this region that I have ever seen."

Asked whether the "high TCE contamination found in the city's monitoring well was caused by the base," a base spokesperson is quoted as saying:

"It's not important. We're investigating and whatever we find, we are going to fix it up" (1).

Although the spokesperson's comment may have been sincere, it does not address the understandable concerns of those who are wondering whether they or their families have been unknowingly exposed to unsafe water, or the concerns of property owners worried that safe or not, their homes may now be difficult to sell. Obviously, this spokesperson's comment does not inspire confidence. And yet, responding to a reporter's question about this TCE situation is not easy. The spokesperson may have felt reluctant to express concern, worrying that concern may imply fault. Further, he may not have been fully aware of the TCE situation himself. Or, he may have been as aware of it as anyone else but uncertain about what the data on TCE and health effects meant.

This story illustrates some of the many complexities associated with risk communication, the process of communicating about physical hazards. Because risk communication is a challenge to nearly everyone's communication skills, practioners need a framework for thinking about how best to respond in such situations. More specifically, risk communicators need an understanding of the reasons why risk communication situations are so challenging, a framework for identifying the particular tensions likely to characterize a risk communication situation, and a repertoire of research-supported approaches for acknowledging and, ideally, overcoming these tensions.

This paper offers risk communicators a sketch of these conceptual tools. To enhance understanding of the difficulties facing risk communicators, it first describes two common, intuitive responses to risk situations. Second, it discusses the strengths and limitations of these responses. Particular attention is focused on showing why these two common responses have led to the development of "rule-based" approaches to risk communication. The paper then shows that although these rule-based approaches are well intended, they are often inadequate. Instead, communicators need a diagnostic framework for identifying clusters of tensions that characterize risk communication situations and tested methods for overcoming these communication obstacles. This diagnostic framework is sketched in the

paper and its use illustrated by examining two communication goals in detail: building trust and explaining complex material. In brief, the paper's organization is:

1. INTRODUCTION
2. INADEQUACIES IN TWO COMMON RESPONSES TO RISK
3. THE PROBLEM-SOLVING (PS) APPROACH TO RISK COMMUNICATION
4. USING THE PS FRAMEWORK TO BUILD TRUST
5. USING THE PS FRAMEWORK TO EXPLAIN COMPLEX MATERIAL
6. CONCLUSION

INADEQUACIES IN TWO COMMON RESPONSES TO RISK

Scholars have described two common responses to risk situations. Fiorino calls these responses the "technical" and the "democratic" (2). On learning about some physical hazard, such as high levels of TCE, technicals tend to focus on the physical hazard itself. They want more information about its nature, the methods used to assess its amount, potential consequences, and so forth. They are interested in the instrumentation used to locate and analyze the hazard and the accuracy of the hazard estimate. In general, "technicals" tend to be people who have had considerable scientific and technical training. The technical response to risk situations is also common when people view themselves as responsible for the existence of some potentially problematic and humanly produced hazard. That is, the officials at a plant accused of emitting large amounts of potential carcinogens into the air and water are apt to react as technicals and want as much information as possible about the physical hazards this situation may or may not pose. They are also apt to focus on ways of defending their actions, and perhaps tend to minimize the severity of the risk.

In contrast, an equally common and understandable reaction to risk situations is that of the "democratic." Fiorino calls this response the democratic because it functions as an alternative to the potentially paternalistic tendencies of the technical orientation. Instead of focusing on whether a physical hazard is severe or not, democratics focus on the people affected by the risk and the extent to which they participated in the decision to have this risk imposed on them. In addition, democratics wonder whether the "imposers" have reaped the benefits of the risky substance's use, and those imposed upon have principally reaped its harms. In the TCE situation, a homeowner living near the base might understandably react as a democratic after reading the *Daily News* story. Such individuals may wonder how long responsible individuals have known about the TCE problem and to what extent these responsible

parties have delayed in alerting them to the existence of this problem. They may at first be worried and frightened. Later, they may become angry, believing they were lied to and treated unjustly. In general, democratics tend to be people whose backgrounds in science and technology are less extensive than those of technicals, though this is not always the case. Often, simply feeling that a humanly produced risk has been imposed upon one's self or one's family is the context in which people evince a democratic reaction. If an official asserts that the hazard is minimal, such assertions may be met with deep suspicion by democratics.

Strengths and Limitations to Both Responses

Strengths. The strength of the technical response to risk communication situations is its focus on careful inquiry and respect for scientifically derived knowledge. Technicals respect the scientific method and are aware of the enormous strides society has made in conquering infectious disease and overcoming sanitation problems because of advances in microbiology, sophisticated instrumentation, and statistical analysis.

The strength of the democratic approach is its appreciation for issues of justice. Democratics are keenly aware that risk situations are rarely matters of scientific analysis alone. Instead, they require considerable judgment, value analysis, and participation by all affected parties in decision-making processes (3).

Limitations. First, both responses tend to delegitimate or denigrate the merit of the other's concern. The technicals' focus on determining the nature of the hazard makes issues of justice moot: if we do not know how hazardous a situation is, technicals reason, we cannot know whether it is just or unjust. Conversely, the democratic perspective makes the technicals' very reasonable interests seem trivial: the exact nature of the hazard is often viewed as irrelevant by democratics because focus is given to the fact that the rights of unsuspecting parties have been violated, either in a small or a big way.

A second inadequacy in both the technical and democratic perspectives lies with their implications for risk communication. Technicals tend to assume that risk communication is exclusively an educational process, a process of instructing the ignorant. If the less scientifically trained individual understood the TCE situation as fully as the more technically trained individual did, technicals reason, then this fuller understanding would lead to agreement and acquiescence. Similarly, democratics have different but

equally inadequate prescriptions for risk communication. Democratics believe that the principal obstacles to effective risk communication are unequal power relations. If only citizens were granted as much power and credibility as experts, democratics reason, then communication about risk could proceed effectively.

A third limitation lies with the approaches each suggest to risk communication. Both offer sets of rules for communicating. Because technicals think risk communication is principally a matter of education, they offer rules for risk communication such as "speak clearly" and "avoid jargon." Democratics also offer rules. Their rules include "accept the public as a legitimate partner" in risk decision making and "listen to the public's specific concerns" (4). Democratics' rules are premised on the assumption that if only all affected parties in risk situations were granted equal chances for input and respect, the resulting decisions and communication processes would have the best possible chance of being fair to all.

These rules describe ideal behaviors and states toward which risk communicators should strive. However, as tools for communication, rules have limitations. First, they offer practitioners and scholars little aid in determining why a communication encounter failed. Second, they do not help practitioners anticipate and overcome likely difficulties in future risk communication, nor can they help locate research in varied fields that are useful in reducing communication difficulties.

THE PROBLEM-SOLVING APPROACH TO RISK COMMUNICATION

To overcome the limitations of rule-based approaches to risk communication, this paper offers a diagnostic or problem-solving approach. This approach assumes that risk communication situations are like all communication situations except in one respect. Risk communication situations involve threatening and often poorly understood physical hazards (e.g., toxic substances, unfamiliar technologies, environmental pollutants); consequently, they are steeped in suspicion, lack of awareness, misunderstanding, disagreement, and apathy. To communicate effectively in such situations, one cannot follow rules alone. Instead, people need bases for anticipating likely obstacles to effective communication and selecting approaches that reduce these difficulties.

There are at least five general obstacles and goals risk communicators must understand. First, because risk communication situations are frequently riddled with suspicion, participants need strategies

for diagnosing this problem and creating trust. Second, because risk communication situations are characterized by lack of awareness (about whether some substance is cancer-causing or not, or the difference between tornado watches and warnings, or what evacuation route to take in an emergency), there is a need for awareness creation strategies. Third, because risk communication situations involve difficult ideas (such as what irradiation is, or how nuclear generators work, or why water containing a known carcinogen may still be safe to drink), practitioners need strategies for determining why these ideas are difficult and methods of overcoming these difficulties to create understanding. Fourth, because risk communication situations involve frequent disagreements among the well informed, there is a need for skills in gaining agreement. And fifth, because risk communicators must sometimes stimulate action, in emergencies or in efforts to improve health and safety habits, risk communicators need strategies for motivating action.

The problem-solving approach integrates the technical and democratic approaches by acknowledging the importance the technical framework places on informing and persuading and the importance of full and fair participation in risk communication as stressed by the democratic perspective. In addition, it offers a specific and systematic conceptual system for transcending the confines of these two approaches. The problem-solving approach offers no "magic words," but it does provide a framework for communicative problem-solving.

In brief, this approach to risk communication says that to be effective, risk communication requires knowledge, fair processes, and communication skills. It focuses on enhancing practitioners' communication skills by providing conceptual tools for analyzing and responding in risk communication situations. These tools are:

- Specifying goals for risk communication
- Identifying obstacles to each goal
- Presenting research-supported methods for addressing each obstacle.

First, risk communication situations typically involve pursuing one of five goals. To keep these goals easily in mind, the mnemonic CAUSE can be used. That is, risk communication involves building trust or establishing **C**redibility; creating **A**wareness; deepening **U**nderstanding of difficult material; gaining agreement on **S**olutions to complex problems, and moving from agreement on solutions to their **E**nactment:

Build trust:	**C**redibility
Create awareness	**A**wareness
Deepen understanding	**U**nderstanding
Gain agreement on solutions	**S**olutions
Motivate action	**E**nactment

Communication theorists have maintained for centuries that these goals are generally best pursued in this order (5). That is, usually people are uninterested in understanding a subject more deeply if they do not trust those individuals who wish to explain it or believe that a partnership of trust and mutual respect has not been established. Similarly, it makes little sense to urge action if there is not first agreement on the nature of a situation and the desirability of a given action. Thus, individuals need to determine first if communication in a given situation has faltered principally because of suspicion, lack of awareness, and so forth. Communication goal selection should be determined by an analysis of which obstacles to communication in a given case are the greatest.

Once a communicator has determined that, for example, lack of trust is the principal obstacle and trust-building or credibility management must be pursued, the problem-solving framework provides sets of heuristics for determining more precisely the ways in which that goal might best be achieved. These analyses of principal obstacles to enhancing credibility, awareness, understanding, agreement, and action are developed by reviewing research in a wide range of fields including communication, rhetoric, social psychology, instructional design, educational psychology, science education, counseling, negotiation, marketing, and disaster response. Knowing about the more frequent obstacles to these communication goals provides communicators with inquiry or diagnostic tools. Instead of applying rules for risk communication unreflectively, communicators using this problem-solving framework know (a) what goal they have selected and why; (b) what obstacles are likely, given this goal, and (c) what research-supported options are available for addressing that goal.

To illustrate the problem-solving framework, I present discussions of obstacles and options associated with the pursuit of two goals: building one's own credibility and deepening others' understanding of complex material. In another work, I discuss obstacles to the other three goals (enhancing awareness, gaining agreement, and moving people from agreement to action), and methods of addressing these obstacles (6).

USING THE PS FRAMEWORK TO BUILD TRUST

Risk communicators confront suspicion frequently. In fact, government and industry officials sometimes seek advice on risk communication because they have just been shouted down at a public meeting or chastised by angry citizens. One response to such situations is to see them as a sort of warfare between members of the public and governmental or corporate representatives. This response is dangerous. It implies that the public comprises irrational groups to be subdued and overcome instead of people to work with in partnerships. As Peter Sandman has said, to earn trust, one first has to grant it (7).

We chiefly build reputations for trustworthiness by making our actions consistent with our words and by showing, over time, respect for the goals and perspectives of others. No words by themselves build trust. However, there are better and worse responses to difficult questions, responses that can provide a foundation for trust-building. Research shows that the credibility of a speaker is threatened when an audience suspects that person's motives, competence, or willingness to act in their best interests (8-10). Risk communicators need to consider which of these obstacles is predominant in a given situation and focus on addressing it first. The following are responses to three principal obstacles in establishing trust. They are effective, in the long run, only if matched by trust-building, responsible actions.

In fact, there are two rules of risk communication:

First and Last Rule: Be sincere. People approach risk situations already irritated, frustrated, and alert to any hint of deception. If they sense they are being misled, patronized, or put off, your cause is lost. The only way to appear sincere is to be so. In addition to being sincere, though, communicators can analyze risk situations, determining if suspicions about motives, competence, or willingness are the principal problems.

Suspicion of Motives

Option A: Show understanding of and respect for an audience's concerns. Suspicion about motives can be minimized to some extent if risk communicators simply acknowledge the existence and understandability of one another's concerns. People do not like having their feelings ignored, trivialized, or dismissed without explanation. People's perceptions of a situation may or may not be accurate, but the fact that they are upset, worried, concerned, or angry can be acknowledged and respected. If a plant

official is asked whether emitted chemicals could cause harm to children playing near the plant, that official might show understanding of and respect for these concerns by saying:

> "Your question is an important one. I will tell you what I know about the health effects of benzene. I can also give you information on how to reduce your children's probable exposure to benzene, and steps you can take to gather additional information on benzene exposure from public health officers, environmental scientists, and physicians."

In situations of this sort, it is often appropriate to acknowledge that nearly anyone is interested in avoiding physical harm and that currently available information gives cause for concern. Acknowledging the understandability of concern and providing health effects information are two steps that assist affected parties in taking steps to protect themselves and their families. These steps do not also have to imply fault or wrongdoing.

