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Lessons Learned in Applying the U.S. EPA Proposed Cancer Guidelines to Specific Compounds

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An expert panel was convened to evaluate the U.S. Environmental Protection Agency's "Proposed Guidelines for Carcinogen Risk Assessment" through their application to data sets for chloroform ($CHCl_3$) and dichloroacetic acid (DCA). The panel also commented on perceived strengths and limitations encountered in applying the guidelines to these specific compounds. This latter aspect of the panel's activities is the focus of this perspective. The panel was very enthusiastic about the evolution of these proposed guidelines, which represent a major step forward from earlier EPA guidance on cancer-risk assessment. These new guidelines provide the latitude to consider diverse scientific data and allow considerable flexibility in dose-response assessments, depending on the chemical's mode of action. They serve as a very useful template for incorporating state-of-the-art science into carcinogen risk assessments. In addition, the new guidelines promote harmonization of methodologies for cancer- and noncancer-risk assessments. While new guidance on the qualitative decisions ensuing from the determination of mode of action is relatively straightforward, the description of the quantitative implementation of various risk-assessment options requires additional development. Specific areas needing clarification include: (1) the decision criteria for judging the adequacy of the weight of evidence for any particular mode of action; (2) the role of mode of action in guiding development of toxicokinetic, biologically based or case-specific models; (3) the manner in which mode of action and other technical considerations provide guidance on margin-of-exposure calculations; (4) the relative roles of the risk manager versus the risk assessor in evaluating the margin of exposure; and (5) the influence of mode of action in harmonizing cancer and noncancer risk assessment methodologies. These points are elaborated as recommendations for improvements to any revisions. In general, the incorporation of examples of quantitative assessments for specific chemicals would strengthen the guidelines. Clearly, any revisions should retain the emphasis present in these draft guidelines on flexibility in the use of scientific information with individual compounds, while simultaneously improving the description of the processes by which these mode-of-action data are organized and interpreted.

Key Words: **chloroform; dichloroacetic acid; proposed cancer guidelines, U.S. EPA; mode of action; margin of exposure; harmonization; modeling, toxicokinetic; evidence, preponderance of; risk assessment.**

In April 1996, the U.S. Environmental Protection Agency (EPA) published a set of "Proposed Guidelines for Carcinogen Risk Assessment" (U.S. EPA, 1996), the first revision since the original guidelines in 1986 (U.S. EPA, 1986). The proposed guidelines were developed as part of an interoffice program by a technical panel of the Risk Assessment Forum within EPA's Office of Research and Development. The proposed guidelines take into consideration the complexities of the carcinogenic process and the rapid pace of ongoing research in carcinogenesis. They acknowledge that insights gained from this research should be used to make more scientifically based assessments of the carcinogenic potential of chemical and physical agents with an emphasis on using mode-of-action information to project dose-response relationships. Although it is important that these guidelines remain general and flexible, it is anticipated that supplemental technical guidance documents will be developed when necessary. Following publication of the proposed guidelines in the Federal Register in April 1996, EPA requested public comments. The agency also expressed interest in developing chemical-specific case studies to illustrate and assess the strengths and limitations of the proposed guidance.

In September of 1996, an expert panel was convened by the

The opinions expressed herein are those of the Expert Panel members and do not necessarily reflect the views of their respective affiliations or the sponsoring organizations.

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Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI) to develop two case studies related to the application of the EPA's revised cancer-risk-assessment guidelines (ILSI, 1997). The two compounds evaluated by this panel were chloroform ($CHCl_3$) and dichloroacetic acid (DCA). These compounds were selected because there are significant sources of human exposure. Both occur as by-products of water chlorination and have been used or suggested for use as markers of the occurrence of trihalomethanes (THMs) and haloacetic acids (HAAs) in water supplies. Drinking water is the major route of exposure for the public to these compounds. Occupational exposures do occur with $CHCl_3$ which is also present at low concentrations in the atmosphere from a variety of natural and anthropogenic sources (IPCS, 1994). DCA is a metabolite of several chlorinated organic solvents and has been used as an anti-diabetic drug and for the treatment of lactic acidosis (IARC, 1995).

There were three major objectives in convening the panel:

- To review the available data relevant to an assessment of the carcinogenicity of $CHCl_3$ and DCA, including bioassay data, information on mutagenicity, metabolism, toxicokinetics, target organ toxicity, and modes of carcinogenic action.
- To apply the guidance provided in the "Proposed Guidelines for Carcinogen Risk Assessment" (U.S. EPA, 1996) and to make recommendations regarding appropriate approaches for assessing the potential carcinogenic risk of these two compounds.
- To provide a critique of the risk assessment process outlined in the "Proposed Guidelines for Carcinogen Risk Assessment" and a commentary on specific issues encountered in applying the guidelines to $CHCl_3$ and DCA as case studies.

The activities of the panel were supported by a group of sponsoring organizations and oversight was provided by a steering committee (see Acknowledgments). Ten individuals with expertise in the diverse disciplines required for cancer risk assessments were nominated for panel membership by the steering committee. The expertise represented in the group included the fields of pathology, mutagenesis, carcinogenesis, hepatic and renal toxicology, pharmacokinetics, and general risk-assessment. The panel membership did not include epidemiologists because review of the epidemiological data was considered outside the scope of the charge. These ten experts and a senior ILSI/HESI staff scientist are the authors of this perspective.

During the next 12 months, the panel met face-to-face five times and regularly through conference calling to complete their report. Following review by four outside experts, the final report was published in November 1997. For details regarding the charge to the panel and the specific recommendations for the two case-study compounds, readers are referred to the panel's full report (ILSI, 1997). This article highlights the expert panel's experience in applying the guidelines to real-world data sets (point (c) above). In the development of this critique, the panel has reflected on several questions: What was learned about the ability to step through a cancer-risk assessment following these guidelines? Do the guidelines strike an appropriate balance between flexibility and structure? What important components of the risk assessment process are missing or ambiguously defined in these guidelines? Lastly, what recommendations might the panel provide for future assessments?

TABLE 1
Topics Highlighted in Working with the Proposed Guidelines

Qualitative Considerations Related to Data Base Evaluation
Working with a Diverse Expert Panel
Evaluating Cancer, Noncancer and Intermediate Endpoints
Considering Plausible Modes of Action
Defining the Weight of Evidence
Quantitative Considerations Related to Risk Assessment
Toxicokinetic, Case-Specific and Biologically Based Dose Response Models
Applying Extrapolation Models from the 'Point of Departure'
Selection of Appropriate 'Point of Departure' for Extrapolation
Use of Quantal vs. Continuous Endpoints for Risk Assessment
Providing a Working Definition of 'Margin of Exposure
Use of Toxicokinetic Models to Assist in Margin of Exposure Analysis
Risk Management Considerations
Blurring the Distinctions between Risk Assessment and Risk Management
Striving for Consistency in Cancer and Noncancer Risk Assessment
Developing a Library of Case Studies
Consistency of Mode of Action Inferences with Epidemiological Data

Organization of Topics

The guidelines outline the manner in which broad data sets on the carcinogenic action of a compound can be organized and evaluated to produce integrated carcinogenic risk assessments. These assessments center on developing consensus regarding the mode or modes of carcinogenic action of specific compounds. The mode of action concept entails a strongly supported, scientific understanding of a plausible sequence of steps leading from exposure to cancer induction. Once the mode of action is established, it leads to specific options for low-dose and interspecies extrapolation. The panel's deliberations fell into three broad categories (Table I). First, there were the largely qualitative considerations associated with evaluating data related to each proposed mode of action. Second, there were semi-quantitative and quantitative considerations into which the mode of action provides insight: (a) target-tissue dose metrics, (b) appropriate toxicokinetic and dose-response models, and (c) the methods for determination of acceptable exposure levels. Third, several risk management considerations were noted in relation to differences between these guidelines and past practices. Each of these specific topics is discussed separately, followed by a brief summary.

Qualitative Considerations Related to Database Evaluation

In previous U.S. EPA cancer risk assessments, the single decision point for proceeding with a quantitative risk assessment was determination of a statistically significant increase in tumor incidence in an acceptable chronic animal bioassay. The broader array of biological data were often analyzed only to assess relevance, i.e., was the response in animals expected in humans? In general, these other data did not directly influence the typical quantitative assessment. The default approach consisted of a linearized, multistage (LMS) model with body weight scaling for interspecies extrapolation, regardless of the degree of understanding of the mode of action of the carcinogen in question. Incorporation of mode of action at both qualitative and quantitative levels necessitates a methodical organization and evaluation of existing data to determine consistency with specific hypotheses. This new approach to the cancer dose-response assessment process requires a closer interaction than was the case previously among the individuals from various biological and mathematical disciplines who contribute to cancer risk assessment.

Working with a Diverse Expert Panel. About half of the ILSI HESI panel consisted of biologically oriented scientists. These individuals faced the task of explaining their specific decision-making strategies to other panel members whose training and experience emphasized a more quantitative approach to cancer risk-assessment problems. The former group tended to be skeptical of the quantification of cancer risk assessment. As was the experience of this panel, the breadth of expertise that should be represented on such expert panels will foster improved appreciation of the complementary skills required in organizing a mode of action-based argument and then applying it to quantitative dose-response assessments.

