Mode of Action Frameworks in Toxicity Testing and Chemical Risk Assessment

Mary Elizabeth Meek

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Mode of Action Frameworks in Toxicity Testing and Chemical Risk Assessment

Het gebruik van analytische kaders gebaseerd op de werking van stoffen bij het testen van toxiciteit en de risicobeoordeling

(met een samenvatting in het Nederlands)

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Mary Elizabeth Meek

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te Kingston, Ontario, Canada

Promotoren: Prof. dr. W. Slob
Prof. dr. M. van den Berg

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Chapter 1

Introduction

Objective:

Recently, legislative mandates worldwide are requiring systematic consideration of much larger numbers of chemicals. This necessitates more efficient and effective toxicity testing, as a basis to be more predictive in a risk assessment context. This in turn requires much more emphasis early in the design of test strategies on both potential exposure and mechanism or modes of toxicity and a resulting shift in focus from hazard identification to hazard characterization. This enables grouping of substances and development of predictive computational tools.

It also requires a much better common understanding in the regulatory risk assessment community of the nature of appropriate data to inform consideration of mode of action and resulting implications for dose-response analysis and ultimately, risk characterization. This requires a shift in focus from the previously principally qualitative considerations of toxicological science to the necessarily more predictive and quantitative focus of risk assessment. It also has implications for appropriate communication and training of risk assessors.

Analytical frameworks such as those for human relevance of hypothesized mode(s) of action (MOA/HR) and chemical specific adjustment factors (CSAF) are important components in this evolution. They serve to better coordinate and integrate input of both the research and regulatory communities in the translation of relevant mechanistic data into quantitative characterization of risk. They also present essential pragmatic tools in interim strategies to advance common understanding in these diverse communities of appropriate application of data from evolving technologies.

The background to the critical role of these frameworks is introduced. Research related to their development is described in the body of the thesis. The critical role of the analysis which they promote in the evolution of more focussed, efficient and effective, public health protective approaches is addressed in the discussion. Recommendations for relevant next steps are also presented.

Historical Regulatory Context:

Modern chemicals legislation was introduced in Europe and North America in the 1970's. In the intervening years, its focus has expanded with respect to the media of interest (air, water, products), environmental as well as human health effects and incrementally greater numbers of substances. The need to adopt a life cycle approach to effectively manage the harmful effects of chemicals has also been increasingly recognised.

Introduction and/or renewal of early chemicals legislation focussed principally on information requirements for New Chemicals. Resulting systematic consideration of New Chemicals prior to their introduction into commerce encouraged the chemical industry worldwide to minimize intrinsic hazard in the development of their products. On the other hand, Existing Chemicals, i.e., those which were in commerce at the time of introduction of relevant legislation, were "grandfathered". That is, systematic consideration of all as a basis to identify priorities for risk management was not required, though a limited number were identified early for assessment.

This resulted in detailed assessments being conducted for several hundred identified "Priority" chemicals within Canada and Europe over the past few decades. In Canada, for example, it involved in depth evaluation of 69 substances (including complex mixtures and groups) identified as priorities under the original and first

renewal of the Canadian Environmental Protection Act (CEPA -1988 and CEPA – 1999). This transpired in two mandated five year timeframes between 1989 and 2000 [Meek, 2001; Meek, 1997; Meek, 1996; Meek and Hughes,1997; Meek and Hughes, 1995; Meek et al.,1994]. These assessments were followed by the implementation of risk management measures for a significant proportion that were deemed to present a risk to the environment or human health.

More recently, mandates have required systematic consideration of priorities for risk management from amongst all of the hundreds of thousands of existing chemicals used worldwide. This is legitimately based on the likelihood that unconsidered Existing Chemicals present potentially greater risk to health and the environment than those introduced as New Chemicals following the advent of modern legislation.