Option B: Offer to work toward mutually satisfactory solutions, rather than impose a preformulated one. Insisting on a preformulated solution, regardless of its merit, implies that an individual is not truly concerned with listeners' problems and increases feelings of suspicion. Risk assessors sometimes face hostility if they wait for lengthy periods of time before releasing their recommendations to the public and then insist that the recommendations are a thorough response to the situation at hand. People who were not party to the decision-making process become suspicious of any solution in which they played little or no role. As Bradbury and Cottrill have argued, one needs to involve the public as early as possible in decision making about local risks (11, 12). In addition, when asked about risk situations, communicators can respond in ways that show interest in working toward mutually agreed upon solutions. For instance, if someone complains about strong odors coming from a nearby manufacturing facility, a spokesperson interested in showing a desire to work toward a mutually satisfactory solution might say:

> "The odors from the plant ARE strong sometimes. We work at the plant, and we are interested in minimizing our exposure to emissions. You would help us if you would report times when you find these odors objectionable. The more information we have the better we can address this problem. Also, you may want to attend meetings of the Local Emergency Planning Committee. Our plant officials belong to this group, which includes citizens, officials from other area plants, and local government representatives.

This group meets to ensure that all manufacturing facilities in this area handle emissions responsibly."

Option C: Call for a fair hearing, just as you have given your audience. If you have a record of listening to others' concerns and responding to them fairly, you earn a right to request a fair hearing when accusations are made against you. In essence, you can gently show that the suspicion directed toward you may be unwarranted. For example, if concerns have been raised about the amount of sulfur dioxide a plant has been emitting but plant officials have a good record of cooperating with governmental requests for emission controls, the plant officials can more legitimately ask to be treated fairly, just as they have treated others. So, they might respond by saying:

"You say that the sulfur dioxide levels are too high. That's what EPA officials have said, based on estimates of our emissions, rather than actual measurements. We think it is important to get accurate information about the amount of sulfur dioxide actually being emitted before millions of dollars are spent solving a problem that may not be as severe as has been estimated. We will fix the problem if there is one, but first, it seems fair that those who say the problem exists support their claims."

Option D: Offer complete messages. Discuss both benefits and harms of substances you are asked about. Many risk communication publications note the importance of discussing both the benefits and harms of a potentially hazardous substance or policy. For example, physicians who oppose mandatory testing of health-care workers for AIDS may seem overly defensive if they discuss only the potential harms associated with this policy option – and then only the harms to themselves. Being willing to discuss both potential benefits and harms for health-care workers and the public may enhance the average person's willingness to think carefully about this issue and minimize suspicions about your motives.

Suspicion about Competence

Suspicion about the competence of a speaker concerns the relevance of that individual's experience and training to a new assignment. Once again, it is easiest to establish oneself as credible through actions, in this case by performing the task in question reliably over a period of time. However,

there are also messages one can send to enhance perceptions of competence. Clark discusses some of these messages (13).

Option A: Describe your personal successes and relevant background in solving similar problems in the past. Parents who move to a new city and find the playground equipment dangerous may be viewed as competent to address this problem if they discuss their past successes in improving playground safety at their old neighborhood. Similarly, a military officer who has detonated bombs at Aberdeen Proving Grounds in Maryland should describe his past experiences doing so, if people wonder whether he will handle a current situation effectively. Information about relevant personal experience and expertise supports the impression that these individuals have the competence necessary to address these problems.

Option B: Explain how judgments were reached. Clark notes that we tend to find experts more credible if they describe not only the conclusions they have reached, but also the reasoning processes that led to these conclusions (13). If your group has debated long and hard before deciding that building an incinerator is the best of several plans for dealing with increased municipal waste, your decision is more apt to be viewed as competent if it is compared with the other options considered and the advantages and disadvantages of each option discussed.

Option C: Indicate knowledge of and appreciation for local expertise. Farmers have more opportunities to experience an insecticide's effects on human skin than scientists do: scientists study the substance; farmers live with it. Consequently, before giving precautionary advice, a scientist commenting on adverse effects of insecticides might acknowledge the existence of this important set of local information and indicate interest in learning as much as possible about it from area farmers.

Suspicion about Willingness

Option A: Provide names and phone numbers to call, so concerned citizens can monitor progress in resolving some problem. A risk communicator may be seen as trustworthy and competent but not sufficiently motivated to solve a problem as quickly as affected parties might like. To enhance the perception that one is willing to help others, invite them to call or write you to monitor your progress.

Option B: Describe ways you personally benefit from serving your audience's best interests. Safety officers sometimes earn awards or salary bonuses for actions that enhance employee and public safety. If you will benefit in multiple ways from actions you take to investigate safety matters, say so. You might also discuss your personal pride in being able to overcome safety problems that affect many people.

Option C: Locate power in entity larger than oneself. Another way of showing willingness to address problems is to note that you are required by law to do so. Federal law currently requires that states develop plans for monitoring lead in school drinking water supplies. School principals interested in showing parents that they are taking steps to monitor lead levels might note that they see such activity as part of their job and that, in addition, they are required to view matters in this manner by federal law.

Option D: Speak with confidence in your position. If you think the water is safe to drink, say so. Make sure your posture, manner, and vocal tone look and sound confident as well. Your behavior can enhance or undermine perceptions of your credibility.

One might reasonably object to any of these sample messages, saying they sound simplistic or could be viewed as rule-like themselves. But the problem-solving approach to risk situations is not a new list of messages or guidelines. The framework rests in the diagnostic process. Communicators need to ask themselves why trust (or any of the other four communication goals such as awareness of new information or understanding of complex material) is lacking. They then can consider the traditional sources of trust breakdown (doubts about another's motives, competence, or willingness) and select communication approaches designed to minimize these difficulties. By making themselves aware of their own diagnostic processes, risk communicators also can do a more effective job of evaluating the effectiveness of their approaches.

USING THE PS FRAMEWORK TO EXPLAIN COMPLEX MATERIAL

Because so many physical risks are difficult to understand, risk communicators also need effective approaches to explaining difficult ideas. Currently, most textbooks on technical speaking and writing provide textual forms such as analogies, comparison and contrast schemes, diagrams, examples, and the like. This focus on form ignores the crucial step of diagnosis. Communicators need to determine why an idea is difficult to understand before they can design an effective explanation of it.

Prior to offering explanations, risk communicators should recall that people do not learn complex material unless they are ready and interested in doing so (remember the CAUSE mnemonic, where Understanding can occur only after Credibility and Awareness are established). Patients awaiting surgery are sometimes given the option of watching the procedure, conducted on some other patient, on video; some patients choose to watch, others do not. The former feel a desire for graphic information; the latter do not. Similarly, people are more apt to learn complex information about environmental hazards when they themselves request explanations. Lectures forced on people or offered "for their own good" may not be understood or appreciated.

Nevertheless, good explanations of difficult material are often useful. To produce them, risk communicators need to know the principal reasons why technical material may be difficult to comprehend. To identify these sources of difficulty, I reviewed research in instructional design, educational psychology, reading, document design, and science education (14, 15). Three obstacles were identified. Specifically, comprehension usually falters over hard words (e.g., the distinction between specificity versus sensitivity in testing; the meaning of teratogenesis), hard to envision structures or processes (classification principles for viruses, the process of photosynthesis, tumor development), and ideas that are hard to understand because they are hard to believe (e.g., that there are much larger quantities of "natural carcinogens" in wholesome foods than there are humanly produced carcinogenic pesticide residues). Risk communicators can enhance comprehension of difficult ideas if they first determine the main reason why an idea is hard to understand and then address that difficulty by selecting research-based approaches for overcoming that particular difficulty.

Hard Words and Concepts

Research in instructional design shows that when people struggle to understand the meaning of a term or concept, they are in fact struggling to distinguish the term's critical (always present) features from its variable (frequently present but necessary) features. In several decades of research, these strategies have been found effective in helping people master the meaning of difficult terms and concepts (16).

Option A: Substitute a more easily understood term if doing so will not mislead. Journalists writing about "false positives" in cancer detection tests avoid confusion by referring to them not as false positives but as "false alarms." Similarly, Fischhoff calls false negatives "misses" (17).

Option B: If the difficult term is really the best choice, use it and then define it by its *critical* (always present) attributes rather than its *variable* (frequently associated with the term but not crucial to its meaning) attributes. Radiation is frequently associated with harm or danger, but because radiation is not always dangerous to health, "harmful" is a variable, not a critical, feature of its meaning.

Option C: Give examples and "nonexamples" of the term's use. The critical or essential features of a term become more evident to people as they consider a range of examples of the term's use and a range of nonexamples, or instances they might think are examples but are not. Learning that radiation is a broad term describing a wide array of electromagnetic energy forms allows one to understand that harm comes from radiation in some contexts but not all (radiation from the sun is harmful if one receives too much of it while sunbathing; X-ray radiation is not harmful in small doses but is in large ones). People's notions of radiation are further refined when they examine "nonexamples," instances one might think are examples of radiation but are not. Thus, if I wrongly begin to think that radiation is a synonym for any sort of energy, someone can correct me by noting that mechanically produced energy is not radiation. In this case, "mechanical energy" is a nonexample of radiation.

Hard to Envision Structures or Processes

Research in educational psychology shows that when people struggle to understand a complex structure or process (e.g., the periodic table, the human reproductive system), they can be viewed as attempting to build a mental model of such phenomena (18). They begin with crude approximate models and refine them. Educational psychologists have identified two general strategies that facilitate mental model building. They are as follows:

Option A: Provide a sense of "the big picture." Good explainers have large repertoires of strategies for helping people understand the key points, major components, or the "gist" of complex structures and processes. They use, for example, simplifying previews ("there are four principal questions in risk assessment"), diagrams, and model-generating analogies ("the Earth is like a greenhouse"). The broad claims these tactics produce often frustrate experts because of their ultimate inaccuracy. However, these broad claims are useful to the lay person. People (like scientists) come to understand complex structures and processes by building a series of mental models, each a better approximation than the last. Thus, for example, when asked to explain why cancerous tumors develop in humans, a toxicologist might begin by saying that "four of the body's defense mechanisms must fail

for a malignant tumor to develop" (19). This statement oversimplifies, but it provides an initial framework for people to use in constructing more refined understandings of malignant tumor development, especially if they initially believed a single disease, cancer, was caused by a single type of exposure to a carcinogen. The goal in explaining difficult information is not to make precise claims that only the latest research can support, but instead to anticipate likely confusions and facilitate further learning. In many cases, scientists and top science journalists do such explaining quite well. A recent article in the New York *Times* science section described the human immune system as a "defense system" against the onset of disease. This broad and familiar analogy was used throughout the article. For instance, viruses such as chicken pox and cytomegalovirus were described as human-like invaders "lurking latent in the body after an initial infection," and the puzzling HIV was described as slipping "into some types of cells and wait[ing] there quietly" (20). These images of defense systems and "lurking" viruses obviously anthropomorphize and probably oversimplify, but they also provide vivid models of highly complicated processes, models people can mull over and refine with further study.

Option B: Use text features that highlight connections among main points. In addition to providing a broad approximation of how something works or what its principal components are, good explanations of complex structures and processes use carefully chosen transitions, headings, and reviews to highlight key links and components in complex structures and processes (21). A lecture that began by saying four of the body's defense mechanisms have to fail for a cancerous tumor to form could reinforce this point by having the lecture itself be divided into four parts, one on each of these four failing mechanisms. A good lecture would also help people build their understanding of these phenomena by continually reinvoking the general model of "defense systems" failing, discussing for example, the various ways in which these mechanisms might fail (e.g., one life form being killed; another being disabled) and perhaps noting the remarkable fact that such a series of failures is relatively rare.

Hard to Understand Because Hard to Believe

In communicating about risk, you may find yourself needing to explain ideas that are difficult to understand chiefly because they are counterintuitive. For instance, you may want to help people understand how studies of the effects of huge quantities of some substance ingested by rats can tell us anything about the effects of much smaller quantities ingested by humans over decades. Or, you may want to explain the implausible idea that even when TCE levels are substantially above the legal limit, TCE-tainted water may still be safe to drink.

According to research in science education, the best way of explaining aspects of the physical world about which people have powerful lay or alternative notions is to treat these notions as one would any other respected but erroneous idea (22, 23, 24). Specifically, when an idea is difficult to understand because it is difficult to believe, explainers must follow these four steps:

Step A: State the erroneous but plausible notion.

Step B: Acknowledge its apparent plausibility.

Step C: Demonstrate its inadequacy by noting inconsistencies between it and evidence familiar to the audience but not yet considered.

Step D: Present the more accepted view and demonstrate its greater adequacy.

Research shows that science teachers often wrongly begin explanations of counterintuitive ideas by simply stating the correct notion (22). This approach does not help students recognize, consider, and reject intuitively appealing lay notions. Like teachers, risk communicators may wrongly assume that an idea is difficult to understand principally because of the terms in which it is expressed or because the structures and processes it involves are hard to envision. Instead, the larger obstacle to understanding may be a powerful, but tacit lay mental model. Just as scientists do not give up their own theories easily, neither does anyone else. In fact, because lay notions about the physical world seem to be reinforced by daily experience, they are very difficult to give up. However, it is possible to address these obdurate notions and sometimes overcome them, using Steps A through D above.