The interdisciplinary composition of the panel was important in the early stages, where individuals reviewed the data related to their own areas of expertise. At a later stage, the emphasis on mode of action required a careful evaluation of the relationship between various non-neoplastic precursor lesions and cancer for both $CHCl_3$ and DCA. The panel representation in toxicology and pathology was especially important for these evaluations. Individuals with background in pharmacokinetic modeling aided in integrating the broader data base to show consistency of responses across dose routes, dosing vehicles, and animal species. The practical experience of several panel members in risk assessment was instrumental in translating qualitative mode of action concepts into integrated, quantitative relationships for the dose response evaluations.

Evaluating Cancer, Noncancer, and Intermediate Endpoints. Evaluation of the available data on the two case-study chemicals closely followed the order outlined in the guidelines: "Analysis of Tumor Data," "Analysis of Other Key Data," and "Mode of Action-Related Endpoints," including "Direct and Secondary DNA Effects," "Non-mutagenic and Other Effects," and "Identification of Mode(s) of Action." In the panel's experience, it is essential that these sections be comprehensive and presented in sufficient detail to serve as a basis for consideration of a range of hypotheses for the mode of action. If a single, most-plausible mode of action emerges from this analysis, it must be supported by and developed from the broader discussion.

Evaluation of known or suspected metabolic pathways and the toxicology of stable metabolites and transient reactive intermediates must be one of the early steps in identifying and integrating the relevant data base. Oxidative and reductive pathways of cytochrome P450-mediated metabolism form reactive intermediates with $CHCl_3$ that might contribute to carcinogenesis (Pohl *et al*, 1977; Pohl and Krishna, 1978; Testai and Vittozzi, 1986; Tomasi *et al*., 1985; Wolf *et al*., 1 977). The consequences of metabolism through these pathways were evaluated carefully during the panel's deliberations. In addition, evidence for the mutagenicity of metabolites such as glyoxalate from DCA (Marnett *et al*., 1985; Sasaki and Endo, 1978; Sayato *et al*. 1987; Yamaguchi and Nakagawa, 1993), free radicals formed by cytochrome P450-mediated $CHCl_3$ reduction (Pegram *et al*, 1997; Testai and Vittozzi, 1986; Tomasi *et al*., 1985), and glutathione conjugates from $CHCl_3$ (Green, 1983; Thier *et al*., 1993) had to be examined, along with the mutagenicity studies of the parent compound. Comparison with other chemicals with well-established modes of action can provide important perspective. For instance, experience with CCl_4, which is metabolized solely through a reductive pathway, provided insights for assessing the role of the reductive pathway in the toxicity, mutagenicity, and carcinogenicity of $CHCl_3$.

Precursor effects of the chemical on the target organs for neoplasia, including target tissue toxicity, must be cataloged and organized. Important considerations in associating such precursor effects with cancer are the dose-response relationships and establishing whether these other effects are obligatory for or simply incidental to carcinogenesis. Typically, the information used to evaluate associations of precursor response with tumors is derived from different studies. Data on intermediate or key precursor events are usually acquired in shorter-term toxicity or mechanistic studies while information on carcinogenicity is obtained in two-year bioassays that may or may not include interim sacrifices, stop-recovery studies, etc. In fact, many cancer risk assessments in the past were necessarily based on bioassays that provided little data other than tumor incidence. The panel placed emphasis on evaluating the bioassay studies for evidence of association of precursor effects with tumor formation. If correlations could not be assessed in these chronic studies, evaluation of precursor effects in subchronic studies employing similar dosing regimens to the chronic study became the preferred basis for comparison.

To evaluate precursor lesions in the target tissues, pathologists on the panel obtained histopathology slides from the $CHCl_3$ cancer bioassay (Jorgenson *et al*. 1985) and from a drinking water study on DCA (DeAngelo *et al*., 1991). With

$CHCl_3$, these evaluations were important in establishing that: (1) cytotoxicity was present at intermediate time points (12 and 18 months) and at the termination of the study, and that (2) there was a dose-response relationship between drinking water concentrations of $CHCl_3$ and kidney toxicity. For DCA, a careful, retrospective evaluation of the histopathological changes in the liver was important in reaching the conclusion that hepatotoxicity occurred at all dose levels used in the chronic bioassays. All dose levels examined produced toxicity and were considered to be in excess of a maximally tolerated dose. Emerging emphasis on mode of action will require retrospective evaluation of the non-tumor lesions in affected tissues on a more routine basis. This emphasis will likely necessitate convening pathology review panels to assess archived samples for noncancer endpoints, just as these panels have been used previously for assessing tumors. In newly designed bioassays, evaluations of suspected target tissues should be incorporated at specified intervals during the study, in all dose groups.

In most cases, the panel reviewed the primary literature on these compounds to ensure that positions reached were supported and not simply developed from past reviews or evaluations. This activity required a great deal of effort and led to a certain amount of isolation of experts within their areas of expertise. Such deliberations could be streamlined by preparation of relevant documentation prior to convening the expert panel. With the background data organized and summarized, expert panels could devote more energy to interpretation of the data for hazard evaluation and dose-response analysis. In our deliberations, a great deal of the panel's effort revolved around organizing the available data and discussing the consistency of the broad data bases in pointing to specific hypotheses for modes of carcinogenic action for these two compounds and their metabolites.

Considering Plausible Modes of Action. Reaching agreement on the mode of action is central to the application of the proposed cancer guidelines. The panel's approach to deciding whether there was a single mode of action or multiple plausible modes of action was to specify the various possibilities and evaluate the support for or the evidence against each particular mode of action. For example, with $CHCl_3$, potential modes of action included an obligatory role for cell injury with compensatory hyperplasia as a precursor to carcinogenicity and other modes of action related to the mutagenic potential of either reduced free radical metabolites or glutathione conjugates. These two general modes of action—cytotoxicity and mutagenicity—are not mutually exclusive. They could both occur simultaneously and contribute differentially at different doses. In addition, effects may be dose-dependent, i.e., high-dose effects might not occur at low doses (Counts and Goodman, 1995).

The panel considered data on mutagenicity of $CHCl_3$, brominated THMs, and CCl_4 in evaluating the genotoxic, DNA-reactive potential of $CHCl_3$ and its metabolites. A glutathione conjugation pathway converts brominated THMs to mutagenic intermediates in Salmonella transfected with copies of glutathione-*S*-transferase-θ (Pegram *et al*., 1997). However, $CHCl_3$ is not mutagenic in these bacterial assays and inferences from the Pegram *et al*. studies with brominated THMs cannot be extended to $CHCl_3$. In addition, comparisons between the hepatotoxicity of $CHCl_3$ and CCl_4, which is only metabolized by reductive pathways, indicated that these compounds act on the liver by different modes of action. The evidence for a role of mutagenic intermediates in the carcinogenicity of $CHCl_3$ was explicitly examined and found to be very weak (Testai *et al*., 1990; Testai and Vittozzi, 1986; Tomasi *et al*., 1985). The available studies clearly support a dominant role for oxidative metabolites in the toxicity of $CHCl_3$ (Ade *et al*., 1994; De Biasi *et al*., 1992; deGroot and Noll 1989; Testai *et al*., 1990; Testai and Vittozzi, 1986).

Mode of action is broadly defined in the guidelines. For instance, they state in one section, "Thus, mode of action analysis is based on physical, chemical, and biological information that helps to explain critical events in an agent's influence on development of tumors." "Critical events" refers to those biological processes altered by chemical exposure that influence carcinogenesis. Quantitative dose-response assessments will be more straightforward when the mode of action also serves to define the active form of the chemical. The mode of action statement for $CHCl_3$ would be stated in two parts. First, $CHCl_3$ forms oxidized metabolites that cause cell damage in tissues with high concentrations of the relevant metabolizing enzyme. Second, metabolite-mediated cytotoxicity leads to cell death, regenerative hyperplasia, and higher probabilities of cell mutation and cancer. High rates of compound metabolism and metabolite-mediated cytotoxicity are thus regarded to be key steps in $CHCl_3$ carcinogenicity. Uncertainties in either the mode of action or in the biologically active agent (i.e., parent chemical, reactive metabolite, stable, freely circulating metabolite, etc.) would lead to uncertainties in the dose response assessments and in any subsequent risk estimates.

In establishing a plausible mode of action, a dual approach should be emphasized. The first part is the articulation of competing hypotheses and the second part is the evaluation of evidence for each hypothesis, to determine whether one is much more plausible than the others. For $CHCl_3$, the panel agreed unanimously that a mode of action involving obligatory cytotoxicity as a precursor to cancer in target tissues was most plausible, i.e., much more strongly supported by the comprehensive data set than any of the other modes of action. To paraphrase the conclusion, there should be no significant carcinogenic risk from $CHCl_3$ at concentrations below those that cause cell damage. The data on DCA were less informative in developing clear hypotheses for potential carcinogenic modes of action, in part because the cancer studies were conducted at doses that caused overt hepatotoxicity. Although evidence suggested an essential relationship between toxicity, regeneration,

and carcinogenicity, no single hypothesis for the mode of action for DCA emerged as the most plausible.