For example, under the Canadian Environmental Protection Act (CEPA), precedent-setting provisions were introduced to systematically identify priorities for assessment and management from amongst the approximately 23, 000 substances used commercially. This work was to be completed within a mandated 7 year time frame between 1999 and 2006. This necessitated the development of innovative methodology including evolution of the previously linear or sequential steps of risk assessment and risk management to a more iterative approach where the need for, and focus of, potential control options are identified at as early a stage as possible. It has also required development of assessment products that efficiently dedicate resources, investing no more effort than is necessary to set aside a substance as a non-priority or to provide necessary information to permit risk management.

More recently, in Europe, a law entitled the **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical substances (REACH) entered into force on 1 June 2007. Its objective is to effect greater parity between the consideration of New and Existing Substances. This much broader consideration of Existing Chemicals, as required under legislation in both Canada and Europe, necessarily has implications for the efficiency of both testing and assessment.

***Evolution of the Paradigm for Consideration of Hazard in Chemical Risk Assessment*:**

Risk assessment i.e., the characterization of the potential adverse effects of human exposures, is the requisite basis for the development and implementation of control measures that are protective of public health (i.e., risk management), Traditionally, it has been considered to be composed of four different elements: *hazard identification* (i.e., the intrinsic capability of a chemical to do harm), *dose-response assessment, exposure estimation* and *risk characterization.* The latter is a synthesis of relevant data from all of the component steps with a clear delineation of uncertainties and their implications for risk management.

This paradigm, proposed initially by the U.S. National Research Council in the now infamous "Red Book" (NRC, 1983), is more than 25 years old. It is, perhaps, in need of revisiting, given the essential shift in focus in chemicals risk assessment necessitated by evolution in regulatory mandates. Specifically, the emphasis on *hazard identification* must necessarily shift to *hazard characterization.* The latter involves a comprehensive, integrated judgment of all relevant information supporting conclusions regarding a toxicological effect including human relevance, but most importantly, taking into account mechanistic information. This shift in focus from *hazard*

identification to *hazard characterization* is essential as a basis to avoid labor intensive testing strategies which provide no or minimal data on mechanistic underpinnings of observed toxicological effects. Continued reliance on studies designed to identify hazard in animals at high doses without accompanying relevant mechanistic data necessarily limits capability to predict risks to the public from exposure to chemicals and the adequacy of resulting measures to protect public health.

Currently, toxicological studies focus on specific systems or types of effects. Indeed, much effort and resources are invested currently in conducting and considering the adequacy of toxicological studies on individual endpoints in experimental animals (e.g., cancer, reproductive and developmental effects, etc.) as a basis to *identify* hazard. There is, however, very little consideration at early stage in their design of relevance to the prediction of risk. Rather, standardization has been emphasized as a basis to ensure comparability of outcome. This has led often, to focus in assessment on features of standardized study design based on criteria for their technical adequacy in test guidelines as established by organizations such as the US Environmental Protection Agency (USEPA) or OECD (Organization for Economic Cooperation and Development). This includes aspects, for example, of whether the study adhered to the principles of good laboratory practice, versus their relevance to risk assessment. This is a function, likely, of the need for simplicity.

Since studies focus on particular systems or types of effects, weight of evidence determinations in hazard identification relate to particular effects rather than being integrated across systems. For example, consistency is considered in the context of whether similar effects (e.g., cancer or reproductive) have been observed in other studies or species. The types, specific site, incidence and severity of these effects and the nature of the exposure- or dose-response relationship are also taken into account in assessing weight of evidence for the observed effect. In assessing potential to induce tumors, for example, aspects that add to the weight of evidence include observation of uncommon tumor types, occurrence at multiple sites by more than one route of administration in multiple strains, sexes and species, progression of lesions from preneoplastic to benign to malignant, including metastases and comparatively short latency periods. Consideration of contribution of the nature of changes in other systems in the same animals, might, however, permit more informative and predictive integration across biological systems, providing greater mechanistic insight.