For instance, assume that the base spokesperson in the TCE story, presented at this paper's beginning, has established his credibility and a relationship of trust with area residents. Assume further that these residents now have requested an explanation of whether or not their drinking water is dangerous because of the high levels of TCE found near their homes. Assuming the spokesperson knew that his audience had a "lay theory" about this situation of the sort "if the levels of TCE are higher than what EPA says they should be, then this is a dangerous situation," he might respond this way:

> [*Step A: State the lay notion*]. The amount of TCE found in a monitoring well near the base is certainly high: 876 parts per billion. I share your concern and interest in this problem. Here is what I know about it.

[*Step B: Acknowledge the plausibility of the lay view, in this case, the reasons for concern about TCE*]. TCE or trichloroethylene is an industrial solvent used for cleaning. It is used to clean machinery and aircraft parts. Certain amounts of TCE have been found to cause cancer in laboratory animals, and so EPA wants to be very cautious about having this substance in or near our drinking water supply. However, scientists do not know precisely how or whether this chemical affects human health. To be on the safe side, EPA officials have set the maximum allowable amount for TCE at a very low level, that of 5 parts per billion.

[*Step C: Demonstrate the inadequacy of the lay view.*] The water you are drinking comes from Rohrers Island production wells, NOT from the monitoring wells where the high levels of TCE were found. Your drinking water contains TCE but in amounts lower than 5 parts per billion, the standard set by EPA.

[*Step D: State the alternative view and explain its greater adequacy.*] This very small amount of TCE is quite different from what was found in the monitoring well near the base. So, this finding of extremely small amounts of TCE in your drinking water suggests to me that your water is safe to drink. Because I live nearby, I drink it myself, as does my family. As far as our best measuring efforts show, our drinking water has not been affected by the high TCE levels found in the monitoring wells.

The bottom line for you is that your water is safe to drink, but you will want to see this TCE situation solved. The bottom line for us is that we need to be even more vigilant than we have already been in determining the nature of any TCE problem in this area. I am one of the people charged with making progress in this regard. Here is my phone number. You should call me if you have any additional questions or if you wish to see how we are doing in terms of getting this TCE situation resolved.

Explaining why high levels of TCE in a monitoring well are not necessarily a cause for alarm is difficult: the health effects of TCE are not yet understood. Given the facts in this case, however, it seems reasonable to discuss both the reasons why there should be some concern and why, in this case, the drinking water is probably safe. This kind of explanation should be accompanied by efforts to provide

listeners with some control over this situation (in this case, a phone number to call) because this is their right and because, in the long run, we are all safer from such risks if their existence and resolution are everyone's concern.

I offer this sample message not as a set of "magic words" for this kind of situation, but rather as a text to be analyzed for its strengths and limitations. It may or may not be an ideal response to this situation. Again, explaining complex material is most appropriate only when mutual trust is well established and listeners are genuinely interested in deepening their comprehension of something confusing.

CONCLUSION

The problem-solving approach to risk communication has several advantages over rules-based approaches. First, it recognizes the range of problems characterizing risk situations. They rarely call solely for educating the uneducated or solely for encouraging full and fair participation by all stakeholders, as the technical and democratic responses suggest. Instead, this framework says risk situations are characterized by breakdowns in **C**redibility, **A**wareness, **U**nderstanding, agreement about **S**olutions, and **E**nactment of effective response (the CAUSE mnemonic). Second, the problem-solving approach locates key obstacles to these five communication goals and offers message-generation principles likely to overcome identified difficulties. These principles are supported by decades of research in fields such as rhetoric, communication, educational psychology, instructional design, and science education (14-16; 18, 21-24). Finally, the framework integrates research from diverse fields. It does so by focusing on ways similar risk communication difficulties can be reduced with analytic communication skills.

In the long run, risk communication, like risk management, will be most effective if it is viewed as every affected party's responsibility. Effective communication skills are one essential element of good risk communication, but they must be accompanied by knowledge and a commitment to justice. The more we can recognize the understandability of people's concerns and the power their concerns provide in solving challenging problems, the more we can work together to create rational and fair procedures for the management of hazardous substances and situations.

REFERENCES

1. J. Dougherty, "Toxic Waste in Well Water Near Base," (Dayton, OH) *Daily News* (June 15, 1990).

2. D.J. Fiorino, "Technical and Democratic Values in Risk Analysis," *Risk Anal.* **9**, 293-299 (1989).

3. National Research Council, *Improving Risk Communication* (National Academy Press, Washington, DC, 1989).

4. V.T. Covello and F.W. Allen, *"Seven Cardinal Rules of Risk Communication,"* (Office of Policy Analysis, U.S. Environmental Protection Agency, Washington, DC, 1988).

5. G. Campbell, *The Philosophy of Rhetoric*, Book I, Chapter IV (1776). Reprinted in J.L. Golden and E.P.J. Corbett (eds.), *The Rhetoric of Blair, Campbell, and Whately* (Holt, Rinehart and Winston, NY, 1968).

6. K.E. Rowan, "Goals, Obstacles, and Strategies in Risk Communication: A Problem-solving Approach to Improving Communication About Risks," *J. Appl. Comm. Res.* **19**, 300-329, (1991).

7. J.C. Davies, V.T. Covello, and F.W. Allen, (eds.) *"Risk Communication: Proceedings of the National Conference,"* (The Conservation Foundation, Washington, DC, 1986), p. 49.

8. D.J. O'Keefe, *Persuasion: Theory and Research* (Sage, Newbury Park, CA, 1990).

9. J.K. Burgoon, "The Ideal Source: A Re-examination of Source Credibility Measurement," *Central States Speech J.* **27**, 200-206 (1976).

10. K.E. Andersen and T. Clevenger, Jr., "A Summary of Experimental Research in Ethos," *Speech Monographs* **30**, 59-78 (1963).

11. J.A. Bradbury, "The Policy Implications of Differing Concepts of Risk," *Sci. Technol. Human Values* **14**, 381-399 (1989).

12. Personal communication with C.A. Cottrill, Science Communication Analyst, Environmental Criteria and Assessment Office (U.S. Environmental Protection Agency, Cincinnati, OH, 1993).

13. R.A. Clark, *Persuasive Messages* (Harper & Row, New York, 1984), pp. 189-213.

14. K.E. Rowan, "A Contemporary Theory of Explanatory Writing," *Written Comm.* **5**, 23-56 (1988).

15. K.E. Rowan, "The Art of Explanation: Strategies for Enhancing the Comprehension of Science," in B. Lewenstein (ed.), *When Science Meets the Public* (American Association for the Advancement of Science, Washington, DC, 1992), pp. 131-143.

16. M.D. Merrill and R.D. Tennyson, *Teaching Concepts: An Instructional Design Guide* (Educational Technology Publications, Englewood Cliffs, NJ, 1977).

17. B. Fischhoff, "Risk: A Guide to Controversy," in National Research Council (eds.), *Improving Risk Communication* (National Academy Press, Washington, DC, 1989).

18. R.E. Mayer, "What Have We Learned About Increasing the Meaningfulness of Science Prose?" *Sci. Ed.* **67**, 223-237 (1983).

19. B. Rensberger, "Cancer: The New Synthesis," *Science* **84**, 5, (September, 1984), pp. 28-33.

20. G. Kolata, "Tests Show Infection by AIDS Virus Affects Greater Share of Cells, *The New York Times* (December 29, 1992).

21. R.E. Mayer, "Signalling Techniques that Increase the Understandability of Prose," *J. Ed. Psych.* **76**, 1089-1105 (1989).

22. M.G. Hewson and P.W. Hewson, "Effect of Instruction Using Students' Prior Knowledge and Conceptual Change Strategies on Science Learning," *J. Res. in Sci. Teaching* **20**, 731-743 (1983).

23. J.A. Shymansky, and W.C. Kyle, "A Summary of Research in Science Education – 1986," *Sci. Ed.* **72(3)** (1988).

24. K.E. Rowan, "When Simple Language Fails: Presenting Difficult Science to the Public," *J. Tech. Writ. Comm.* **21**, 369-382 (1991).

Tri-Service Initiatives in Risk Communication: A Training Experience

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ABSTRACT

In response to rising concerns about health and environmental risks and ever increasing requirements for public meetings, the U.S. Army Environmental Hygiene Agency (AEHA) recognized the need for a structured course in risk communication. Past practices by the Tri-Services in attempting to communicate risk to the public, other government agencies, and the media have proven to be deficient in "getting the message across" effectively without distortion. The central cause of this deficiency is due to lack of a systematic approach to communicating risk and heavy reliance on past experiences and observations.

After review of many risk communication philosophies, the AEHA contracted with Dr. Vincent Covello of Columbia University to develop and implement a program of instruction in the basics of risk communication. The first workshop was held in August 1991. In January 1992, a second presentation to management level staff of the AEHA, the Army Environmental Center, USAF Armstrong Laboratories, and the Navy Environmental Health Center resulted in the development of a Tri-Service Workplan Schedule for five presentations of the course.

For 1993, the AEHA has concentrated its risk communication efforts to reach Army NPL Site Commanders, Environmental Coordinators, and Public Affairs Officers, while continuing to provide access for Tri-Service oriented courses.

The NAS Risk Paradigm as a Medium for Communication

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ABSTRACT

Many journalists and other recipients of risk assessment information are familiar with the National Academy of Sciences risk assessment paradigm. From time to time, paradigm concepts such as "risk characterization" and the risk assessment/risk management distinction appear in news features or community group discussions on environmental issues. With knowledge of the paradigm common to scientists, journalists, and other interested parties, the paradigm is a potentially important medium for communication between risk scientists, journalists, and the public. Specifically, the paradigm offers widely accepted organizing principles for presenting risk information, a common language for addressing a variety of issues and concepts, and a flexible analytical system that accommodates the diversity of scientific information and policy perspectives that characterize the risk assessment process. In addition, the paradigm outlines important relationships and distinctions between risk assessment and risk management. Informed and creative use of these features of the paradigm can guide and simplify interviews between journalists or community groups and their scientific sources, clarify presentation of risk science information, and promote collaboration between risk scientists, journalists, and others to ensure complete, objective and fair comment on risk issues of interest to the public.

THE NAS RISK PARADIGM AS A MEDIUM FOR COMMUNICATION

The program for this conference demonstrates the important role the National Academy of Sciences (NAS) paradigm (1) has assumed over the last 10 years as a medium for communicating risk science information. Many in the risk assessment community use the paradigm as a framework for discussing principles and for developing risk assessments for some of the chemical agents reviewed in this conference. Risk assessors also use the paradigm to present their work to scientists, decision-makers, and the public. The paradigm is used broadly, providing an organizing system not only for EPA's risk assessment guidelines (2,3), but also for a wide range of professional organizations (4,5).

LISTENING TO RISK SCIENCE INFORMATION

These uses of the paradigm – development and presentation of the science of risk assessment – represent the vantage point of those who generate risk assessments. A comparably useful, but often overlooked, use of the paradigm represents the vantage point of the recipient of risk assessment: the paradigm also is a useful aid for receiving, understanding, and mastering risk science information and translating that information for others in the communication chain. In this sense, the paradigm empowers the recipient by offering a diagnostic tool for studying the science used in the risk assessment process or any particular assessment.

My theme today is that the paradigm is a useful medium for communicating risk science information from scientists to journalists and from journalists to the public, or from scientists to community groups, because the paradigm offers a straightforward system for asking and answering questions about risk science issues.

The paradigm as a "listening device" is important because of the great – and ever increasing – number and diversity of participants in risk conversations. Some of the participants are scientists who contribute relevant scientific information (e.g., practicing biologists, geologists, statisticians). Other participants are decision-makers in regulatory agencies (including EPA and other federal and state agencies), industrial organizations, the military, community organizations, and public interest groups. These recipients use the risk assessment, along with other non-scientific considerations, to make individual and institutional judgments on the use or nonuse of agents that may pose a risk to human health or the environment; they gauge their judgments and reactions – concern, confusion, outrage, indifference – in part, on the basis of the completeness, clarity, and comprehensibility of the risk science information presented to them.

The deliberately limited scope of this presentation is important. As stated at the outset, this presentation considers the NAS paradigm as a medium for communicating risk science information and analyses. Consistent with the 1983 NAS report, science here refers mainly to the physical sciences and natural sciences, with information and analyses drawn from the social sciences having important roles in the overall regulatory decision. Other systems take different approaches and often offer other advantages. However, for this conference on the NAS paradigm, this presentation focuses not on the overall

regulatory decision, but only on scientific information used for risk assessment as discussed in the 1983 report.

RISK CONCEPTS

Risk assessment is often regarded as confusing, excessively complicated, and needlessly controversial. Indeed, some observers describe it as a "black box," a place where government hides policy decisions, or a "political tool." Given the complexity, diversity, and uncertainty that characterize both the information content and the practices of risk assessment, such impressions are not surprising.

Together, all of these factors explain why misunderstanding and miscommunication are so prevalent that they act as a bar to effective risk communication. This is where the NAS paradigm enters the picture. By offering a simple but flexible system designed to accommodate all of the information and policies that are necessary to describe risk and make related regulatory decisions, the paradigm provides a medium for communication between the various participants in the risk conversation.