Defining the weight of evidence. A challenge in applying the mode of action concept to specific data sets occurs in assessing the adequacy of the weight of evidence for a particular mode of action and in the manner of comparing competing hypotheses for the mode of action. The guidelines are not explicit in terms of presentation, organization, and weighting evidence for competing modes of action. Much of the guidance addresses factors relevant to interpretation of the adequacy of investigations of empirical associations, such as epidemiological studies of cancer incidence or mortality and cancer bioassays. Broad criteria for judging the adequacy of weight-of-evidence on mode of action are presented. They include "mechanistic relevance of the data to carcinogenicity, number of studies of each endpoint, consistency of results in different test systems and species, and similar dose-response relationships for tumor and mode of action-related effects." It is appropriate for expert panels to evaluate the data supporting modes of action on a case-by-case basis. Nonetheless, the determination of the adequacy of the weight of evidence for a mode of action will be pivotal in characterizing the dose-response relationship, predicting its shape in the low-dose region and using this information in the risk assessment for many compounds. Consideration of a general framework for assessing the weight of the evidence for a particular mode of action could serve as an important guide for future efforts, although the panel did not endorse a rigid set of rules for this evaluation.

A Workshop on "Issues in Cancer Risk Assessment" was held in January 1998 in Hanover, Germany. At the workshop, a framework was proposed to assist in making judgments about the sufficiency of available data in supporting proposed modes of carcinogenic action (IPCS, 1998). A well-crafted outline of the criteria applied in supporting hypotheses for mode of action will increase transparency in this new risk assessment process. Some suggestions for considering adequacy of evidence for a mode of action may also arise from other illustrative case studies applying these U.S. EPA guidelines (Barton *et al*., 1998; Bogdanffy *et al*., 1999, in press; Conolly *et al*., 1997, 1998). Inclusion of examples of the weighting of multiple modes of action would also be helpful in guideline revisions.

Due to the importance of mutagenicity as a potential mode of action, a generic framework for evaluation of mutagenicity studies also should be considered. Results of genetic toxicity studies in a large database, such as that available for $CHCl_3$, will inevitably be mixed. The manner in which the weight of evidence for genotoxicity should be evaluated and presented is not addressed in the guidelines. For the assessment of the genotoxicity of $CHCl_3$ and DCA, the panel used a comprehensive, quantitative weight of evidence approach to evaluate large, heterogeneous genetic toxicology databases published by the International Commission for Protection against Environmental Mutagens and Carcinogens—ICPEMC (Lohman *et al*., 1992). This evaluation scheme produces a numerical value that is easily compared. These scores are relative DNA reactivity scores. The maximum positive score is 100 and the maximum negative score is –100. For over 100 chemicals evaluated and classified on this basis, the highest positive score obtained was 49.7 (triazaquone) and the lowest negative score was –27.7 (ethanol). Other methods of evaluating the preponderance of evidence might also have been chosen.

Results from over 40 studies on $CHCl_3$ yield a quantitative net negative score (-14.3), indicating that the weight of evidence supports a non-genotoxic classification (Brusick *et al*., 1992; Lohman *et al*., 1992). The more limited database for DCA required direct evaluation of the results of individual studies. The weight of the evidence for the ability of DCA or its metabolites to induce effects on DNA did not support assigning a genotoxic mode of carcinogenic action to DCA, since the bulk of the mutagenicity data were negative or equivocal (Herbert *et al*., 1980; DeMarini *et al*., 1994; Matsuda *et al*., 1991; Meier, 1988). The panel noted that capacity of a particular chemical to cause genotoxicity is dependent on a variety of factors, e.g., dose/concentration of test material and the type of test system. The fact that a compound causes genotoxicity under some limited set of experimental conditions does not necessarily mean that carcinogenic effects of the compound would be related to mutagenicity. Ideally, the emphasis on mode of action in these cancer risk assessment guidelines should impact the nature of future genotoxicity testing. A weight of evidence evaluation of genotoxicity studies might then place more emphasis on studies conducted in the target organ of concern than on results from the overall battery of *in vitro* and *in vivo* tests.

Quantitative Risk Assessment Considerations

As described in the guidelines, agreement regarding the mode of action leads to specific options for dose response analysis in the range of observation and extrapolation. Dose response modeling is divided into two components: analysis in the range of observation and analysis in the range of extrapolation. In the range of observation, whenever data are considered sufficient, the guidelines prefer a biologically based dose response (BBDR) or case-specific dose-response (CSDR) model to relate dose to response. Otherwise, as a standard default procedure, a suite of models is fitted to the response data and a specific model chosen based on the fit to the data. The lower 95% confidence limit on a dose associated with an estimated 10% increased tumor incidence or a 10% increase in a nontumor response causally related to the carcinogenicity (LED_{10}) generally serves as the point of departure for extrapolating the relationship to environmental exposure levels. The guidelines specify that other points of departure may be more appropriate for certain data sets and may be used instead of the LED_{10}. With $CHCl_3$, the panel used an ED_{10}, a central estimate

of the dose associated with a 10% increase in tumor incidence (or nontumor precursor response). The panel believes it important to maintain flexibility in the choice of the point of departure on a case-by-case basis. In each case, the choice of a particular point of departure should be clearly supported in the body of the assessment.

Extrapolation to lower dose levels, if required, also relies on a BBDR or CSDR model if such models can be supported by sufficient data. Otherwise, default approaches are applied in accordance with the established or presumed mode of action of the agent. These options include approaches that assume linearity or non-linearity of the dose-response relationship or others that consider both possibilities. The default approach for linearity is to extend a straight line to the zero dose/zero response intercept. This process is a simplification of the linearized multistage (LMS) modeling recommended in EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986); the new default has been proposed to avoid a degree of sophistication associated with the LMS that is unwarranted for a default. The guidelines indicate that genotoxic compounds would have a linear dose-response relationship. This assertion may not necessarily be true and possibilities of non-linear, genotoxic modes of action as well as linear, non-genotoxic modes of action have to be considered. These approaches would have to be supported clearly in the arguments associated with defining mode of action. Flexibility in the guidelines will aid in insuring that all pertinent data are carefully considered in the dose-response assessment.

The default approach recommended by the guidelines for non-linearity is a margin of exposure (MOE) analysis rather than a model that attempts to estimate the probability of effects at low doses. This MOE approach has been introduced to accommodate cases in which there is sufficient evidence of a non-linear dose response, but not enough evidence to construct a mathematical model for the relationship. The MOE is likely to be the most commonly used method for non-linear modes of action and needs to be more carefully developed in the guidelines (see III.b. below).

Constructing toxicokinetic, case-specific, and biologically based dose response models. Toxicokinetic (TK) models are important in quantitatively organizing available data on absorption, distribution, metabolism, and elimination (Clewell and Andersen, 1985; Leung, 1991; Page *et al*., 1997). Thus, TK modeling is likely to be important in early stages of the evaluation of the relevant studies. For example, estimates of tissue dosimetry from different routes of administration may be required to infer the appropriate target tissue doses associated with toxicity/carcinogenicity and to help distinguish among possible modes of action. Although not emphasized in the proposed guidelines, the range of possible modes of action should influence the design of the TK, BBDR, or CSDR models that have a role in the quantitative portions of the risk assessment. TK models are most useful when the definition of mode of action specifies the appropriate target tissue dose metric. The TK model can then be formulated to predict these tissue doses. In the absence of knowledge of the metric for tissue dose, TK models can be used to assist in parent chemical defaults or in making inferences about the active form of the compound, as done with methylene chloride (Andersen *et al*., 1987). For $CHCl_3$, a likely mode of action was the accumulation of reactive, oxidized metabolites, leading to cell toxicity. This mode defines the measure of tissue dose, i.e., concentrations of metabolites produced through the oxidative pathway that will be calculated by the TK model. With DCA, the absence of a consensus on the mode of action is an impediment to defining the dose measure that would be estimated by a TK model with this compound.

In future revisions of the guidelines, it would be helpful to explicitly emphasize the concept that both a dose metric and a biological mechanism need to be captured in the mode of action statement (see Fig. 1). An advantage of a two-part, dose metric-toxic effect composite definition for the mode of action is that it aids in defining the defaults and preferred options for the quantitative risk assessment. The guidelines indicate that BBDR or CSDR models are preferred; neither is very well defined. Since both are dose-response models, the dose measure to be incorporated into these models should be specified, if possible, from the mode of action statement. Mutation rates or rates of cell division or cell death in the BBDR or CSDR model can then be related to relevant measures of dose.

CSDR models, by specifying the relevant dose metrics, would also require the risk assessor to justify the use of the default dose scaling of $bw^{0.75}$ that has become the EPA standard. The two-part definition also more clearly indicates the role of quantitative models in the risk assessment. Toxicokinetic (TK) models predict tissue dose metrics associated with a particular mode of action and BBDR models use input from TK models to predict responses at various exposure levels (Fig. 1). The approach with the $CHCl_3$ assessment was based on arguments from the mode of action to support a non-linear extrapolation and use of a TK model for predicting dose to tissue, measured as metabolized dose in target tissues. The tissue doses of $CHCl_3$ were used as the basis for modeling tumor outcome. They also serve as the starting point for extrapolation or for applying adjustment factors as specified in the guidelines.