Traditionally, also, weight of evidence descriptors for cancer and other effects (principally mutagens and developmental/reproductive toxins) such as "carcinogenic to humans", "probably carcinogenic to humans" etc., have been developed by a number of international organizations such as the International Agency for Research on Cancer (IARC) and various national regulatory agencies including the US EPA and Health Canada. These are delineated both as a basis for distinguishing approaches to dose-response analysis in subsequent risk characterization and also as a basis to communicate hazard. These characterizations represent, then, weight of evidence determinations in hazard identification (i.e., intrinsic capability to cause harm) for particular types of effects but are often misinterpreted to be risk-based (where exposure, relevance and dose-response have been taken into consideration). In recognition of this shortcoming, there is trend to providing more narrative and accurate descriptors, which include reference to the conditions under which the effect is observed, as a basis to avoid misinterpretation.

Undue emphasis on *hazard identification* as described above not only leads to potential misinterpretation in the context of risk, but necessarily limits investment of

resources in more relevant and predictive components of risk assessment, such as hazard and risk characterization. Given the need in future to be much more efficient (and resultingly predictive in the context of human health risk), it seems essential to focus early in testing and assessment on assimilation of information that informs in the context of hazard characterization.

As indicated above, h*azard characterization* takes into account not only results of traditional test guideline studies designed to identify hazard for individual endpoints but additionally, mechanistic data, which are considered in the context of "mode" of induction of toxic effects. In fact, an increasingly common understanding of the concept of "mode of action" and its contrast with "mechanism of action" has been a major area of advance in risk assessment. "Mode of action" is essentially a description of the critical metabolic, cytological, genetic and biochemical events that lead to induction of the relevant end-point of toxicity for which the weight of evidence supports plausibility. "Mechanism of action", on the other hand, implies a more detailed molecular description of causality.

A postulated mode of action (MOA), then, is a biologically plausible sequence of "key events" leading to an observed effect supported by robust experimental observations and mechanistic data. Identification of "key" events – i.e., those that are both measurable and necessary to the observed effect is fundamental to the concept and the quintessential element of mode of action analysis. Delineation of the key events in an hypothesized mode of action forces early interdisciplinary collaboration in consideration and development of data. It is also a unifying theme in the various components of risk assessment, imposing more explicit delineation of relevant considerations for human relevance and subsequent dose-response analysis.

Mode of action as considered in this thesis (and the relevant frameworks addressed herein) is comprised of both toxicokinetics (absorption, distribution, metabolism and excretion) and toxicodynamics (interaction with target sites and the subsequent reactions leading to adverse effects). This contrasts with previous specification, for example, by the US EPA (2005), wherein it is stated that processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action. Toxicokinetics is included here as part of mode of action, given that often, the critical (and sometimes rate limiting) early key event (i.e., that which is driving the process) involves metabolic activation to a relevant toxic entity. Toxicokinetics is also included as a basis to integrate rate limiting (key) steps in subsequent dose-response analysis, which is often delivery of the parent compound to the target tissue and/or metabolism to the active agent. It's of interest in this context that while US EPA (2005) indicates that toxicokinetics are excluded, mode of action statements in their assessments reference both toxicokinetic and toxicodynamic aspects.

Information on mode of action is critically important to prediction of risk - in determining relevance of observed effects in animals to humans, transitions in effect at various doses and potentially susceptible subgroups. It is also critical as a basis to address whether or not there is likely to be site concordance of effects between animals and humans. While there is indication that, for example, growth control mechanisms at the level of the cell are homologous among mammals, there is no evidence for nor reason to believe that mechanisms for effects such as cancer induction are site concordant. Rather information on likely variations between animals and humans in toxicokinetics and toxicodynamics, based on understanding of mode of induction will inform in this context. This information is essential also to integrate the results of

studies in animals and humans. For example, it is critical as a basis to interpret (particularly) the significance of negative epidemiological data, taking into account the sensitivity of the study to detect effects at most likely tumour sites in humans (i.e., which are not necessarily those observed in animals). It is also critical in the development of relevant biomarkers in epidemiological studies, to increase their utility as a basis for consideration of the risks to exposure to chemical in both the occupational and general environments.

In *hazard characterization*, then, the weight of evidence of hazard integrating information on mode of action for a spectrum of (often interrelated) end-points is assessed critically but separately in order to define appropriate end-points for and approaches to characterization of dose/concentration–response.