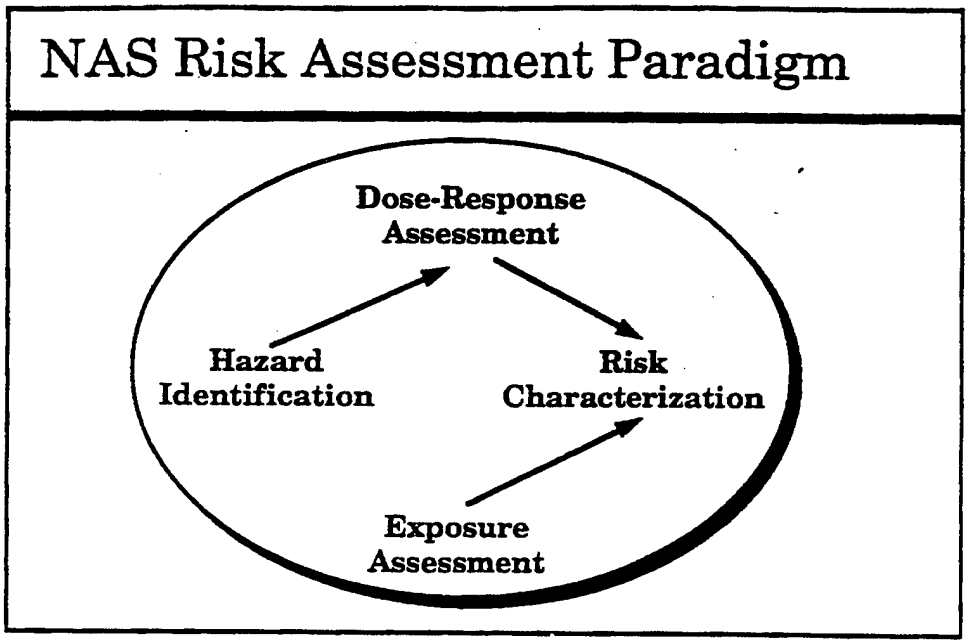
As a starting point, perhaps the most useful aspect of the NAS paradigm is its emphasis on defining terms and distinguishing among risk concepts. The paradigm is useful for distinguishing among the several diverse contexts in which risk is analyzed and discussed: risk assessment, risk management, risk communication, risk perception, risk reduction, comparative risk, and relative risk. All embrace risk concepts, all are sometimes used interchangeably, but each has a somewhat different usage.

As published in 1983, the NAS paradigm focuses on differences between the closely related concepts of risk assessment and risk management (Figure 51). Much of the miscommunication (i.e., confusion, controversy) on risk matters stems from failure to discriminate between these closely related terms or to specify the term used in any particular risk conversation. This presentation uses the term risk assessment as the NAS used the term in its 1983 report, *Risk Assessment in the Federal Government: Managing the Process*:

> ... We use risk assessment to mean the characterization of the potential adverse health effects of human exposures to environmental hazards.
>
> ... The term risk assessment is often given narrower and broader meanings than we have adopted here.

... Broader uses of the term than ours also embrace analysis of perceived risks, comparisons of risks associated with different regulatory strategies, and occasionally analysis of the economic and social implications of regulatory decisions – functions that we assign to risk management. (ibid., p.18.)

Risk Assessment in EPA



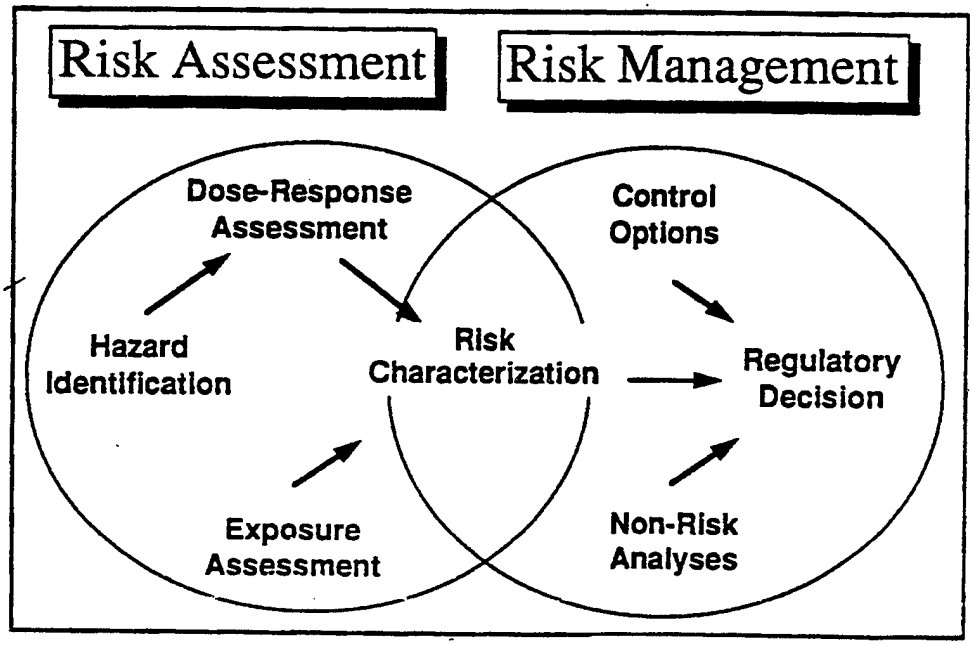


Figure 51. Risk Assessment in EPA.

In limiting this discussion to risk assessment as defined by the NAS in 1983, this presentation excludes information on cost-benefit analysis, risk reduction options, the social and economic impacts of risk reduction, technological feasibility, and public outrage. All of these factors are important and legitimate factors in some risk conversations and in all risk management activities, but not in risk assessment as defined then by the NAS. Indeed, the presence of information on these topics in a risk discussion suggests that the analysis addresses risk management matters and thus goes beyond the NAS description of a science-based risk assessment.

STRUCTURAL FRAMEWORK

The NAS paradigm offers several principles to guide comprehension and further communication of risk assessment information. The paradigm defines four fields of analysis, which the recipient should look for in each assessment:

- identification of hazard (i.e., a demonstrated relationship between an agent and an injury, ideally in human epidemiology studies, but usually in laboratory animal studies);
- relationship between dose and response in the foregoing studies;
- assessment of exposure, actual or projected, in populations that are expected to be exposed; and
- characterization of risk, a summary of qualitative and quantitative information, with special emphasis on uncertainties, limitations, alternatives, and identification of policy choices.

Informed recipients of risk information look for analyses in each of these four areas and for three general kinds of information in each analysis: (1) scientific data, (2) uncertainties and data gaps, and (3) clearly identified policy preferences regarding the use and (nonuse) of available scientific theories, principles, and methods for predicting human risk.

An example is instructive. Hazard identification may rely on several well-conducted, corroborative studies establishing an undisputed adverse health effect in laboratory animals exposed to Agent X. Despite this strong database, in the absence of related human studies, uncertainty exists about the effect of Agent X in humans. To address this uncertainty, some participants assume the data are predictive of potential human risk, while others may assume the data are not predictive for humans. Such

uncertainties and policy preferences mark each aspect of the assessment; all require highlighting and discussion as a major feature of risk characterization.

The emphasis on risk characterization, like the emphasis on the risk assessment/risk management distinction, is one of the major contributions of the paradigm toward more effective risk communication. The converse of this feature demonstrates its importance.

Incomplete and oversimplified presentations of risk information often offer a single value (e.g., "ten to the minus 6" or "30 excess cancer cases"). This provides no information on the source of the estimate, its data content and quality, uncertainties in the data, alternative analyses, or policy input. It does not reveal whether human studies, animal studies, cost considerations, opinion polls, or all of these factors lie behind the number.

Risk characterization calls for full disclosure of these factors in describing risk, including uncertainties and policy choices, along with the underlying scientific information. As explained in the NAS report, "the final expressions of risk derived in this step will be used by the regulatory decision-maker when health risks are weighed against other societal costs and benefits to determine appropriate action" (p. 36).

DIAGNOSTIC PRINCIPLES

These paradigm principles suggest several criteria for defining and identifying incomplete communication and miscommunication about risk.

- Establishing definitions. Although distinctions between risk assessment and risk management seem obvious, risk assessment and risk management are often confused, inadvertently or deliberately. This confusion precludes meaningful communication. Risk assessment (as defined by the NAS) draws primarily on the natural and physical sciences with the objective of describing risk in scientific terms, whereas risk management utilizes information on social and economic considerations, as well as scientifically defined risk information, to fashion regulatory options.

- Characterizing risk. Risk assessments should always be presented with enough information to inform users of the information content of the analysis, and related uncertainties and limitations. A bare number reveals little about the kind of data used (e.g., animal or human studies), the uncertainties in the data (e.g., whether the cancer in humans exposed to multiple chemicals is due to chemical X or chemical Y), and the relevance of animal data to human risk.

- Science policy. Incomplete scientific information is often supplemented by policy judgments about the meaning and use of the information for risk assessment. Such science policy depends on many factors, including the nature of the available data and related gaps, social and political philosophies, and peer involvement and peer review, among other things. Policy judgments should be identified as such and explained.

The authors of the NAS report introduced a concept that distinguishes not only the information content of risk assessment and risk management, but also related differences in the policy components of environmental decision-making. Referring to choices regarding uncertainties and unknowns in scientific data and methods, the authors explained that "such judgments made in risk assessment are designated risk assessment policy, that is policy related to and subservient to the scientific content of the process, in contrast with the policy invoked to guide risk management decisions, which has political, social, and economic determinants" (pg. 37). This approach helps systematize the policy components of the risk assessment and risk management processes.

If risk characterization is thorough and complete, use of the paradigm simply serves as a receiving medium for the recipient to collect and review information on the assessment. Often, however, risk characterization is incomplete. For example, an assessment may be stated only as a number (e.g., 10^{-6} additional cancer cases), without any other information. In this situation, the recipient learns about the assessment through these questions. These questions go to the core of the assessment by disclosing the scientifically defined "known" and "unknown" aspects in each of the four paradigm analyses, and the related policy choices.

SUMMARY

Effective communication on environmental risk issues requires commonly understood principles, concepts, and terminology. Without agreement among all members of the communication network – scientists, journalists, and community groups – miscommunication and confusion result. Consistent, informed use of the 1983 NAS risk assessment paradigm can assure clear, comprehensible presentation and reception of risk science information. Use of the paradigm ensures that risk management information and concerns – what will it cost, how shall we regulate – will be discussed separately from questions regarding the scientific assessment – what does science tell us about the nature and extent of injury to human health and the environment.

REFERENCES

1. National Research Council, *Risk Assessment in the Federal Government: Managing the Process* (National Academy Press, Washington, DC, 1983).

2. U.S. Environmental Protection Agency, "Guidelines for Carcinogen Risk Assessment," *Federal Register* **51**: 32992-34003, et seq. (1986).

3. U.S. Environmental Protection Agency, "Guidelines for Exposure Assessment," *Federal Register* **57**: 22888-22938 (1992).

4. American Chemical Society, *Chemical Risk: A Primer* (1984).

5. American Industrial Health Council, "Presentation of Risk Assessments of Carcinogens" (1989).

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POSTER ABSTRACTS

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IN VITRO CYTOTOXIC EFFECTS OF HYDRAZINE HYDRATE TO WB344 AND 743X CELL LINES

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Hydrazine toxicity studies recently gained importance due to the change in the Threshold Limit Values (TLV) recommended by the American Conference of Government Industrial Hygienists (ACGIH) from 0.1ppm to 0.01ppm occupational exposure. *In vitro* studies assessed detrimental levels of hydrazine. For comparison, the 743X male Chinese hamster lung cells were used to represent the respiratory tract and WB344 rat liver epithelical cells as hepatocytes. The study examined cytotoxicity in cell metabolic processes and intracellular protein synthesis by means of growth curves, colony-forming efficiencies (CFE), and the Sulfarodamine-B (SRB) assays. The growth curves were completed using 0.25mM hydrazine hydrate. Cell counts reflected that hydrazine-dosed WB344 cells double at a rate 15.6% faster than controls. The 743X-dosed cells double at a rate 26.3% slower than controls. The CFE used five incremental doses of hydrazine and reflected significantly reduced growth only at the highest dose of 0.3mM in both cell lines. The EC_{50} for intracellular protein synthesis were 0.9mM for WB344 and 1.2mM for the 743X cell lines. Membrane damage was indicated by enzyme leakage in the lactate dehydrogenase/aspartate amino transferase (LDH/AST) assay. Doses of 0.5, 1.0, and 2.0mM hydrazine were used over a 24-h exposure period. There was a dose-dependent increase in LDH leakage, but insignificant AST leakage in both cell lines. Hydrazine hydrate demonstrated a cytotoxic effect in both cell lines.