The panel extended a physiologically based pharmacokinetic (PBPK) model for $CHCl_3$ developed by others (Borghoff *et al*., 1994; Corley *et al*. 1990; Gargas *et al*., 1990; Lilly, 1996) to calculate doses of $CHCl_3$ metabolites in the target tissues, i.e., the centrilobular regions of the liver acini and the renal cortex. These calculated doses of metabolites were correlated with tissue responses. The panel did not develop a BBDR or CSDR model for either compound and did not believe it was possible to construct such a model based on the data available at that time. Despite the recommendation for the use of BBDR or CSDR models in the guidelines, there are no examples where

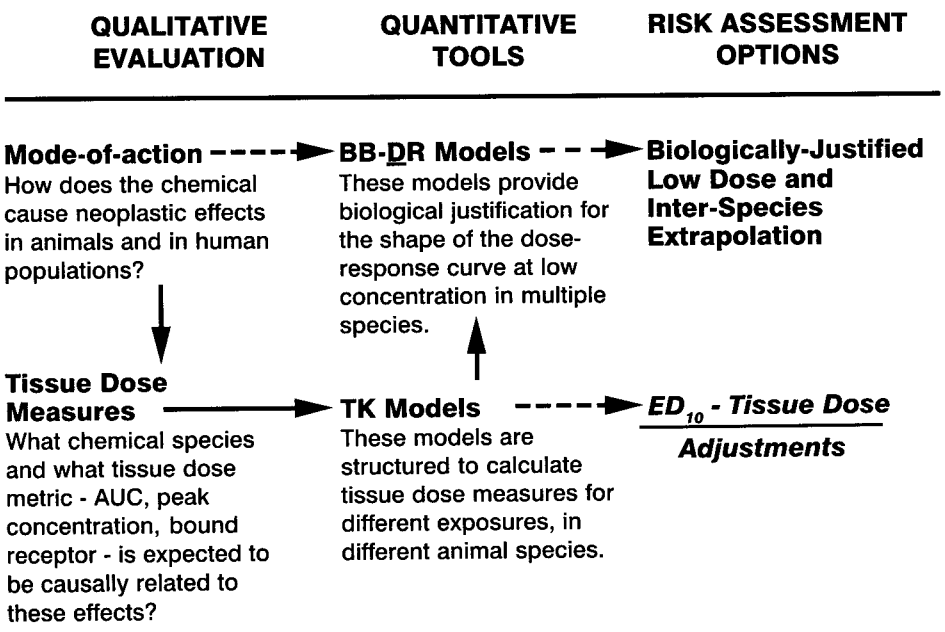

FIG. 1. Schematic of the qualitative and quantitative aspects of applying mode-of-action considerations in cancer risk assessment. An optimal approach consists of unambiguous characterization of the mode of action and the tissue dose associated with the toxic responses. In this case (Path 1), TK models estimate tissue dose over a range of exposure conditions and provide input into BB-DR models that predict precursor effect and tumor incidence from biological understanding of the processes involved. The process used with $CHCl_3$ (Path 2) produced estimates based on measures of tissue dose (see text).

such models have been formally adopted for cancer risk assessment.

Applying extrapolation models from the 'point of departure'. Simplification of the linear extrapolation model is welcomed and should help in more meaningfully informing the risk management community and the public concerning the lack of precision in cancer risk estimations that are based frequently on extrapolation over many orders of magnitude. Nonetheless, the product of the risk assessment (i.e., excess numbers of cases of cancer per unit of the population) will likely continue to be interpreted, particularly by the public, as implying a greater degree of precision than is warranted. The precision of these estimates varies from compound to compound. There is greater confidence in estimation of risk expressed as excess cases of cancer, when the difference between levels to which the general public is exposed and those that have induced adverse effects is within an order of magnitude, e.g., arsenic in drinking water (Clewell *et al*., 1998). Less confidence would be accorded to risk estimates for compounds that induce adverse effects in toxicological studies in animals at doses 6 to 7 orders of magnitude greater than the predicted exposure of humans (e.g., melamine) (U.S. EPA, 1988) Indeed, in this latter case there may well be no risk at relevant exposure concentrations. There is no provision in the current guidelines to reflect these different degrees of confidence in the database, either in determining the adequacy of the MOE or in pursuing linear extrapolation from the point of departure. The proposed guidelines state that, "The default assumption of linearity is also appropriate as the ultimate science policy default when evidence shows no DNA reactivity or other support for linearity but neither does it show sufficient evidence of a non-linear mode of action to support a non-linear procedure." Provision of a single option for these two very different situations, when the mode of action is clearly consistent with a linear default or when available data are simply inadequate to support a specific mode of action, does not reflect the degree of confidence in the accuracy of the extrapolation. Though controversial, the panel felt that there should be flexibility to permit adoption of a non-linear approach (rather than the default assumption of linearity) for analysis in the range of extrapolation, if the available data are consistent with non-linearity and inconsistent with direct genotoxicity, and even though there may be no clear consensus hypothesis of a specific mode of action. In fact, the panel's recommendations following review of the database on DCA provide such an example.

For DCA, a formal quantitative analysis of the dose response was not pursued, due to the lack of adequate studies to evaluate carcinogenicity quantitatively. For instance, the studies in which DCA-induced tumors appear to have used doses in excess of current definitions of maximally tolerated doses (Bucher *et al*., 1996; Foran, 1997). Because carcinogenicity was associated with target organ (liver) toxicity (Daniel *et al*., 1992; Sanchez and Bull, 1990), overt pharmacological responses and dose-dependent elimination (Cornett *et al*., 1997; Gonzalez-Leon and Bull, 1996; Gonzalez-Leon *et al*, 1997a,b), a linear approach to extrapolation did not appear to be appropriate for DCA. In addition, pharmacologic studies in humans at comparably high doses showed no evidence of hepatotoxicity (Stacpoole *et al*, 1979, 1997). A provisional MOE analysis was conducted to provide guidance for the prioritization of further work with DCA. The panel used the no-observed-adverse-effect level (NOAEL) for toxicity/carcinogenicity in the rodent studies (DeAngelo *et al*, 1991, 1996) and derived a MOE based on relatively crude estimates of human exposure

(IARC, 1995; WHO, 1996). The MOE estimated from this approach (greater than 1300) was considered adequate, based on the absence of tumors at doses of DCA that do not cause toxicity, pharmacologic actions, or impaired clearance.

The MOE approach does allow conservatism to be incorporated into the value adopted as the recommended MOE. A greater margin of exposure would be recommended in cases with these kinds of mechanistic uncertainties. This option, recommending an MOE to account for uncertainty in the mode of action, was preferred over the option currently in the guidelines that advocates adoption of both a linear and non-linear extrapolation in a case such as that for DCA. The panel believed that presentation of both approaches, as currently recommended in the guidelines, provides excessive credibility to the linear option, which would likely become the preferred choice for the risk manager. Cancer slope factors derived from the linear option give estimates of population risks that provide inappropriate risk-communication information to the public. The MOE does not provide an analysis as easily abused for estimating specific population risks.

Selection of the appropriate 'point of departure' for extrapolation. After considerable debate about the point of departure for extrapolation, the panel endorsed latitude in the selection of points of departure. With rich data sets, an LED_{10} utilizes more of the data and, together with the ED_{10}, provides an indication of variability. With sparse data sets, the LED_{10} becomes highly model-dependent and may give a greater impression of accuracy than merited. Depending on the data sets, either an ED_{10} or an LED_{10} might be preferred for the point of departure, and there should be flexibility for making this determination on a case by case basis. However, both central values and upper and lower confidence limits should always be presented. The panel strongly endorsed graphical depiction of the point of departure and confidence bounds. In some cases, use of a NOAEL or LOAEL might be desirable. The panel's analyses used a tissue dose-based ED_{10} for $CHCl_3$ and a NOAEL for DCA. The latter option was pursued with DCA since the information on dose-response was not sufficiently robust to establish a point of departure that offered any advantage over a NOAEL or LOAEL.

Use of quantal vs. continuous endpoints for risk assessment. In contrast to previous reliance on analysis of quantal endpoints (i.e., incidence of tumors), analysis of continuous precursor responses will become increasingly important under the new guidelines. Some issues, such as conversion of continuous data to quantal data (in the form of the number of animals showing an ″adverse″ change in a variable) or definition of adversity for changes in a continuous variable, such as cell proliferation for $CHCl_3$, have not been addressed in the proposed guidelines. Dose response curves for tumor incidence and those for induced cell proliferation with $CHCl_3$ were very similar (Health Canada, 1999). The panel's tumor-based ED_{10} was 71.3 mg $CHCl_3$ metabolized/liter kidney cortex/h for the increased incidence of kidney tumors in the Osborne-Mendel rat. This dose measure was derived from a composite PBPK model for $CHCl_3$ disposition (ILSI, 1997). An ED_{10}/LED_{10} for increased cell proliferation would have been the preferred point of departure, since cell proliferation, a secondary response following cytotoxicity, is considered to be an obligatory precursor for cancer. Cell proliferation data were reported as group means ± SD (Larson *et al*., 1994a, 1994b, 1995; Templin *et al*, 1996). Estimating an ED_{10} for cell proliferation requires defining a level of increase in cell proliferation in an individual animal that would be considered as adverse. Lacking the full data sets, the panel conducted the evaluation based on tumor incidence.