Dose- or exposure-response (dose/exposure-response) assessment, involves quantitation of the probability that an exposure may result in a health deficit in a population. This is necessarily based on characterization of hazards that are considered critical and relevant to humans (i.e., those that are biologically relevant at lowest doses). Advances in common understanding of the contrast of "mode of action" (a less detailed description with emphasis on critical key events) with "mechanism of action" (the molecular basis) and the pivotal role of "key events" in this context are instrumental in encouraging more predictive testing and assessment as a basis for better informed dose-response assessment. This includes taking into account the shapes of the dose-response curves for the various key events (not just the adverse effect, itself) and considering on the basis of the mode of action analysis, which of these key events is likely to be rate limiting at various doses.

The toxicokinetic and toxicodynamic aspects considered in a mode of action analysis are also potentially informative in quantitating interspecies differences and variability within humans. Indeed, there is a continuum of increasingly data (mode of action)-informed approaches to account for interspecies differences and human variability, which range from default ("presumed protective") to more biologically based ("predictive"). The least data-informed option is incorporation of straight default values, which incorporate no chemical or species-specific considerations. The basis for such defaults is largely unknown. Cited support remains nebulous, though they are sometimes justified, taking into account uncertain retrospective analyses of available data on empirical relationships (Dourson and Parker, 2007).

Where data permit, categorical defaults, which permit more refinement through delineation of categories based on, for example, characteristics of the compounds themselves or of the species in which the critical effect has been determined, can be developed. The latter include allometric (i.e., surface area to body weight) scaling for different species or the approaches to development of reference concentrations for inhalation for various types of gases/particles adopted by the U.S. EPA (Jarabek, 1994). Additional data permit replacement of kinetic or dynamic components of interspecies or interindividual variation with chemical specific adjustments, based on comparative kinetic and dynamic parameters between animals and humans or within humans. More quantitative toxicokinetic data may permit development of a physiologically-based pharmacokinetic model (PBPK) which estimates a "biologically effective dose" based on the mode of action and quantitative physiological scaling taking into account, relevant chemical-specific physical chemical properties and biological constants. Though rarely the case, where there is fuller quantitative characterization of toxicokinetic and toxicodynamic aspects, a case specific or biologically-based dose-response model can be adopted.

The approach along this continuum adopted for any single substance is necessarily determined principally by the availability of relevant data. Availability of relevant data has often been limited in the past, owing to the (perhaps unwarranted) focus on the desire to repeat high dose studies designed to address hazard identification.

Increasingly, for a limited number of Existing Chemicals identified as priorities for assessment under early chemicals legislation, mode of action data have been developed as a basis to reduce uncertainties in the areas of greatest inference in risk assessment: namely, extrapolations across and within species (as a basis to identify susceptible subgroups) and doses. For a limited number, biologically motivated case-specific models or fully biologically based dose-response models that integrate significant amounts of data have been developed. These advances and data have been informative not only in the context of the individual chemicals, themselves, but also in identifying patterns of effects associated with particular modes of action and their implications for both human relevance and dose-response analysis.

In the vast majority of cases, however, even for substances for which there are significant amounts of data on mode of action, default approaches to extrapolation of dose-response and consideration of interspecies differences and human variability are adopted in regulatory risk assessment. These approaches are described here.

Traditionally, then, default approaches in dose/exposure-response extrapolation are distinguished for effects for which it is believed that there may be a probability of harm at all levels of exposure (e.g., interaction with DNA leading to cancer) versus those for which it is believed that there is a level of exposure below which effects will not be observed. The former approach is justified on the theoretical basis that a single molecule could be sufficient to induce harm, if it interacts with the appropriate target.

These different assumptions generally lead to two distinct default approaches, the first of which can result in a (highly uncertain) estimate of risk at various levels of exposure (i.e., low dose risk estimates) and the second which results in development of a "safe" dose (acceptable, reference or tolerable intakes). For the latter, this is a level to which it is believed that a population can be exposed over a lifetime without adverse effect. Both approaches to dose-response assessment are generally based on only two to three data points in the experimental range, that is, in groups of animals exposed to doses which exceed considerably those associated with most human exposures. The relatively high exposures in toxicological studies designed to identify hazard have traditionally been justified on the basis that only small numbers of animals per group are surrogates for a much larger human population.