EFFECT OF DICHLOROACETIC ACID AND TRICHLOROACETIC ACID ON CELL PROLIFERATION IN LIVER AND PRECANCEROUS LESIONS OF B6C3F1 MICE

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Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are formed during chlorination of drinking water and are present in finished water. Trichloroethylene and tetrachloroethylene, common contaminates of ground water, are metabolized to DCA and TCA. Dichloroacetic acid and TCA are hepatocarcinogens in mice and appear not to be genotoxic, so that a proposed mechanism is enhanced cell proliferation in precancerous cells resulting in their clonal expansion. Dichloroacetic acid at 0.26, 0.86, or 2.6 and TCA at 0.32, 1.06, or 3.2 g/l were administered in the drinking water to female B6C3F1 mice. Five days prior to sacrifice, the mice had pumps containing 30 mg/mL bromodeoxyuridine (BrDU) implanted subcutaneously and were sacrificed at 5, 12, or 33 days after the start of exposure. Dichloroacetic acid produced a dose-related increase in the BrDu labeling index (LI) after five days of exposure, but not after 12 and 33 days. After five days of exposure, all three dose levels of TCA increased the LI greater than DCA. Similar to DCA, TCA exposure for 12 or 33 days did not affect the LI. The effect of DCA and TCA upon cell proliferation in precancerous cells is in progress to determine whether they remain sensitive to exposures longer than five days. In conclusion, DCA and TCA can induce cell proliferation in the liver of B6C3F1 mice, however, the liver is sensitive for only a limited duration. Precancerous hepatocytes could, therefore, remain sensitive to DCA and TCA for a longer period which would result in their clonal expansion (i.e., tumor promotion).

ACTIVATION OF ras ONCOGENE IN MOUSE LIVER TUMORS INDUCED BY DICHLOROACETIC ACID, TRICHLOROETHYLENE, AND TETRACHLOROETHYLENE

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[2]Environmental Health Research and Testing, Inc., Lexington, KY

Trichloroethylene and tetrachloroethylene are common contaminants of ground water. Dichloroacetic acid is a metabolite of trichloroethylene and is found in chlorinated drinking water. These three chemicals have been demonstrated to induce liver tumors in B6C3F1 mice. Liver tumors were induced in male B6C3F1 mice and analyzed for mutations in the ras protooncogene. Hepatocellular adenomas and carcinomas in dichloroacetic acid- and trichloroethylene-treated mice had the same incidence of point mutations in H-ras gene as found in untreated mice. In tetrachloroethylene-treated mice these tumors had a lower incidence of mutations in the ras gene family in spite of the occurrence of point mutations in K-ras, which were not present in "spontaneous" mouse liver tumors. In dichloroacetic acid-treated animals mutations occurred only in the 61st codon, while the two chloroethylenes also had mutations in codon 13 and 117. The mutation spectrum in the 61st codon was different for these three chemicals compared to spontaneous tumors.

In conclusion, the mechanism of dichloroacetic acid and trichloroethylene appear similar, resulting in tumors containing mutations in H-ras at the same yield as spontaneous tumors. However, the different spectrum of mutations in the H-ras gene in tumors from these two agents compared to vehicle controls suggest that they promote different precancerous hepatocytes to tumors that occur in untreated animals. Tetrachloroethylene appears to induce tumors by a different mechanism which does not involve mutations in the H-ras gene but does to a limited extent involve the K-ras gene.

ROUTE OF ADMINISTRATION DETERMINES WHETHER CHLOROFORM ENHANCES OR INHIBITS CELL PROLIFERATION IN THE LIVER OF B6C3F1 MICE

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Chloroform administered by gavage has been shown to induce liver cancer in B6C3F1 mice whereas, when administered in the drinking water, chloroform inhibited liver cancer in mice. The effect of chloroform administered by these two routes upon cell proliferation in mouse liver was determined. Female B6C3F1 mice were divided into five groups. Five days prior to sacrifice, an osmotic minipump containing 30 mg/mL bromodeoxyuridine was implanted subcutaneously. After five days of treatment, the livers from Group 1 which were treated daily with 263 mg/kg chloroform in corn oil by gavage, had a labeling index (LI) of 81.1 $\pm$ 6.2; Group 2: 1800 ppm chloroform in the drinking water, a LI of 0.61 $\pm$ 0.25; Group 3: 1800 ppm chloroform in the drinking water and daily 10 mL/kg corn oil by gavage, a LI of 0.29 $\pm$ 0.14; Group 4: 10 mL/kg corn oil daily by gavage, a LI of 17.2 $\pm$ 2.51 and Group 5: untreated controls, a LI of 9.15 $\pm$ 1.56. After 12 and 33 days of chloroform administered by gavage, the Li decreased to 61.8 and 22.8, respectively and by drinking water approached control level. Therefore, chloroform administered (1) by corn oil gavage enhanced cell proliferation and (2) in the drinking water inhibited cell proliferation, which corresponds to its enhancement or inhibition of hepatocarcinogenicity by these two routes. The co-administering of corn oil by gavage with the chloroform in the drinking water did not affect the inhibition of cell proliferation. Thus, the different routes of administering chloroform determined these contrasting effects.

DIFFERENCES IN THE TISSUE PARTITIONING OF HCFC-123 AND ITS ANALOG, HALOTHANE, IN THREE MAMMALIAN SPECIES

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Through the Montreal Protocol, governments of the United States and other nations have agreed to cut the production of compounds which deplete atmospheric ozone. The niches held by many of these chemicals must be filled with replacement compounds. The fire extinguishants have potential replacements which include hydrochlorofluorocarbon 123 (1,1,1-trifluoro-2,2-dichloroethane, HCFC-123), a structural analog of halothane, (1,1,1-trifluoro-2-chloro-2-bromoethane). Over the past 30 years, much data on the pharmacology, toxicology, metabolism, and pharmacokinetics of halothane have been derived in the human. Only recently have data on HCFC-123 begun to appear from rodent surrogates. A favorable comparison of the biologically relevant parameters of halothane and HCFC-123 will greatly assist in the assessment of risk involved with human exposures to HCFC-123. In an attempt to identify a proper surrogate species for modeling purposes and to quantitate differences in tissue partitioning between these two compounds, we have assessed differences in the tissue:air partitions of these two compounds in the rat, monkey, and human.

CRITICAL REVIEW OF RODENT LIVER TUMOR PROMOTION DATA FOR DEHP

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Di-2-ethylhexyl phthalate (DEHP), a non-genotoxic chemical, produces liver tumors at high dietary doses in both rats and mice. Tumor initiation in rodent liver has not been shown, suggesting that DEHP may have promoting effects on spontaneously initiated rodent liver cells. Indeed, DEHP was a liver tumor promoter in mice at doses which produced liver tumors.

More than 10 *in vivo* studies have looked at the liver tumor promotion potential of DEHP in rats. Although many of these studies were negative, they were less than optimal to assess for tumor promotion. More sensitive test conditions indicated that DEHP is a liver tumor promoter in rats with thresholds at <100 mg/kg/day. A weight-of-evidence evaluation of *in vitro* data (gap junctional intercellular communication and JB6 epidermal cells), *in vivo* markers (inhibition of mitogens and alterations in receptors or membrane fluidity) and structure–activity relationships indicate that DEHP is a liver tumor-promoting agent in the rat. The putative toxic form of DEHP appears to be its phthalate monoester, MEHP.

By understanding the species differences in mechanism of action for DEHP, it is concluded that cancer risk assessment using the Linearized Multi-Stage model is inappropriate for DEHP. An alternative risk assessment approach such as a NOAEL/Uncertainty Factor can be justified. This alternative approach indicates that DEHP presents a negligible cancer risk for man.

DETERMINATION OF HEPATOCELLULAR GLUTATHIONE USING MONOCHLOROBIMANE IN A MULTIWELL FLUORESCENT PLATE READER

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Monochlorobimane (MCB) is a fluorophore that emits at a wavelength of 470 nm when enzymatically bound to GSH by glutathione-*S*-transferase. Flow cytometric and biochemical analysis has indicated that MCB has a relatively high specificity for GSH with minimal reaction with protein sulfhydryls. Using a multiwell fluorescence plate reader, GSH-MCB associated fluorescence in primary rat hepatocytes and a rat hepatocyte cell line (WB-344) was accurately reflected in control, GSH depleted and chemically exposed cells. Primary hepatocytes depleted of GSH were found to be more sensitive to a C_6 halogenated fatty acid compound when compared to identically exposed normal hepatocytes. A newly developed 380 nm excitation filter with a 485 nm emission filter yielded the highest MCB fluorescence sensitivity. The results of this study indicated that MCB can be used in a multiwell fluorescence plate reader to screen the effects of chemicals on cellular GSH.

EVALUATING LOW LEVEL ACUTE TOXICITY TO HUMANS FROM AIRBORNE CHEMICALS: THE INTERPLAY OF TOXICOLOGY, AIR MODELING, AND PUBLIC HEALTH

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Risk assessment has become increasingly widely used in environmental regulatory processes. This has led to the need to develop health-based criteria for forms of toxicity that have not previously been considered. One example of this is recent attempts to evaluate low level acute toxicity. In the absence of existing dose–response values it is necessary to select an approach and develop the needed criteria.

Low-level acute toxicity refers to effects such as tearing, coughing, or headache that may occur as the result of a short (minutes to hours) exposure to a chemical. These kinds of effects are commonly involved in nuisance complaints about indoor and outdoor air quality. Several types of end points that may be identified are respiratory irritation, eye irritation, and central nervous system effects. The chemicals involved vary widely including aldehydes, acid aerosols (sulfuric acid), sulfur dioxide, ozone, hydrocarbons, and chlorinated hydrocarbons.

To evaluate such concerns, an approach to exposure assessment must be selected such as air modeling of hourly concentrations. In addition, chemical specific health-based criteria must be developed to compare to the modeled concentrations. The consistency of averaging times used in the exposure assessment and the dose-response assessment is critical.

The development of criteria involves a combination of science and policy. Scientific information helps to direct the process but inevitably there are information gaps. These gaps may be scientific and potentially resolvable. Realistically, however, there are never likely to be sufficient scientific resources to resolve all the questions for the large number of chemicals involved. Other gaps may be scientific but unethical to study in humans, such as studies with particularly sensitive populations. Finally, gaps may exist because information is unknowable, for instance where people will actually be and what they are doing in the future.

This poster reviews some of these issues and approaches to addressing them. It includes a case study of one approach to developing a low-level acute toxicity criterion for sulfuric acid.

THE EFFECT OF BODY FAT AND BODY WEIGHT FROM NAVY SUBPOPULATIONS ON DOSE METRICS USED IN RISK ASSESSMENT

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Physiological and biochemical characteristics of different populations can affect their potential lifetime cancer risks due to chemical exposure. The purpose of this study was to explore differences among United States Navy subpopulations (male underwater demolition trainees and specialists, male and female aviators, male and female fleet sailors), human EPA default assumptions, and the general non-military population. Variations in clinical blood variables, body fat (%), and body weight (kg) were considered using physiologically based pharmacokinetic (PBPK) models. Statistical analysis was done on various blood variables, body fat (%), and body weight (kg) for the above populations. The mean and variance for body fat and body weight were used in a Monte Carlo simulation which subsequently was used as input for PBPK models for perchloroethylene and methylene chloride. Perchloroethylene and methylene chloride were chosen to represent chemicals with high and low fat solubility, respectively. Simulations were performed to generate dose metrics which were statistically analyzed to compare populations. Using the various dose metrics, potential excess lifetime cancer risks for the above populations were calculated and compared statistically. The analysis demonstrates that the population characteristics for body fat and body weight can be important determinants of risk.

INCORPORATING BIOLOGICAL RESPONSE INFORMATION INTO RISK PREDICTION

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Quantitative Structure–Activity Relationship (QSAR) methods applied to statistically well-designed training sets have long shown promise for predicting different chemical/species/biological-response end point combinations, based on analogies in chemical structure. We present a new approach to health effects prediction that takes (chemical/species/response) triples as the basic data points to be analyzed and seeks predictively useful clusters using analogies among all three components, instead of only the first one which is used traditionally. This allows partial information about the biological response profile of a chemical in different species and *in vitro* test systems to be included in sharpening the predictions for specific end points and species, such as cancer in humans. The practical implementation of this idea has been accomplished using an artificial intelligence machine learning technique (recursive partitioning) that has technical and conceptual advantages over the parametric statistical modeling methods (e.g., partial least squares) used in state-of-the-art QSAR models. This presentation will (i) review recent QSAR methodology and results for predicting genotoxic and nongenotoxic effects of halogenated hydrocarbons; (ii) describe a prototype computer program, called STEM, that has been designed to support data analysis of (source/target/effect) combinations using machine learning methods and exploiting available biological response information; and (iii) compare the two approaches on several example data sets. We find that STEM successfully discovers biologically meaningful, predictively useful rules for making quantitative risk predictions.