Providing a working definition of 'margin of exposure'. According to the guidelines, an MOE analysis is conducted when the mode of action dictates a non-linear approach in the absence of sufficient information for a BBDR or CSDR model. The MOE is calculated as the ED_{10}, the LED_{10}, or another point of departure divided by the "environmental exposure of interest," implying, obviously, that exposure data are available. The adequacy of the calculated MOE should then be evaluated in light of the uncertainties in both the exposure and the toxicity estimates. In the guidelines, the definition of "environmental exposures of interest" is the actual or projected exposures, or regulatory levels of specific interest with respect to a risk management requirement. Indeed, exposure and associated exposure-assessment uncertainties have to be considered in their own right in MOE evaluations. The U.S. EPA's published *Guidelines for Exposure Assessment and Risk Characterization Guidance* (U.S. EPA, 1992, 1995) provide guidance for these considerations. Full characterization of exposure of the general population to $CHCl_3$ and DCA was not within the scope of the charge to the panel. Exposure was discussed in a cursory fashion, more as a basis for acquiring experience in developing MOEs for the specific case studies than for establishing a precedent for these types of calculations. In this process, several questions became apparent. What criteria are used to determine representative exposures for the general population, to compare with calculated points of departure? Are these estimates media-specific or are they estimates of total exposure? Presumably, the answers vary depending upon the purpose of a particular assessment. Little consideration appears in the new guidelines regarding close integration of exposure estimates into the MOE. Confidence in the exposure assessment is an absolutely critical component in determining adequacy of the MOE. A smaller MOE might be acceptable if estimates of exposure were necessarily based on worst-case considerations. However, a small MOE would be of considerable concern if estimates of exposure were relatively certain. The guidelines correctly indicate that characterization of exposure should include a description of the strengths and limitations (uncertainties) of the data and methods. These same

limitations, related to exposure characterization, should be factored into evaluating the adequacy of the MOE.

Use of toxicokinetic models to assist in margin of exposure analysis. TK models can be applied to calculate the tissue dose associated with a toxic or carcinogenic response. These models estimate tissue doses at the point of departure in the animals and at human exposure levels. With $CHCl_3$, the point of departure was expressed in terms of the rate of formation of reactive metabolites per volume target tissue in the liver or kidney. A target tissue dose using the same metric could also be calculated for ambient exposures in humans. The ratio between the tissue dose at the point of departure in the animals and the tissue dose at ambient human exposure levels becomes the MOE. This approach was adopted in the panel's work on $CHCl_3$. Alternatively, the tissue dose in animals at the point of departure could have been converted to an administered dose equivalent and compared to the human exposure level, a procedure that accounts for animal but not human toxicokinetics. The procedure based on tissue dose has the advantage of allowing the evaluation of multiple exposure routes in the human exposure assessment. The administered-dose approach could give incorrect estimates for compounds, such as DCA and $CHCl_3$, which have dose-dependent pharmacokinetic behavior near the point of departure for the extrapolation.

Risk Management Considerations

Blurring the Distinction between Risk Assessment and Risk Management

The purpose of an MOE analysis is to provide the risk manager with all available information on how much reduction in risk may be associated with reduction in exposure from the point of departure. This background information supports the risk manager's decision of an acceptable MOE for the statute guiding a particular decision. It is stated that: "A margin of exposure analysis explains the biological considerations for comparing the observed data with the environmental exposure levels of interest and helps in deciding on an acceptable level of exposure in accordance with applicable management factors." While this evaluation is considered to fall under risk management, "the risk assessor is responsible for providing scientific rationale to support the decision." Thus, contrary to previous proposals to clearly delineate risk assessment and risk management (NRC, 1983, 1994), these new guidelines actually blur the distinction between them. More responsibility is now placed in the hands of the risk manager to understand the uncertainties of the chemical's database. The risk manager has to evaluate the adequacy of the MOE against various factors; almost all of these decisions would be best informed by the risk assessment.

The guidelines delineate some of the factors that are to be considered in assessing adequacy of the MOE. They include the slope of the dose-response curve at the point of departure, the nature of the response (i.e., precursor lesion, frank toxicity or tumors), the nature and extent of human variability, the persistence of the agent in the body, and the relative species-sensitivity between humans and experimental animals (interspecies variability). If human variability and relative species-sensitivity cannot be estimated from available data, each should be considered to be at least 10 fold. However, the guidelines specify that it should not be assumed that "numerical factors are the sole components for determination of an acceptable margin of exposure." In the context of these particular factors, the perceived role of the risk manager in determining the adequacy of the MOE is unclear. Indeed, the risk assessors should address these issues to ensure that the toxicological database is adequately and consistently taken into account in the development of recommended standards, to insure protection of public health. For example, most risk managers are unlikely to be trained to consider the sufficiency of biological data necessary to replace defaults. Some obvious factors do fall into the category of risk management, including sociopolitical aspects related to public perceptions concerning adversity of particular types of effects.

In practice, the guidelines will result in departure from current practices. Presently, quantitative risk-management goals are provided as concentrations or levels considered acceptable by the risk assessor to the risk manager. This change in procedure will likely create concerns among risk managers who may be uncomfortable in fulfilling this vaguely defined new role. While it is not entirely clear how the transfer of these responsibilities to risk managers will be realized in practice, the guidelines, at a minimum, necessitate greater interaction between risk assessors and risk managers. While this discourse should be beneficial in ensuring that risk assessments are better tailored to meet the needs of risk management, it will present a challenge to the integrity of the risk assessment process, which needs to consider the relevant scientific aspects in determining adequacy of the MOE.

Striving for Consistency in Cancer and Noncancer Risk Assessment

One of the implications of the proposed guidelines' emphasis on mode of action and the potential for use of precursor lesions (i.e., noncancer endpoints) is the convergence of cancer and noncancer risk-assessment approaches. For example, where carcinogenicity is secondary to another type of toxicity, the MOE analysis should be similar to approaches used for these noncancer responses. The potential to use similar points of departure for dose response analysis with cancer and noncancer effects also offers greater opportunity for congruence. Increasing use of the benchmark dose/benchmark concentration in noncancer risk assessment dovetails with the recommended point of departure for cancer (i.e., the ED_{10} or LED_{10}). The use of the LED_{10} is essentially a benchmark dose for cancer.

The need for harmonization of cancer and noncancer approaches has been recognized (Barton and Andersen, 1998,

Conolly, 1995). The cancer risk assessment approach in the United States differs from that of other countries, where risk characterization is based on a limiting critical effect, either cancer or some other endpoint, and determined based on consideration of the entire data set. This approach not only conserves resources, but permits integration across endpoints in cases where cancer and noncancer effects share common modes of action. The present practice in the United States with noncancer endpoints uses a point of departure (BMD, NOAEL, LOAEL, etc.) and applies dosimetric corrections (U.S. EPA, 1994) and uncertainty factors (UCFs) to derive exposure recommendations that should be without significant public health consequences. The introduction of these uncertainty factors occurs in the risk-assessment process itself, and follows well defined but still evolving procedures (Dourson and Stara, 1983; Dourson *et al*, 1992, 1996).

For noncancer assessments, UCFs are selected based on judgment by experts within the EPA. In general, the factors used by EPA are intended to account for the specific areas of uncertainty (Dourson, 1994). The list below recapitulates U.S. EPA policy. They are described here to show consistencies and inconsistencies in applying the factors to cancer and noncancer endpoints.

- Intrahuman: 10-fold when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans
- Animal to human: For reference doses (RfDs), 10-fold when extrapolating from valid results of long-term studies on experimental animals. For reference concentrations (RfCs), reduced to 3-fold when a NOAEL-human equivalent concentration (HEC) used as basis of estimate.
- Subchronic to chronic: 10-fold when extrapolating from less than chronic results on experimental animals.
- LOAEL to NOAEL: 10-fold when deriving an RfC or from a LOAEL instead of a NOAEL
- Incomplete database: 10-fold factor when extrapolating from valid results in experimental animals when the data are "incomplete"

Several of these factors may not be relevant for cancer. For example, the LOAEL to NOAEL extrapolation is not necessary in most cases since data should be sufficient to derive a modeled estimate of the point of departure. However, with compounds such as DCA, for which available data on the dose response for cancer are exceedingly poor, even a factor of this kind may be relevant.