In the development of acceptable, reference or tolerable daily intakes (ADIs, RFDs, TDIs) the experimental data are assessed to determine a level without adverse effects (the no-observed-adverse-effect level or NOAEL). Alternatively, a curve is fitted that best fits the central estimates of the relationship defined by these experimental data points and confidence intervals are calculated. Reference or tolerable intakes are commonly adopted for organ-specific, neurological, immunological, and reproductive-developmental effects and carcinogenesis not induced by direct interaction with genetic material. Without information on mode of action, however, there is no reason to believe that this or the alternative (i.e., linear extrapolation) is more appropriate.

Development of a reference, acceptable or tolerable intake is traditionally based, then, on an approximation of the threshold, through division of a no- or lowest-observed-(adverse)-effect-level [NO(A)EL or LO(A)EL] by uncertainty factors (Dourson

1994, IPCS 1994, Meek et al. 1994). The NOAEL is the highest level of exposure that causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism in toxicological studies. Uncertainty factors address interspecies differences, human variability and inadequacies of the database.

Increasingly, the benchmark dose or concentration (BMD/BMC) —an estimated dose (or its lower confidence limit) associated with a particular effect level (e.g., 5 or 10% incidence or inhibition) for the critical effect—is adopted in lieu of an no or lowest observed effect level. BMDs are estimated from fitted dose-response models which describe the dose-response curve as a whole. This offers a number of advantages from the perspective that BMDs/BMCs are not limited to the doses tested experimentally, are less dependent on dose spacing, take into account more of the shape of the dose-response curve, and provide flexibility in determining appropriate levels for biologically significant changes. However, it should be noted that the quantitative impact of the use of a No- or Lowest-Observed (Adverse) Effect Level versus a benchmark dose or concentration on derived reference doses is rather limited in comparison with that potentially resulting from relevant data on mode of action (kinetic or dynamic aspects) to address components of uncertainty factors.

Alternatively, the magnitude by which the N(L)OAEL or BMC/BMD exceeds estimated exposure (i.e., the margin of exposure or safety) is considered in light of various sources of uncertainty (see, for example, Chapter 2).

Though commonly referenced as uncertainties, elements addressed in development of the reference or tolerable dose or concentration or against which the adequacy of the margin of exposure is judged include both uncertainty and variability. The nature of the (principally) variability addressed includes interspecies differences and intraspecies (interindividual) or human variability. Inadequacies of the database such as missing data on specific endpoints (uncertainty) are also commonly taken into account.

The default "uncertainty" factor for interspecies differences can be considered to convert the no or lowest effect level or benchmark dose/concentration for animals (derived from a small group of relatively homogeneous test animals) into the no or lowest effect level or benchmark dose/concentration anticipated for an average representative healthy human. It is generally 10 fold. Although data on adverse effects in humans can be used directly without the need for a factor for interspecies differences, the paucity of such data results in the vast majority of risk assessments being based on studies in experimental animals. The default uncertainty factor for human variability converts the no or lowest effect level or benchmark dose/concentration for the average human into a no or lowest effect level or benchmark dose/concentration for susceptible humans. It is also generally 10 fold.

These default values have been used for over 40 years (with limited reconsideration) by both national agencies and international bodies such as the Joint FAO/WHO Committee on Food Additives and Contaminants (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as a basis to derive ADIs, TDIs or RfDs for the general population. There is, however, very limited support for these default values whose use continues to be justified based principally on uncertain retrospective historical analyses of limited databases.

At present there is no clear consensus on appropriate methodology for dose/exposure-response assessment of chemicals for which a probability of harm at

any level of exposure is assumed (e.g., carcinogens that interact directly with genetic material and germ cell mutagens). Options include:

1) expression of dose/exposure–response as potency in or close to the experimental range,
2) estimation of risks in the low-dose range through linear extrapolation from an effective dose,
3) calculation of the margin of exposure, and
4) advice that control measures should be introduced to reduce exposure to the maximum extent practicable (Younes et al., 1998).