ACUTE DELAYED NEUROTOXICITY EVALUATION OF TWO JET ENGINE OILS USING A MODIFIED NAVY AND EPA PROTOCOL

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The acute delayed neurotoxicity potential of two jet engine oil formulations was evaluated. The major component of each oil is a mixture of hydrocarbon-based esters. One formulation contained 3% tritolylphosphate (TTP) isomer additive, whereas the second contained 3% of the ortho derivative of tritolylphosphate (TOTP), a known neurotoxicant. This study was initiated with three objectives. The first was to determine if either of the two jet engine oils had the potential to produce delayed neuropathy. A second objective was to determine if the Navy repeated-high-dose of 420 mg/kg/day for five days was sufficiently sensitive to determine neurotoxicity. The last was to compare the results of the former Environmental Protection Agency (EPA) single dose "limit test" of 5 g/kg with the new standard of 2 g/kg. The assays performed indicated that the jet engine oil containing TOTP produced delayed neuropathy, whereas the jet engine oil containing TTP did not. A repeated dosing regimen of the oil containing TOTP at doses greater than 420 mg/kg/day (1000 and 2000) produced organophosphorus-induced delayed neuropathy (OPIDN), whereas hens dosed at 420 mg/kg/day were asymptomatic. No potential for OPIDN was indicated in hens treated with the oil containing TOTP at a single dose of 2 g/kg, but hens dosed at the previous EPA limit test dose of 5/kg had significant brain neurotoxic esterase (NTE) inhibition and axonopathy by 30 days posttreatment. (Supported by Department of the Air Force Contract No. F33615-90-C-0532)

A CONSENSUS METHOD FOR SETTING COMMUNITY EXPOSURE GUIDELINES

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Through a series of consensus workshops, a panel of industrial toxicologists has significantly refined a previously published method for determining acceptable concentrations for volatile chemicals in air (see Lewis, SC, et al, *Regulatory Toxicology and Pharmacology*, 11:314-330, 1990). The consensus method was used to derive Community Exposure Guidelines (CEGs) for 13 organic compounds. Community Exposure Guidelines were operationally defined as "ambient concentrations to which the members of the community, including sensitive members of the population, could be exposed continuously throughout their lifetime with no adverse health effects expected." The consensus method was comprised of three phases: (1) a review of the toxicology data base for the selected chemical, its "critical" effect, and the NOAEL for that effect; (2) determination of the most appropriate adjustment factors (aka, uncertainty or safety factors); and (3) derivation of the CEG. Using flexible adjustment factors, selected from a plausible range of values, allowed for a greater input of scientific judgment. Further refinements are clearly possible, and input from the broader scientific community is actively invited.

HUMAN POLLUTANT EXPOSURE AND MUNICIPAL WASTE COMBUSTION: A CASE STUDY

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Since 1985, combustion has been increasingly utilized for the volume reduction of generated municipal solid wastes. Combustion, like other waste management methods, has the potential to cause adverse human health effects; some of the health risks posed by this waste option are associated with exposure to the combustor emissions. To examine the human health risks associated with incineration, the U.S. EPA conducted an exposure assessment at an operating co-incinerator which accepts both municipal sewage sludge and refuse (residential, commercial, and industrial). This exposure assessment was limited to 10 stack pollutants emitted into the atmosphere; the emission data were taken from two separate but recently conducted emissions tests. Atmospheric dispersion of the pollutants from the incinerator stack and their subsequent deposition by wet and dry mechanisms onto environmental media was modeled using local meteorologic data. Human exposure from inhalation of the 10 pollutants was estimated. Then, utilizing the *Methodology for Assessing Health Risks Associated with Indirect Exposure to Combustor Emissions (EPA/600/6-90/003)* and site-specific data from the area around the facility, we modeled human exposure based on contact with a pollutant through the consumption of contaminated terrestrial animal tissue, fish, plants, and drinking water. The estimated human lifetime average daily dose (LADD) was greater for the contaminated food and drinking water pathways (combined) than inhalation. In addition, this poster presentation will highlight some of the assumptions used in particular for mercury exposure.

DERMAL PENETRATION MODELING

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For many compounds, the stratum corneum presents the major barrier for penetration into intact, healthy skin. Entry into and diffusion through the stratum corneum can be estimated by an analysis of the physicochemical characteristics of a compound, such as its octanol/water partition coefficient and molecular weight. For some compounds, and under some conditions, additional factors may be important in determining penetration into the systemic circulation. We have developed a general physiologically based mathematical model for the penetration of compounds through the skin into the vasculature that includes a parallel polar pathway through the stratum corneum (for large or hydrophilic molecules), and an aqueous layer in series with the stratum corneum, which may be a significant barrier for very lipid soluble compounds. This composite model also incorporates clearance of a compound into the capillary bed of the skin, which is an important determinant of overall penetration for rapidly penetrating compounds and under some conditions (such as abraded skin). The model is able to describe quantitatively changes in skin capillary blood flow and permeability, and to predict changes in penetration rate under altered physiological conditions. It can take into account the concentration of substance already in bloodstream, allowing it to be readily incorporated into a whole-body pharmacokinetic model. The model is being incorporated into a user-friendly computer program to facilitate the estimation of dermal penetration coefficients from physicochemical properties for use in a risk assessment context.

IMPACT OF RELATIVE BIOAVAILABILITY FACTORS ON RISK ASSESSMENT OF INORGANICS

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A survey of EPA risk assessments of 40 inorganics revealed that relative bioavailability from drinking water and diet is often not considered in derivation of Reference Doses (RfD). Indeed, U.S. EPA has not developed guidelines for derivation of relative bioavailability factors (RBF). This study examined data on cadmium (Cd) with the objective of exploring approaches to derivation of RBFs that would facilitate consistent integration of bioavailability information in risk assessment. An analysis of 39 studies containing data on three indices of bioavailability of Cd in rats (percent absorbed dose, rate of accumulation in kidney and liver) revealed that bioavailability of Cd in drinking water and diet were not different (p, 0.31-0.95). This observation does not support distinct RfDs for Cd in drinking water and diet derived by U.S. EPA. Several data qualify factors that may impact on assessments of RBFs for inorganics were identified. Lack of media-specific difference in bioavailability of Cd in rats may be explained by *ad libitum* exposure to Cd in water or normal chow. Human exposure to inorganics in food and water resembles the *ad libitum* conditions used in animal experiments, suggesting that absorption of inorganics in humans may be determined more by the nature of the total diet than by the medium of consumption; assessment of relative bioavailability of inorganics in humans generally involves single dosing of fasted subjects. An important challenge to estimating relative bioavailability factors for inorganics is to develop test guidelines that establish minimal design and reporting criteria for future studies.

PARTITION COEFFICIENT DETERMINATION FOR MIXTURES OF VOLATILE CHEMICALS IN HUMAN BREAST MILK AND BLOOD

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Exposure of nursing infants to toxic chemicals in breast milk is of great concern to mothers who may be occupationally exposed to solvents or fuels. An automated vial equilibration method was developed to determine simultaneously human milk/air and blood/air partition coefficients (PC) for several volatile organic chemicals. Partition coefficients were determined for up to 12 chemicals per mixture using gas chromatography coupled with headspace autosampling and cryofocusing. Milk and blood samples from nine donors were analyzed using this method. Our mixture method was validated by comparing PC values from single chemical vial equilibration studies with the mixture PC values in rat blood and human milk. Blood PCs compared favorably to reported literature values. Milk/air and blood/air PCs for 22 chemicals are reported. (Supported by Department of the Air Force Contract No. F33615-90-C-0532)

INHALATION UPTAKE AND METABOLISM OF VINYL CHLORIDE (VC) AND TRICHLOROETHYLENE (TCE) MIXTURES

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Vinyl chloride (VC) and trichloroethylene (TCE) are two common ground water contaminants that are often found together as chemical mixtures. Also, VC may arise from bacterial degradation of TCE. Therefore, it is necessary to better understand and predict the possible toxicological interactions such as potentiation, synergism, and antagonism. The pharmacokinetics and metabolism of VC and TCE as individual chemicals and as mixtures at varying ratios were investigated and a physiologically based pharmacokinetic (PBPK) model was developed. This was accomplished by exposing Sprague-Dawley rats to VC and TCE from 100 to 10,000 ppm in a closed chamber recirculating system for a 6-h period. Samples were taken from the chamber and gas chromatography with FID was used to determine the concentration of the VC and TCE. Estimates of metabolic constants derived using PBPK modeling are VC: K_m = 0.07 mg/L (1 uM), V_{maxc} = 3 mg/hr/kg bw TCE: K_m = 0.25.

EVALUATION OF TOXICITY BIOASSAYS FOR THEIR APPLICATION IN ASSESSING THE TOXICITY OF COMPLEX CHEMICAL WASTES

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Traditional engineering technology has concentrated on assessment of hazardous waste mixtures by chemical and geophysical measures. One way to offset the problems associated with the use of these measures alone is to supplement them with toxicity tests. Tests utilizing living organisms to evaluate toxicity provide a direct measure of environmentally relevant toxicity. The complexity of hazardous chemical wastes requires that appropriate and relevant toxicity tests be selected to identify potential toxicity from chemical contaminants in environmental samples.

Identically prepared samples (326) collected from 37 sites located in 29 states were evaluated using aquatic macroinvertebrates, algae, and bacteria. EC50 and LC50 toxicity results were produced in 185 (57%) of the samples. Independently, the 96-h algal chronic and 48-h *Daphnia* acute tests identified 158 (85%) and 139 (75%) of the samples, respectively, that contained toxic constituents. Collectively, the algae and *Daphnia* tests identified toxicity in 177 (96%) of the samples demonstrating toxicity. The bacteria test responded to 67 (36%) toxic samples. However, the bacteria showed no response to 118 (64%) of the samples which were toxic to either, or both, algae and *Daphnia*.

Additional toxicity tests using bacteria were performed to determine if a rough mutant of *Escherichia coli* would prove more sensitive than *Photobacterium phosphoreum*. Fifteen elements and compounds and nine Superfund site samples were evaluated. Both organisms demonstrated greater sensitivity to a few samples but, generally they demonstrated comparable sensitivity to the toxicants tested.

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EFFECTS OF ABNORMAL BREATHING ATMOSPHERES ON THE METABOLISM OF METHYLENE CHLORIDE

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The Navy is interested in the effects of deep sea diving on the expression of toxicity. Factors present in the deep sea diving environment that are anticipated to affect toxicity are hyperbaric pressure, low oxygen concentrations in the breathing gases, high heat loss, and high ergonomic work load. As part of the effort to evaluate the effect of these conditions, experiments have been conducted to determine the effects of altered oxygen and carbon dioxide concentrations on the metabolism of methylene chloride. A closed, recirculating gas-uptake system similar to that described by Andersen et al. was used to expose F344 rats to controlled gas mixtures containing 10 to 1000 ppm of methylene chloride. On-line gas chromatography was used to determine the concentration methylene chloride and carbon monoxide produced by methylene chloride metabolism as a function of time after the introduction of a known amount of methylene chloride into the system. These data allowed the calculation of the fraction of methylene chloride metabolized to carbon monoxide by cytochrome p-450 oxidation system. Low oxygen concentrations reduce the fraction of methylene chloride metabolized by cytochrome p-450 oxidation. The pharmacokinetics of this alteration are describable in terms of shifts in PBPK model parameters.

RISK EVALUATION: THE 1989 ALAR INCIDENT

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This paper reconstructs, evaluates, and compares the two principal risk assessments in the Alar incident of 1989. Alar is the trade name for daminozide, a growth regulator that was used to hold maturing red apples on trees so that apples would ripen uniformly and remain crisp in storage. Daminozide is converted to unsymmetrical dimethylhydrazine (UDMH) when treated apples are cooked or metabolized. Unsymmetrical dimethylhydrazine is an animal carcinogen; daminozide is not. As a result of the Natural Resources Defense Council's (NRDCs) risk assessment of UDMH, the public dramatically curtailed the purchase of apples and apple products.

The Environmental Protection Agency (EPA) estimated the lifetime risk of cancer in children exposed to UDMH for 1.5 years as 9×10^{-6}. The NRDC estimated the lifetime risk of cancer in children exposed to UDMH for 6 years as 910×10^{-6}. These two risk assessments cannot be compared directly because they used different durations of exposure, different values for cancer potency factor and lifespan, and different equations for estimating risk. When common values are used in the EPA's risk equation, the EPA's recalculated risk estimate for 6 years of exposure in children is 36×10^{-6}. When common values are used in the NRDC's risk equation, the NRDC's recalculated risk for 6 years of exposure in children is 32×10^{-6}. There is no significant difference between these recalculated risk estimates.