An intermediate approach was taken by the panel with $CHCl_3$ in which an attempt was made to specify the combination of factors that need to be considered in arriving at a composite value for the MOE. The ED_{10} for tumors was used as the starting point. A factor of 10 was considered sufficient to address interindividual variation in determining the adequacy of the margin of exposure. For interspecies variation, a factor of 3.1 was considered sufficient, because the starting point is a measure of tissue dose and PBPK models are available for estimating tissue doses in exposed humans. An additional factor to account for severity of effect was considered inappropriate because the dose response curve for tumors closely paralleled that for the intermediate endpoint. While specific toxicokinetic calculations of human tissue doses were not performed by the panel, the acceptable tissue dose (71.3 mg $CHCl_3$ metabolized/liter kidney cortex/h divided by the proposed uncertainty factor) would be used with a human PBPK model to estimate the maximal exposure level. While the panel did not complete development of a human PBPK model, there appears to be sufficient data to allow development of such a model that would incorporate parameters related to the distribution and activity of the $CHCl_3$-metabolizing CYP2E1 in human liver and kidney. The approach adopted by the panel for $CHCl_3$ may provide a useful example in future revisions of the guidelines in this regard. However, it is important to emphasize that, where dosimetric adjustments are made to the point of departure, this must also be taken into consideration in evaluating the MOE (i.e., a value of less than 10 would be adequate to address interspecies variability). This implementation of the interspecies uncertainty considerations, analogous to the interspecies UCF with noncancer endpoints, is consistent with the approach used in the dosimetry corrections with the RfC calculations (U.S. EPA, 1994).

As the proposed guidelines are applied to a larger number of compounds, such as the work with vinyl acetate and formaldehyde (Bogdanffy *et al*., 1999; Conolly *et al*., 1997, 1998), more detailed guidance can be developed on the components necessary for determining the adequacy of MOE values. Cancer risk assessment methodology should be continually updated as experience is gained with noncancer RfD and RfC evaluations, particularly as noncancer uncertainty factors evolve from a "presumed protective default" to predictive values derived from evaluations of relevant toxicity data bases (Dourson *et al*., 1996; Renwick, 1993). Revisions to these guidelines should reflect recent developments in noncancer risk assessment, contributing to greater consistency between cancer and noncancer assessments.

Developing a Library of Case Studies

Experience gained from exercises conducted by panels such as this one and others should help in establishing a case library to facilitate future improvements of these guidelines. However, there is a potential drawback to an arrangement where outside groups bring assessments to the EPA for comment and concurrence. If agency personnel have not wrestled with the guidelines and adopted specific approaches for the quantitative assessments, they may be in a position to criticize efforts of other groups without providing a clear expectation of the contents required in a quantitative assessment under these new guidelines. Therefore, it would be helpful to the evolution of these guidelines if EPA

staff also were to conduct several quantitative case studies. Joint efforts of EPA staff and outside parties could also be very helpful by bringing diverse perspectives to these case studies. Such efforts will provide EPA valuable experience in deciding when a mode of action has sufficient scientific support and in assessing the appropriate MOE to be used for specific cases. This panel's experience emphasizes the value derived from working with the issues in the guidelines and carrying specific quantitative examples through the process. Another valuable lesson from conducting these case studies is the ability to define more clearly the nature of the data needs for quantitative mode of action-based risk assessment. The experience with case studies conducted by multiple stakeholders should greatly refine our conceptions of the types of hazard identification and dose-response studies that provide optimal information for mode of action-based risk assessment. This experience should be useful in guiding experimental design and also in guiding EPA and others in investing in toxicology and dose-response assessment related research.

In the introductory section of the proposed guidelines, under "Weighting Evidence of Hazard," the administrator of the U.S. EPA writes, "In this proposal, decisions come from weighing all the evidence. This change recognizes the growing sophistication of research methods, particularly in their ability to reveal modes of action of carcinogenic agents at cellular and subcellular levels as well as toxicokinetic and metabolic processes. The effect of the change on the assessment of individual agents will depend greatly on the availability of new kinds of data on them, in keeping up with the state of the art. If these new data are not forthcoming from public and private research on agents, assessments under these guidelines will not differ significantly from assessments under former guidance" (U.S. EPA, 1996). The converse is also true. If compelling data are forthcoming and assessments do not differ in any significant manner from defaults, there will be little support for these studies in the future. A partnership needs to exist where case studies completed by agency personnel and those completed by outside panels and groups would be jointly evaluated to distill the lessons learned and to facilitate future mode of action based assessments.

The two compounds evaluated by the ILSI HESI panel provide interesting examples for this library of case studies. With chloroform, there was both compelling evidence for a non-linear mode of action and available data for developing a TK model for use in quantitative dose-response evaluations. DCA, however, lacks convincing evidence for a consensus mode of action, although there is a body of evidence showing various precursor responses in the liver. The panel's judgment was that this compound should be treated by non-linear dose-response models, while retaining the flexibility to apply uncertainties in mode of action to influence the MOE calculations.

Consistency of Mode of Action and Epidemiological Data

Since the publication of the panel's report, the U.S. EPA has published a NODA (notice of data availability) for several disinfection byproducts (DBPs) including $CHCl_3$ (U.S. EPA, 1998). In this document, EPA proposed a nonzero maximum-contaminant-level goal (MCLG) for $CHCl_3$. The basis of this action was a thorough review of available literature and included reference to the conclusions of the ILSI HESI panel report. This NODA and its recommendations generated significant controversy (U.S. EPA, 1998a,b). The controversy was partially due to the epidemiological observation that some populations exposed to water containing DBPs, which include $CHCl_3$, appear to have increased risks of certain tumors, including bladder and colorectal cancer (Cantor *et al*., 1987; Cragle *et al*., 1985; Young *et al*., 1987; McGeehin *et al*., 1993; King and Marrett, 1996; Doyle *et al*., 1997; Freedman *et al*., 1997; Hildesheim *et al*., 1998; Cantor *et al*., 1998). Since the circulation of the initial NODA and public comment, EPA has withdrawn the proposed non-zero MCLG, on the basis of various procedural and risk management considerations (U.S. EPA, 1998b).

While evaluation of the epidemiological data was not included in the scope of our deliberations, the debate engendered by the NODA is an important controversy that will influence the continued development of the new carcinogen risk-assessment guidelines. The panel recognizes that the dosimetry and mode of action data for $CHCl_3$ from animal studies do need to be considered in light of epidemiological studies. However, the converse is also true—epidemiological inferences have to be interpreted in light of compelling toxicity data from other species. The conclusion from animal studies, completely justified and unanimously supported by the panel, is that toxicity is a prerequisite to the induction of tumors by $CHCl_3$. In the absence of high-dose, overt toxicity, $CHCl_3$ poses no significant carcinogenic risk to humans. The concentrations of $CHCl_3$ found in drinking water are very much below concentrations associated with toxicity. Importantly, the target sites in animals for $CHCl_3$ toxicity and carcinogenicity are not the sites where excess risks have been found in the human populations exposed to water containing DBPs. In addition, no other mechanistic data indicate that intestinal or bladder tissues would be targets in humans due to specific levels of enzymes or accumulation of toxic metabolites. Increased cancer incidence in these populations may be related to factors including, but not limited to, water disinfection by-products. However, the animal studies unanimously indicate that this increase is unlikely to be associated directly with $CHCl_3$ exposures.

The toxicology of the various chemical classes of DBPs are sufficiently distinct from each other that $CHCl_3$ cannot be considered a good representative of all of these chemicals. Some brominated THMs can cause tumors in the large intestine in rats. Information gleaned from toxicity studies of all the various THMs should be incorporated into the design of future

epidemiological studies of human populations exposed to DBPs. As a starting point, better interaction between toxicologists and epidemiologists in study design and reporting could provide more emphasis on the consistencies and discrepancies between animal studies and observations in human populations. This type of collaboration could aid in formulating testable hypotheses regarding the role of specific DBPs in human disease or the possibility that interactions occur among the various DBPs. The panel's evaluations clearly support the recommendation of a nonzero MCLG for $CHCl_3$. However, the panel did not evaluate other THMs and made no conclusions regarding appropriate risk assessment approaches or exposure limits for these compounds. For regulation of $CHCl_3$ alone, we emphasize that non-linear extrapolation is appropriate and a nonzero MCLG would be entirely in order.

SUMMARY

In this perspective, the ILSI HESI panel has reflected on its experience in applying the proposed EPA guidelines for carcinogen risk assessment to $CHCl_3$ and DCA. The panel consisted of ten experts from diverse fields in toxicology and risk assessment. The practical experience of several members in risk assessment was particularly useful in our deliberations. The flexibility of the guidelines concerning mode of action and encouraging utilization of these data for dose response assessments is a marked improvement and a welcome change. These guidelines serve as a sound template for incorporating state-of-the-art mode of action data into carcinogen risk assessments. Some points in the guidelines require further development, including more careful linking of dosimetry with the biological mode of action. Factors influencing calculations of the MOE for carcinogens are vaguely enumerated in the guidelines and are not closely tied to current practices in noncancer risk assessment. Better coordination of these cancer guidelines with practices for noncancer risk assessment should improve the quantitative dose response assessments and MOE assessments for carcinogens. Expert panel efforts such as this one will incrementally develop a case library to guide future cancer risk assessments. The value of participation of a broadly based collection of experts in these early assessments provides a learning experience for the agency in conducting the assessments and for panel members in appreciating the breadth of disciplines required to complete a contemporary cancer risk assessment. It is hoped that the EPA itself might complete risk assessments on specific compounds in the early stages of guideline development in order to take an informed, proactive stance on specific issues related to guideline implementation. While some quantitative considerations remain to be clarified in revision, the essential flexibility in assessing new scientific data present in these guidelines should not be compromised.

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REFERENCES

Ade, P., Guastadisegni, C., Testai, E., and Vittozzi, L. (1994). Multiple activation of chloroform in kidney microsomes from male and female DBA/2J mice. *J. Biochem. Toxicol.* **9**, 289–295.