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INFLUENCE OF OXYGEN CONCENTRATION AND ENZYME INDUCTION ON METABOLISM OF HCFC-123 *IN VITRO*

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HCFC-123, a candidate fire extinguishant, is an analogue of halothane with identical hepatotoxic metabolites. The following study examined the conditions favoring the rate of formation of these metabolites. Control, phenobarbital (PB)-, and pyridine-induced microsomes were added to flasks containing 0 to 21% oxygen. HCFC-123 was added to each flask, the reactions initiated by addition of 1 mM NADPH, and terminated by rapid heating. Metabolites were analyzed by gas chromatography. Incubations conducted anaerobically, and using pyridine- or PB-induced microsomes, resulted in a 4- and 2.5-fold increase, respectively, of the rate of formation of HCFC-133a, the major reductive metabolite, when compared to control rates. The rate of formation of chlorodifluoroethylene, the other reductive metabolite, was only 10% that of HCFC-133a. Rates of trifluoroacetic acid (TFAA) formation were about 8-fold higher in normoxic incubations conducted with enzyme-induced microsomes. Elevated TFAA was also observed in incubations conducted using enzyme-induced microsomes exposed to 2 to 5% oxygen. The induction of cytochrome P-450 isozymes thus results in increased rates of TFAA formation at oxygen concentrations that are similar to those found in the centrilobular region of the liver lobule. (Supported by Department of the Air Force Contract No. F33615-90-0532)

TOTAL P.05

PHYSIOLOGICALLY BASED SIMULATION OF PERCHLOROETHYLENE (PCE) PHARMACOKINETICS IN HUMANS

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Perchloroethylene (tetrachloroethylene, PCE) is a commercially important solvent used in dry cleaning and as a degreasing agent that commonly occurs as a ground water contaminant. To assist in the determination of the potential risk to humans exposed to PCE, a physiologically based pharmacokinetic (PBPK) model describing the kinetics of PCE in humans was developed and used to simulate a number of different human exposure data sets found in the literature. Mean values and error estimates for human blood/air and tissue/air partition coefficients were determined in the laboratory. Physiological parameters for humans, including estimates of parameter variability, were obtained from the physiological literature or from the published PCE kinetic data. A classical pharmacokinetic model describing trichloroacetate (TCA) pharmacokinetics was combined with the PBPK model of PCE to provide for a method of estimating the amount of PCE metabolized to TCA. Simulation of PCE in exhaled air and blood provided good estimates of the experimental data. The model simulations of blood and urinary levels of TCA provided reliable estimates of the laboratory data and confirmed the very low level of PCE metabolism in humans. Human metabolism was described with an apparent $V_{max} \approx 12.6$ mg/hr and $K_m \approx 7.7$ mg/L. While most human data sets were adequately described by the PBPK model, some segments of the simulations deviated from the data, especially the exhaled breath washout phase after longer or higher inhalation exposures to PCE. Incorporation of Monte Carlo simulations utilizing measured parameter variability provided a range of model predictions with error estimates that encompassed the actual human exposure data. (Supported by Department of the Air Force Contract No. F33615-90-0532)

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EVALUATION OF SHIPBOARD FORMATION OF A NEUROTOXICANT (TRIMETHYLOLPROPANE PHOSPHATE) FROM THERMAL DECOMPOSITION OF SYNTHETIC AIRCRAFT ENGINE LUBRICANT

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Gas turbine engine synthetic lubricants that are composed of trimethylolpropane triheptanoate and tricresyl phosphate have been shown to form a neurotoxicant, trimethylolpropane phosphate (TMPP), during thermal decomposition. TMPP is thought to inhibit GABA-mediated inhibitory response and producing epileptiform seizures. Thermal decomposition of the lubricant produces TMPP under laboratory conditions but TMPP has not been detected in the work place following actual fires. It is possible that thermal decomposition of these synthetic lubricants may result in contamination of a lubricant storage space aboard ship producing a dermal hazard to shipboard personnel involved in clean-up operations after a fire. This study examined whether TMPP is produced during an actual shipboard fire by placing the synthetic lubricant in a fire environment aboard the ex-U.S.S. Shadwell, Mobile, AL. Efforts were made to duplicate the shipboard storage environment of the lubricant in order to simulate what might actually result if a fire occurred. Both biological and chemical analyses were performed on the thermally decomposed lubricant. Under the conditions of this study, the formation of TMPP during a shipboard fire was confirmed.

DERIVATION OF A SITE-SPECIFIC RISK VALUE FOR INSOLUBLE INORGANIC MERCURY IN SOIL AND SEDIMENT

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As part of the ongoing remedial investigation of the contamination of Lower East Fork Poplar Creek (LEFPC) in Oak Ridge, TN, it was determined that the derivation of an RfD for mercuric sulfide would be pertinent to remediation efforts. Sampling analysis indicated that the mercury at this site is of a tightly-bound, relatively insoluble form. Although risk values for inorganic mercury are available, they are derived from soluble mercurial salts. Because an inorganic mercurial of low solubility would have limited bioavailability, derivation of an alternate risk value based on a relatively insoluble inorganic mercurial seemed valid. The derivation of this risk value made use of data from a chronic feeding study using mice exposed to a diet containing contaminated soil and sediment from 30 LEFPC sampling sites (Revis et al., 1989). The resulting test diets contained mercury concentrations ranging from 0.59 to 1799 ppm. No significant gross or histopathological evidence of toxicity was detected following chronic exposure to mercury levels as high as 1799 ppm (equivalent to 13.07 mg Hg/kg/day), thereby identifying this dose as a No-Adverse-Effect-Level (NOAEL). For derivation of the site-specific risk value, mercuric sulfide was chosen as a surrogate for a sparingly soluble inorganic mercurial, and elemental equivalence was used to derive a risk value of 0.04 mg HgS/kg/day.

* Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-840R21400

ECOLOGICAL RISK ASSESSMENTS OF U.S. ARMY SITES — AN APPROACH

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Recently, the Army has been faced with expanding their Installation Restoration Program to include Ecological Risk Assessments (ERA) of military Superfund sites as mandated by the Defense Environmental Restoration Program (DERP). Generally, the U.S. Army Environmental Center (AEC) is responsible for implementing DERP and ensuring that these sites are remediated to meet local, state, and federal guidelines. The Army's ERA support comes from internal agencies as well as civilian contractors. The U.S. Army Environmental Hygiene Agency (USAEHA) routinely provides oversight and consultative support to AEC; and in certain cases, USAEHA will conduct ERAs. This paper presents the ERA approach applied at Joliet Army Ammunition Plant (JAAP) by USAEHA. Rather than a total modeling approach, ecological risk characterization was based on a combination of both terrestrial and aquatic field and laboratory data. For the terrestrial component, an extensive survey was conducted to identify both plant and animal resident species. Rodents and deer were tested for contaminant bioaccumulation, and sublethal exposures were measured using biomarker assays. Also, soil samples were taken from study areas and screened for toxicity in a battery of ecological toxicity tests. For the aquatic component, water and sediments samples were tested for the contaminants of concern. Water quality was based on quantitative studies of benthic macro-invertebrates. Again, contaminant bioaccumulation was sampled for in resident fish. This information will be correlated to characterize both exposure and adverse ecological effects for use by site managers in the remediation process.

RISK ASSESSMENT AT DOE'S SAVANNAH RIVER SITE

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The recently completed Federal Facility Agreement for the Savannah River Site specifies 84 RCRA/CERCLA RFI/RI units and 304 units that require remedial site evaluations. This poster will present a brief overview of the environmental restoration work that has been conducted at the site, along with case studies of a Baseline Risk Assessment and a Risk Evaluation that were conducted for two units at the site.

A Baseline Risk Assessment was conducted for the SRL Seepage Basins, which received hazardous and radioactive contaminants between 1954 to 1982. Forty contaminants were selected for the quantitative risk assessment. Exposure concentrations for the soil were available, and concentrations in other relevant media were modeled. A combination of quantitative and qualitative analyses were used for the ecological risk assessment. Current scenarios all demonstrated acceptable levels of risk, but the future (on-unit, residential) scenario demonstrated risks above EPA-accepted limits.

The Metallurgical Laboratory Basin at the SRS was closed under the regulatory requirements of RCRA. A Risk Evaluation was conducted to support the integration of the requirements of RCRA and CERCLA. Available data were compiled and analyzed to determine the contaminants associated with the basin and to characterize the site. For each exposure scenario, the resulting exposure pathways were determined to be incomplete because closure activities have the result that the potentially exposed populations would not come into contact with site contaminants. For the ecological risk evaluation, potential receptors were identified. The risk evaluation concluded that the site does not pose a risk to plant or wildlife populations.

RISK-BASED SOIL CLEANUP LEVELS FOR TPH

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Remedial investigations of sites having soils containing hydrocarbons often employ an analysis of total petroleum hydrocarbons (TPH). Many states have set cleanup levels or action levels for TPH in soils of 100 mg/kg or less. Such cleanup goals have been set arbitrarily and are inappropriate, because they are not risk-based and are applied to all hydrocarbon mixtures regardless of chemical composition. The risk-based cleanup goals derived here for soils containing specific hydrocarbon mixtures are 3 to 1900 times higher than the 100 mg/kg value employed by many regulators. Thus, society's resources are being misallocated for little health benefit when cleanups are determined by the default TPH goals currently in use.

Risk-based target cleanup goals for both residential and industrial exposure scenarios for 10 hydrocarbon mixtures commonly found at hazardous waste sites were developed based on the fractional PAH content of each mixture. Reasonable, scientifically defensible cleanup levels for the mixture having the highest fractional PAH content (weathered fuel oil #6) are 8325 mg/kg for residential settings and 19,707 mg/kg for industrial settings. The most conservative cleanup levels that can be derived using this methodology are based on standard EPA Superfund risk assessment assumptions, do not take into account biodegradation, and employ an additional uncertainty factor of ten. For weathered fuel oil #6, the worst-case cleanup level is 274 mg/kg for residential settings and 1076 mg/kg for industrial settings.

AMERICAN SOCIETY FOR TESTING AND MATERIALS — NEW SUBCOMMITTEE ON ASSESSMENT OF RISK TO HUMAN HEALTH AND THE ENVIRONMENT FROM HAZARDOUS WASTE SITES

Ruth Bleyler, Jan Borkowski, Jane Hixson, Michael Kangas, Suellen Pirages, Fred Price, and Van Wilkinson

American Society for Testing and Materials, Philadelphia, PA

The American Society for Testing and Materials (ASTM) is a not-for-profit organization that provides a forum for producers, users, ultimate consumers, and those having a general interest to meet on common ground and write voluntary consensus standards for materials, products, systems, and services. ASTM publishes standard test methods, specifications, practices, guides, classifications, and terminology. ASTM headquarters has no technical research or testing facilities; such work is done voluntarily by 33,000 technically qualified ASTM members located throughout the world.

Subcommittee E47.13 entitled *on Assessment of Risk to Human Health and the Environment from Hazardous Waste Sites* is developing voluntary consensus standards in five general areas. Those areas, each of which include both human health and ecological risk, are (1) data collection, (2) exposure assessment, (3) toxicity assessment, (4) risk characterization, and (5) general topics. Subcommittee E47.13 first met in January of 1992 and presently has about 100 members distributed among industry, the regulatory community, and academia. There are 18 working groups identified within E47.13 that are writing standards, guides, or practices in the area of risk assessment. In addition, Subcommittee E47.13 participates in annual environmental symposia sponsored by Committee E47.

A poster presentation will summarize Subcommittee activities and the ASTM procedures for developing voluntary consensus standards, practices, and guides. Officers and members of the Subcommittee will be present to answer questions.

FUTURE USE CONSIDERATIONS IN THE CLEANUP OF FEDERAL FACILITIES

Douglas J. Sarno

Clean Sites, Alexandria, VA

Clean Sites has been working on an approach for using the future use of land and natural resources to determine the requirements of cleanup for contaminated sites on Federal Facilities. A detailed approach for cleanup decision making has been developed and is currently being pilot-tested at two bases. The approach centers on cooperative decision making between the Air Force and state and federal regulators to establish clear objectives for cleanup based on the actual risks to human health and the environment under the most reasonable long-term use of the site.

The process being evaluated is based on the premise that it is not feasible from a cost or technology standpoint to return all sites to pre-release conditions, and that a great deal of time and money could be saved if an explicit recognition of future use were introduced into the process of identifying cleanup needs for sites. The process relies on site-specific risk assessment to be applied specifically for identifying cleanup levels for the actual alternative uses of the land and natural resources.

COMPARISON AND ANALYSIS OF THREE DIFFERENT BACKGROUND SURFACE-SOIL SITES FOR WRIGHT-PATTERSON AIR FORCE BASE NORTHEASTERN AREA, OPERABLE UNIT 2

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Three sampling sites, to serve as reference/background for surface soil in the Northeastern Area, were identified and sampled. The locations are: (1) approximately 200 feet west of the Wright Brothers Memorial, off Pylon Road; (2) the City of Fairborn's Sandhill Park; and (3) approximately 2,000 feet west of Gate 26C between Douglas Drive and State Route 235. More than 10 samples were collected at each site, and each sample was examined for 23 metals and cyanide.

The results indicate that there are significant differences in compound concentrations between the various locations. For example, beryllium is over 2.0 times more concentrated at the Wright Brothers Memorial site when compared to the Douglas Drive location, and over 3.5 times more concentrated than at the Sandhill Park site. Other compounds also exhibit similar significant inconsistencies among the different sites. Among these are barium, calcium, magnesium, and manganese. Selenium is unique in this study in that it was only detected at the Wright Brothers Memorial sampling location.

These results attest to the possible difficulties encountered when only one sampling location is used as a background reference. The implications are discussed in detail.

SUPERFUND HEALTH RISK TECHNICAL SUPPORT CENTER

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The Environmental Criteria and Assessment Office (ECAO) operates the Superfund Health Risk Technical Support Center (SFTSC) to provide technical support and guidance to regional toxicologists, risk assessors, and remedial project managers who have questions about Superfund health and risk assessment issues. The ECAO's SFTSC is one of seven specialized Technical Support Centers that comprise the Technical Support Project established by the Office of Research and Development and the Office of Solid Waste and Emergency Response. The SFTSC provides the following types of information: chemical-specific toxicological information, provisional toxicity assessments (RfDs, RfCs, cancer weight-of-evidence classification and potency), interpretation and assistance with implementation of *Risk Assessment Guidance for Superfund: Human Health Evaluation Manual* (RAGS/HHEM), support for the Health Effects Assessment Summary Tables (HEAST), review of site-specific risk assessments, and general risk assessment methodology. The SFTSC has responded to requests from all 10 EPA Regions and from 19 states. PAHs, chlorinated solvents, PCBs, and heavy metals are the most frequently requested chemicals. Information on how the SFTSC operates and how users can access the SFTSC is presented.

THE FSIS COMPOUND EVALUATION SYSTEM (CES) — A RISK ASSESSMENT TOOL FOR ASSESSING CHEMICAL RESIDUES IN MEAT AND POULTRY

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The Food Safety Inspection Service (FSIS) is charged with inspecting the meat and poultry supply to ensure that products are safe, wholesome, and properly labeled. We created a ranking system to evaluate chemicals for their potential to be a hazard to human health through meat and poultry. The CES assists the agency in the effective management of its resources and residue program activities. Compounds (drugs, pesticides, and environmental contaminants) are ranked for toxicity and for probability of human exposure. Our approach has three elements:

1. Determining if a compound can cause a residue; if the answer is "Yes", then,

2. Assessing the toxicity of the compound, and

3. Assessing the potential for human exposure resulting from occurrence in meat or poultry.

Toxicity is ranked from A (highest) to D (lowest) and exposure is ranked from 1 (high probability) to 4 (low probability) resulting in a dual ranking from A-1 to D-4. A detailed set of worksheets organizes material and serves as a mnemonic.

METHODS FOR CONVERTING CONTINUOUS RESPONSE DATA FOR DOSE–RESPONSE MODELING

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For noncancer risk assessment, categorical regression and the benchmark dose method have been proposed as alternatives to the traditional reference dose (RfD) approach based on the No-Observed-Adverse-Effect-Level (NOAEL). Both of these proposed methods involve fitting dose–response models that have been developed mainly for quantal data. This poses a problem: the effect being modeled may result in the measurement of a continuous variable (e.g., enzyme activity, body weight). Utilizing dose–response models designed specifically for continuous response data is one solution. It is not entirely satisfactory, however, because multiple end points may be observed or both continuous and quantal data may be available to describe the chemical's toxicity. An alternative is to convert the continuous response to incidence data. One advantage is that a common model can then be used for the analysis so that all of the effects can be incorporated into the risk assessment in a consistent way.

Two approaches for converting continuous data to incidence data are described. The first invokes an assumption that a fixed proportion of the control group (those with more extreme values, e.g., the upper 5%) constitutes the "responders." The location of this subset establishes the values of the variable that represent a "response". An alternative method uses the entire control group to define a "background" distribution. The proportion of responders for a treated group is then established by comparing its empirical distribution with this background distribution.

EFFECT OF ORAL DOSING VEHICLE ON THE BLOOD TO AIR PARTITION COEFFICIENT

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Based on the dissolution or nonbinding association of volatile lipophilic organic compounds with whole blood lipids, the effect of vehicles on the blood:air partition coefficient (BAPC) was investigated. Vehicle related BAPC changes could influence the *in vivo* distribution and elimination characteristics of the compound. Three dosing vehicles, corn oil (C), mineral oil (M), and water (W) and two chemicals trichloroethylene (TCE) and tetrachloroethylene (PCE) were studied. Fischer 344 rats were gavaged with 5 mL/kg of vehicle. Blood samples were taken at 0.5, 1.0, 2.0, and 4.0 h post gavage and exposed (vial equilibration technique) to 800 ppm of chemical at 37 °C for 2.5 (TCE) to 3.5 (PCE) hours. Each sample was analyzed by the head-space gas chromatographic technique and partition coefficients were calculated. The mean control values were 27.66 and 28.13 for TCE and PCE, respectively. The 0.5, 1.0, 2.0, and 4.0 h values for each treatment group (N=6) were: TCE in C 26.12, 27.90, 27.72, 29.20; PCE in C 26.65, 28.45, 28.28, 30.74; TCE in M 27.99, 25.46, 26.80, 28.39; PCE in M 26.74, 26.97, 27.70, 28.75; TCE in W 28.03, 25.18, 25.20, 28.60; and PCE in W 25.97, 28.23, 26.44, 29.41. Water, mineral oil, and corn oil when used as dosing vehicles did not change the rat BAPC for TCE and PCE.

THE RELEVANCE TO HUMANS OF ANIMAL MODELS FOR INHALATION STUDIES OF CANCER IN THE NOSE AND UPPER AIRWAYS

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Although nasal cancer is relatively rare among the general population, workers in the nickel refining, leather manufacturing, and furniture building industries exhibit increased incidences of nasal cancer. To investigate the causes of nasal cancer and to design ameliorative strategies, an appropriate animal model for the human upper respiratory regions is required. The anatomy and physiology of the nasal passages and upper airways of the humans, rats, and monkeys have been described, compared, and assessed for the purpose of determining a relevant animal model in which to investigate potential causes of nasal cancer.

Based on the mode of breathing, overall geometry of the nasal passages, relative nasal surface areas, proportions of nasal surfaces lined by various epithelia, mucociliary clearance patterns, and inspiratory airflow routes, the rat differs greatly from humans and, therefore, is a poor model. In contrast, the monkey exhibits many similarities to humans. Although the monkey does differ from humans in that it exhibits a more rapid respiratory rate, smaller minute and tidal volumes, a larger medial turbinate, and a vestibular wing that creates an anterior vortex during inspiration, the monkey is a more appropriate model than the rat for studying the toxic effects of inhaled substances on the nasal passages and extrapolating the findings to humans.

ESTIMATING THE LACTATIONAL TRANSFER OF VOLATILE CHEMICALS IN WOMEN USING A PHYSIOLOGICAL MODEL

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The transfer of chemicals from breast milk to the nursing infant is of concern for women occupationally exposed to chemical vapors. There is very little information published in the literature on lactational transfer of inhaled organic chemicals in humans or laboratory animals. This study is an initial attempt to better understand if maternal exposure to chemical vapors results in an indirect chemical exposure of the nursing infant. To evaluate the ability of inhaled organic chemicals to transfer to breast milk via the systemic circulation, we developed a human physiologically based pharmacokinetic (PBPK) lactation model. To build the human lactation model, blood and milk samples were collected from nine volunteer donors and used for determining blood/air and milk/air partition coefficients for 19 chemicals using the vial equilibration technique. Other tissue/air partition coefficients (e.g., fat, liver, and muscle) and allometrically scaled metabolic constants were taken from the experimental literature on rats and humans. For each chemical, the lactation model simulated a single nursing schedule over a 24-h period and an 8-h intermittent occupational chemical exposure of the mother at the Threshold Limit Value (TLV). The estimated amount of chemical transferred per day was tabulated and then, if available, compared to proposed exposure guidelines for children. (Drinking Water Health Advisories, U.S. EPA)

DURATION-ADJUSTMENT OF EFFECT LEVELS: COMPARISON OF PHYSIOLOGICALLY BASED (PBPK) MODELS WITH DEFAULT APPROACH

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For dose–response assessment, interspecies extrapolation of exposure levels associated with toxic effects requires an adjustment of the laboratory exposure regimen to that of the assumed human scenario (e.g., 24 h/day). The default "duration-adjustment" is to linearly prorate the animal effect level by the number of hours/24 h and the number of days/week of the animal exposure regimen (e.g., by 6/24 x 5/7). The rationale for this adjustment is that the resultant human concentration should be the C x T equivalent of the animal exposure level. This assumption of C x T exposure equivalency is tenuous because steady-state conditions may not have been reached under some exposure conditions and is not consistent across different toxicity mechanisms (e.g., an effect mediated by peak blood concentration vs. integrated tissue dose). The PBPK models for dichloromethane and perchloroethylene, chemicals with different physicochemical characteristics (partition coefficients), metabolic parameters (V_{max}, K_m, and K_f), effects and presumed mechanisms of toxicity, are exercised at various exposure levels and durations to illustrate discrepancies of the current default equation with respect to internal dose metrics. Sensitivity analyses identify key parameters and suggest revisions to the default approach.

PERMEABILITY CONSTANTS DETERMINED BY PBPK MODELS FOR VAPOR, NEAT, AND AQUEOUS BENZENE

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The permeability constant is a measure of the ability of a chemical to penetrate through the skin. Physiologically based pharmacokinetic (PBPK) models can be used to estimate the permeability constants of chemicals from the blood concentrations achieved for *in vivo* dermal exposure. The permeability constant for benzene was determined for three different dermal exposures in rats: whole-body dermal exposure to benzene vapor, exposure to neat benzene from a closed cell on the dorsal skin, and exposure to saturated solutions of benzene in water from a closed cell. The PBPK models were developed which described each of these dermal exposure methods. The estimated permeability constant for dermal vapor was 0.152 cm/h, for neat benzene 0.0025 cm/h, and for aqueous solutions 0.05 cm/h. The physical form of the chemical influences the rate of absorption. Neat benzene chemically fixes the skin, reducing the rate of penetration. The permeability constant for rat skin from aqueous solutions was one-half the human permeability constant used for dermal risk assessment, 0.111 cm/h.

IRIS (INTEGRATED RISK INFORMATION SYSTEM)

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The Integrated Risk Information System (IRIS) is an on-line data base of the U.S. Environmental Protection Agency that provides health hazard risk information on over 500 substances. Information includes oral reference doses (RfDs) and inhalation reference concentrations (RfCs) for noncarcinogenic effects, and qualitative and quantitative assessments of potential carcinogenicity. Each substance-specific information section is summarized in 3 to 20 pages, providing information on the studies evaluated, uncertainties, assumptions, an indication of confidence, EPA scientists to contact for more information and complete bibliographic citations. The summaries are the result of an EPA review process of the RfD/RfC or Carcinogen Risk Assessment Verification Endeavor (CRAVE) Work Groups. These two work groups of expert Agency scientists review assessments developed within EPA's program offices and the Office of Research and Development. When consensus is reached, summaries of the assessments are made available to EPA and the public on IRIS. IRIS is updated monthly to reflect the most up-to-date Agency health hazard risk information.

IRIS is available on-line on the National Library of Medicine's TOXNET system and is accessed through COMPUSERVE, TYMNET, TELENET or INFONET telecommunications networks or by direct dial. A diskette version is available on 5-1/4 inch high density floppies for IBM or compatible PC from the National Technical Information Service (NTIS). Current plans include development of a more user-friendly, PC-based system for general use.

MONTE CARLO ANALYSIS: A TOOL FOR EXAMINING UNCERTAINTY IN RISK ASSESSMENT

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There can be significant uncertainties in the input parameters used in risk assessment models. Because these models are used to support decision-and policy-making, quantitative examinations of these uncertainties is important. Recently, the use of Monte Carlo analysis to propagate uncertainties through risk assessment calculations has seen increased use. This paper describes Monte Carlo analysis as it is typically used in examining risk assessment uncertainties. Two examples of its application are presented: (1) to determine a more accurate estimate of "reasonable maximum" risk than the use of U.S. EPA's standard default values for exposure factor inputs, and (2) to evaluate the trade-off between extent (and thus cost) of remediation and degree of confidence in achieving adequate protection of health. A personal computer will be available to demonstrate off-the-shelf software for Monte Carlo analysis in risk assessment.

MIXTOX: A DATA BASE ON TOXICOLOGIC INTERACTIONS

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MIXTOX is a personal computer database and stand-alone data retrieval system containing summary information of studies on toxicologic interactions such as synergism and antagonism. The database reflects published articles on environmental chemicals, primarily on binary mixtures, and currently contains ~3000 records representing 437 articles treating 582 chemicals in 1465 chemical pairs. MIXTOX is intended to be a guide to the literature for use in risk assessment and research. The database includes full literature identification, details on the experimental set-up (animal species, exposure conditions), and results (interaction, observed effects, and sites of the effects). Searching is by the chemicals' identifiers using EPA IRIS names, common names and/or CAS numbers, with filtering by interaction type, duration, and species. Output includes study level detail or interaction summaries by chemical pair. Versions are available for IBM PC-compatible and Macintosh-compatible personal computers.

THE REAL COST OF USING HAZARDOUS MATERIALS

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The Hazardous Material Management Cost Estimating Tool enables system program offices and their prime contractors to input hazardous materials cost considerations into the engineering trade-off studies performed during weapon system development, thus assisting them in the selection of those materials and processes which have lower risk to human health and the environment and those which do not require specialized high-cost handling and disposal procedures.

The cost estimating tool encompasses hazardous materials cost elements for all four phases of the system life–cycle–development, production operation, and support and system disposal/decommissioning. The structure of the cost estimating tool is such that if given a specific material or process, the tool will calculate the life-cycle cost of cost elements affected. Cost elements identified for hazardous materials in each phase of the life cycle are procurement, transportation, handling, monitoring, training, personal protection, potential legal/environmental liability, and medical.

The model is tool for hazardous materials evaluation and for estimating the total cost of using hazardous materials in a weapon system. It enables trade-off analyses to determine the alternative cost of using other less hazardous or nonhazardous materials. The ultimate goal is to reduce the kinds and amounts of hazardous materials used in weapon systems and the processes that produce and support those systems. The model includes Air Force, Army, and Navy systems.