Andersen, M. E., Clewell III, H. J., Gargas, M. L., Smith, F. A., and Reitz, R. H. (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* **87**, 185–205.

Barton H. A., Andersen M. E. and Clewell III, H. J., (1998). Harmonization: Developing consistent guidelines for applying mode of action and dosimetry information to cancer and noncancer risk assessment. *Hum. Ecol. Risk Assess.* **4**, 75–115.

Bogdanffy, M. S., Sarangapani,R., Plowchalk, D. R., Jarabek, A., and Andersen, M. E. (1999). A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. *Toxicol. Sci.* **51,** 19–35.

Borghoff, S. J., Murphy, J. E., and Dix, K. (1994). Validation of a chloroform dosimetry model for male and female F-344 rats (abstract). *Toxicologist* **14**, 43.

Brusick, D. J., Ashby, J., de Serres, F. J., Lohman, P. H. M., Matsushima, T., Matter, B. E., Mendelson, M. L., Moore II, D. H., Nesnow, S., and Waters, M. D. (1992). A method for combining and comparing short-term genotoxicity test data (A report from ICPEMC, Committee I). *Mutat. Res.* **266**, 1–6.

Bucher, J. R., Portier, C. J., Goodman, J. I., Faustman, E. M., and Lucier, G. W. (1996). National Toxicology Program Studies: Principles of dose selection and applications to mechanistic-based risk assessment. *Fundam. Appl. Toxicol.* **31**, 1–8.

Cantor, K. P., Hoover, R., and Hartage, P. (1987). Bladder cancer, drinking water source, and tap water consumption: A case control study. *J. Natl. Cancer Soc.* **79**, 1269–1279.

Cantor, K. P., Lynch, C. F., Hildesheim, M., Dosemeci, M., Lubin, J., Alavanja, M., and Craun, G. F. (1998). Drinking water source and chlorination by-products: I. In risk of bladder cancer. *Epidemiology* **9**, 21–28.

Clewell III, H. J., and Andersen, M. E. (1985). Risk assessment extrapolations and physiological modeling. *Toxicol. Ind. Health* **1**, 111–131.

Clewell, H. J., Gentry, P. R., Barton, H. A., Shipp, A. M., Yager, J. W., and Andersen, M. E. (1998). Requirements for a biologically realistic cancer risk assessment for inorganic arsenic. *Int. J. Toxicol.* **18**, 131–147.

Conolly, R. B. (1995). Cancer and noncancer risk assessment: Not so different if you consider mechanisms. *Toxicology* **102**, 179–188.

Conolly, R. B., Casanova, M., and Heck, H. d'A. (1997). Formaldehyde dose-response assessments using DNA–protein cross-link and cell replication data. Poster presented at 1997 Society for Risk Analysis Annual Meeting, Washington, DC.

Conolly, R.B. and Preston, R.B. (1998). Relative roles of cytolethality and mutagenicity in the carcinogenicity of formaldehyde. Poster presented at 1998 Society for Risk Analysis Annual Meeting, Phoenix.

Corley, R. A., Mendrala, A. L., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., and Reitz, R. H. (1990). Development of a physiologically based pharmacokinetic model for chloroform. *Toxicol. Appl. Pharmacol.* **103**, 512–527.

Cornett, R., Yan, Z., Henderson, G., Stacpoole, P. W., and James, M. O. (1997). Cytosolic biotransformation of dichloroacetic acid (DCA) in the Sprague-Dawley rat (Abstract). *Fundam. Appl. Toxicol.*(suppl. 36), 318.

Counts, J. L., and Goodman, J. I. (1995). Principles underlying dose selection for, and extrapolation from the carcinogen bioassay: Dose influences mechanism. *Regul. Toxicol. Pharmacol.* **21**, 418–421.

Cragle, D. L., Shy, C. M., Struba, R. J., and Siff, E. J. (1985). A case-control study of colon cancer and water chlorination in North Carolina. In *Water Chlorination: Chemistry, Environmental Impact and Health Effects* . R. L.Jolley, R. J.Bull, and W. P. Davis (Eds.), pp. 153–159.Lewis Publishers, Chelsea, MI.

Daniel, F. B., DeAngelo, A. B., Stober, J. A., Olson, G. R., and Page, N. P. (1992). Hepatocarcinogenicity of chloral hydrate, 2-chloroacetaldehyde, and dichloroacetic acid in the male B6C3F1 mouse. *Fundam. Appl. Toxicol.* **19**, 159–168.

DeAngelo, A. B., Daniel, F. B., Most, B. M., and Olson, G. R. (1996). The carcinogenicity of dichloroacetic acid in the male Fischer 344 rat. *Toxicology* **114**, 207–221.

DeAngelo, A. B., Daniel, F. B., Stober, J. A., and Olson, G. R.. (1991). The carcinogenicity of dichloroacetic acid in the male B6C3F1 mouse. *Fundam. Appl. Toxicol.* **16**, 337–347.

De Biasi, A, Sbraccia, M., Keizer, J., Testai, E., and Vittozzi, L. (1992). The regioselective binding of CHCl3 reactive intermediates to microsomal phospholipids. *Chem. Biol. Interact*. **85**, 229–242.

de Groot, H., and Noll, T. (1989). Halomethane hepatotoxicity: Induction of lipid peroxidation and inactivation of cytochrome P-450 in rat liver microsomes under low oxygen partial pressure. *Toxicol. Appl. Pharmacol.* **97**, 530–537.

DeMarini, D. M., Perry, E., and Shelton, M. L. (1994). Dichloroacetic acid and related compounds: induction of prophage in *E.coli* and mutagenicity and mutation spectra in Salmonella TA100. *Mutagenesis* **9**, 429–437.

Dourson, M. L. (1994). Methodology for establishing oral reference doses (RfDs). In *Risk Assessment of Essential Elements* (W. Mertz, C. O. Abernathy, and S. S. Olin, Eds.), pp. 51–61. ILSI Press, Washington, DC.

Dourson, M. L., Felter, S. P., and Robinson, D. (1996). Evolution of science-based uncertainty factors in noncancer risk assessment. *Reg. Toxicol. Pharm.* **24**, 108–120.

Dourson, M. L., Knauf, L. A., and Swartout, J. C. (1992). On reference dose (RfD) and its underlying toxicity database. *Toxicol. Ind. Health* **8**, 171–189.

Dourson, M. L., and Stara, J. F. (1983). Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol.* **3**, 224–238.

Doyle, T. J., Zheng, W., Cerhan, J. R., Hong, C. P., Sellers, T. A., Kushi, L. H., and Folsom, A. R. (1997). The association of drinking water source and chlorination by-products with cancer incidence among postmenopausal women in Iowa: A prospective cohort study. *Am. J. Pub. Health* **87**, 7.

Foran, J. A. (1997). Principles for the selection of doses in chronic rodent bioassays. ILSI Risk Science Working Group on Dose Selection. *Environ. Health Perspect.* **105**, 18–20.

Freedman, M., Cantor, K. P., Lee, N. L., Chen, L. S., Lei, H. H., Ruhl, C. E., and Wang, S. S. (1997). Bladder cancer and drinking water: A population-based case-control study in Washington County, Maryland (United States). *Cancer Causes and Control* **8**, 738–744.

Gargas, M. L., Clewell, H. J. III, and Andersen, M. E. (1990). Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes, and chloroethylenes in the rat. *Inhal. Toxicol.* **2**, 295–319.

Gonzalez-Leon, A., and Bull, R. J. (1996). Continuous treatment with dichloroacetate in drinking water alters its metabolism in rats but not in mice (Abstract). *Fundam. Appl. Toxicol.***1108**(Suppl. 30), 217.

Gonzalez-Leon, A., Schultz, I. R., and Bull, R. J. (1997a). Species differences in the toxicokinetics of dichloroacetate and trichloroacetate in F344 rats and B6C3F1 mice after prolonged administration in drinking water. *Fundam. Appl. Toxicol.* (Abstract) **168**(Suppl. 36), 33.

Gonzalez-Leon, A., Schultz, I. R., Xu, G., and Bull, R. J. (1997b). Pharmacokinetics and metabolism of dichloroacetate in the F344 rat after prior administration in drinking water. *Toxicol. Appl. Pharmacol.* **146**, 189–195.

Green, T. (1983). The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium. *Mutat. Res.* **118**, 277–288.

Health Canada (1999). Supporting Documentation for the Health Assessment of Chloroform as a Priority Substance under the Canadian Environmental Protection Act. Ottawa, Ontario, Canada.

Herbert, V., Gardner, A., and Colman, N. (1980). Mutagenicity of dichloroacetate, an ingredient of some formulations of pangamic acid (trade-named "vitamin B15"). *Am. J. Clin. Nutr*. **33**, 1179–1182.

Hildesheim,M. E., Cantor, K. P., Lynch, C. F., Dosemeci, M., Lubin, J., Alavanja, M., and Craun, G. F. (1998). Drinking water source and chlorination byproducts: II. Risk of colon and rectal cancers. *Epidemiology* **9**, 29–35.

International Agency for Research on Cancer (1995). Monographs on the evaluation of carcinogenic risks to humans, Vol.63: Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals. IARC, Lyon, France.

International Life Sciences Institute (ILSI, 1997). An evaluation of EPA's "Proposed Guidelines for Carcinogen Risk Assessment" using chloroform and dichloroacetate as case studies: Report of an expert panel. ILSI HESI, Washington, DC.

International Programme on Chemical Safety (IPCS) (1994). Environmental health criteria 163: Chloroform. International Programme on Chemical Safety, WHO, Geneva.

International Programme on Chemical Safety (IPCS, 1998). Cancer Risk Assessment, 1998 Hannover Workshop Report. World Health Organization, Geneva.

King, W. D., and Marrett, L. D. (1996). Case-control study of bladder cancer and chlorination by-products in treated water (Ontario, Canada). *Cancer Causes Control* **7**, 596–604.

Jorgenson, T. A., Meierhenry, E. F., Rushbrook, C. J., Bull, R. J., and Robinson, M. (1985). Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3Fl mice. *Fundam. Appl. Toxicol.* **5**, 760–769.

Larson, J. L., Wolf, D. C., and Butterworth, B. E. (1994a). Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: Comparison of administration by gavage in corn oil vs. *ad libitum* in drinking water. *Fundam. Appl. Toxicol.* **22**, 90–102.

Larson, J. L., Wolf, D. C. Morgan, K. T., Méry, S., and Butterworth, B. E. (1994b). The toxicity of 1-week exposures to inhaled chloroform in female B6C3F1 mice and male F-344 rats. *Fundam. Appl. Toxicol.* **22**, 431–446.

Larson, J. L., Wolf, D. C., and Butterworth, B. E. (1995). Induced regenerative cell proliferation in liver and kidneys of male F-344 rats given chloroform in corn oil by gavage or *ad libitum* in drinking water. *Toxicology* **95**, 73–86.

Leung, H. (1991). Development and utilization of physiologically based pharmacokinetic models for toxicological applications. *J. Toxicol. Environ. Health* **32**, 247–267.

Lilly, P. D. (1996). A physiologically based toxicity model of orally administered bromodichloromethane. Submitted Ph.D. thesis, University of North Carolina at Chapel Hill, NC.

Lohman, P. H. M., Mendelsohn, M. L., Moore II, D. H., Waters, M. D., Brusick, D. J., Ashby. J., and Lohman W. J. A. (1992). [ICPAEMC report:] A method for comparing and combining short-term genotoxicity test data: The basic system. *Mutat. Res.* **266**, 7–25.

Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., and Ames, B. N. (1985). Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. *Mutat. Res.* **148**, 25–34.

Matsuda, H., Ose, Y., Nagase, H., Sato, T., Kito, H., and Sumida, K. (1991). Mutagenicity of ozonation and chlorination products from p-hydroxybenzaldehyde. *Sci. Total Environ.* **103**, 141–149.

McGeehin, M. A., Reif, J. S., Becher, J. C., and Mangione, E. J. (1993). Case-control study of bladder cancer and water disinfection methods in Colorado. *Am. J. Epidemiol.* **138**, 492–501.

Meier, J. R. (1988). Genotoxic activity of organic chemicals in drinking water. *Mutat. Res.* **196**, 211–245.

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

National Research Council (NRC, 1994). *Science and Judgement in Risk Assessment*. National Academy Press, Washington, DC.

Page, N., Singh, D. V., Farland, W., Goodman, J. I., Conolly, R. B., Andersen, M. E, Clewell, H. J., Frederick, C. B., Yamasaki, H., and Lucier, G. (1997). Implementation of EPA revised cancer risk assessment guidelines: Incorporation of mechanistic and pharmacokinetic data. *Fundam. Appl. Toxicol.* **37**, 16–3

Pegram, R. A., Andersen, M. E., Warren, S. H., Ross, T. M., and Claxton, L. D. (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in *Salmonella typhimurium*: Contrasting results with bromodichloromethane and chloroform. *Toxicol. Appl. Pharmacol.* **144**, 183–188.

Pohl, L. R., Bhooshan, B., Whittaker,N. F., and Krishna, G. (1977). Phosgene: A metabolite of chloroform. *Biochem. Biophys. Res. Commun.* **79**, 684–691.

Pohl L. R., and Krishna, G. (1978). Deuterium isotope effect in bioactivation and hepatotoxicity of chloroform. *Life Sci.* **23**, 1067–1072.

Renwick, A. G. (1993). Data-derived safety factors for evaluation of food additives and environmental contaminants. *Food Addit. Contam.* **10**, 275–305.

Risk Policy Report (1998a). Chloroform controversy raises major science, risk policy stakes. (D. Clarke, Ed.) *Inside Washington* **5**, 18–19.

Risk Policy Report (1998b). Chloroform call sparks scientific concerns about EPA risk policies. (S. Gibb, Ed.), *Inside Washington* **5,** 3–7.

Sanchez, I. M., and Bull, R. J. (1990). Early induction of reparative hyperplasia in B6C3F1 mice treated with dichloroacetate and trichloroacetate. *Toxicology* **64**, 33–46.

Sasaki, Y., and Endo, R. (1978). Mutagenicity of aldehydes in Salmonella. *Mutation Res.* **54**, 251–252.

Sayato, Y., Nakamuro, K., and Ueno, H. (1987). Mutagenicity of products formed by ozonation of naphthoresorcinol in aqueous solutions. *Mutat. Res.* **189**, 217–222.

Stacpoole, P. W., Henderson, G. N., Yan, Z., Cornett, R., and James, M. O. (1997). Pharmacokinetics, metabolism, and toxicology of dichloroacetate. *Drug Metab. Rev*. **30,** 499–539.

Stacpoole, P. W., Moore, G. W., and Kornhauser, D. M. (1979). Toxicity of chronic dichloroacetate. *N. Engl. J. Med.* **300**, 372.

Templin, M. V., Larson, J. L. Butterworth, B. E., Jamison, K. C., Leininger, J. R., Mery, S., Morgan, K. T. Wong, B. A., and Wolf, D. C. (1996). A 90-day chloroform inhalation study in F-344 rats: Profile of toxicity and relevance to cancer studies. *Fundam. Appl. Toxicol.* **32**, 109–125.

Testai, E., Di Marzio, S., and Vittozzi, L. (1990). Multiple activation of chloroform in hepatic microsomes from uninduced B6C3Fl mice. *Toxicol. Appl. Pharmacol*. **104**, 496–503.

Testai, E., and Vittozzi, L. (1986). Biochemical alterations elicited in rat liver microsomes by oxidation and reduction products of chloroform metabolism. *Chem. Biol. Interact.* **59**, 157–171.

Thier, R., Taylor, J. B., Pemble, S. E., Humphreys, W. G., Persmark, M., Ketterer, B., and Guengerich F. P. (1993). Expression of mammalian glutathione-S-transferase 5–5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to halomethanes. *Proc. Natl. Acad. Sci. U S A* **90**, 8576–8580.

Tomasi, A., Albano, E., Biasi, F., Slater, T. F., Vannini, V., and Dianzani, M. U. (1985). Activation of chloroform and related trihalomethanes to free radical intermediates in isolated hepatocytes and in the rat *in vivo* as detected by the ESR-spin trapping technique. *Chem. Biol. Interact*. **55**, 303–316.

U.S. Environmental Protection Agency (1986). Guidelines for carcinogen risk assessment. *Fed. Reg.* **51**, 33992–34003.

U.S. Environment Protection Agency (1988). Final rule on melamine: toxic chemical release reporting; community right to-know. *Fed. Reg.* **53**, 23128–23133.

U.S. Environment Protection Agency (1992). Guidelines for exposure assessment. *Fed. Reg.* **57**, 22888–22938.

U.S. Environmental Protection Agency (1994). "Methods for the Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry." U.S. EPA, EPA/600/8–90/066F.

U.S. Environmental Protection Agency (1995). EPA risk characterization policy and guidance. Science Policy Council, March 1995, Washington, DC.

U.S. Environmental Protection Agency (1996). Proposed guidelines for carcinogen risk assessment: Notice. *Fed. Reg.* **61**, 17960–18011.

U.S. Environmental Protection Agency (1998a). National primary drinking water regulations: Disinfectants and disinfection by-products, notice of data availability; proposed rule. *Fed. Reg.* **63**, 15674–15692.

U.S. Environmental Protection Agency (1998b). National primary drinking water regulations: disinfectants and disinfection byproducts; final rule. *Fed. Reg.* **63**, 69389–69476.

Wolf, C. R., Mansuy, D., Nastainczyk, W., Deutschmann, G., and Ullrich, V. (1977). The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450. *Mol. Pharmacol.* **13**, 698–705.

World Health Organization (WHO) (1996). *Guidelines for Drinking Water Quality*, 2nd ed., Vol.2. WHO, Geneva.

Yamaguchi, T., and Nakagawa, K. (1993). Mutagenicity of formation of oxygen radicals by trioses and glyoxal derivatives. *Agric. Biol. Chem.* **47**, 2461–2465.

Young, T. B., Wolf, D. A., and Kanarek, M. S. (1987). Case-control study of colon cancer and drinking water trihalomethanes in Wisconsin. *Int. J. Epidemiol.* **16**, 190–197.