In Canada in the Priority Substances Program, for example, dose or exposure response is expressed as potency in or close to the experimental range. This approach was adopted primarily in recognition of the potentially misleading precision associated with expression of risk in the low-dose range in absolute terms (as numbers of cases per unit of the population). Expression in this form has potential to obfuscate the considerable uncertainties associated with linear extrapolation from animals over as much as 6 orders of magnitude (Health Canada 1994; Meek et al., 1994). Rather, quantitative measures of potency in the experimental range are considered more in a relative or priority setting context. This is also the case in Europe (EU, 2003). In the U.S. Environmental Protection Agency cancer guidelines (US EPA, 2005), risks in the low-dose range for such cases are estimated through linear extrapolation from an effective dose in the experimental range.

Consideration of endpoints more in the context of their mode of action necessarily blurs the currently rather arbitrary distinction in default approaches to dose-response characterization between cancer and noncancer (Meek, 1997). Rather, extrapolation for either would be based on more comprehensive understanding of the nature of the dose-response relationship for rate limiting key events. Appropriate defaults in the absence of sufficient data to support development of biologically motivated case-specific or fully biologically based dose-response models may involve linear extrapolation for some noncancer effects and development of reference doses or margins of exposure for tumors or precursor lesions.

The Need for Analytical Frameworks for Mode of Action Consideration in Hazard Characterization and Dose-Response Analysis

There is, then, rather a significant gap between the current approaches to toxicity testing and risk assessment and the need to be predictive to meet imposing mandates for priority setting and assessment of large numbers of chemicals, with limited resources. This results from traditional focus principally on hazard identification in (standardized) toxicity testing and (simple) default approaches to dose-response characterization in risk assessment. And while generic default approaches (e.g., subdivision of effect levels from high dose animal studies by often 100 or more fold values or low dose linear extrapolation from much higher doses) are commonly presumed protective, this has not been well tested. The premise is also inconsistent with what is known about the impact of results from targeted and coordinated investigations of mode of action which have supported more data derived approaches (e.g. chemical specific kinetic and dynamic data that indicate that appropriate adjustments may be more or less than default).

This lack of predictive capacity in the context of human health risks owing to the nature of toxicity testing (addressing principally hazard identification) and associated reliance on default approaches in risk assessment, then, presents a considerable barrier to meeting current regulatory challenges. Indeed, the principal reason that the potential predictive capability of (quantitative) structure activity relationship analyses (Q)SARs for human health risk assessment including the (non-automated) Threshold of Toxicological Concern is limited, relates to the absence of underpinning in a mode of action context.

Our reliance on default while resulting principally from the (undue) focus on hazard identification in toxicity testing and the desire for simplicity, is also often a function of the inadequacy of mechanistic data. This information is sometimes collected in a largely unfocussed and uncoordinated manner in a risk assessment context. In addition, owing to the principal reliance on default, there has been inadequate transparency on the types of information that would be more informative (i.e., specification in risk assessment of critical data gaps that would meaningfully inform mode of action considerations).

Moreover, even in cases where there are considerable robust mechanistic data to inform quantitative risk assessment, they are often not used in regulatory applications. This is sometimes a function simply of lack of understanding of the regulatory risk assessment community and/or regulatory pressures to conduct assessments in very limited timeframes. Alternatively, it is often due to a shortage of interdisciplinary consultation of risk assessors (who commonly have backgrounds principally in toxicology), modelers and those that conduct mechanistic investigations. Improved communication between the various groups and interdisciplinary training would seem to be critical in this context. Occasionally, it is related to a lack of transparency in the separation of science judgment from science policy choices (i.e., with default being considered to be more public health protective).

The focus of this thesis, then, is to review the state of development of analytical frameworks for consideration of the weight of evidence for the human relevance of mode of action and implications for dose-response analyses in the context of their essential contribution as a basis to increase common understanding between the risk assessment and research communities. Additionally, their evolving and expanding content and use is considered in the context of essential more progressive toxicity testing strategies to meet expanded regulatory mandates (Figure 1).

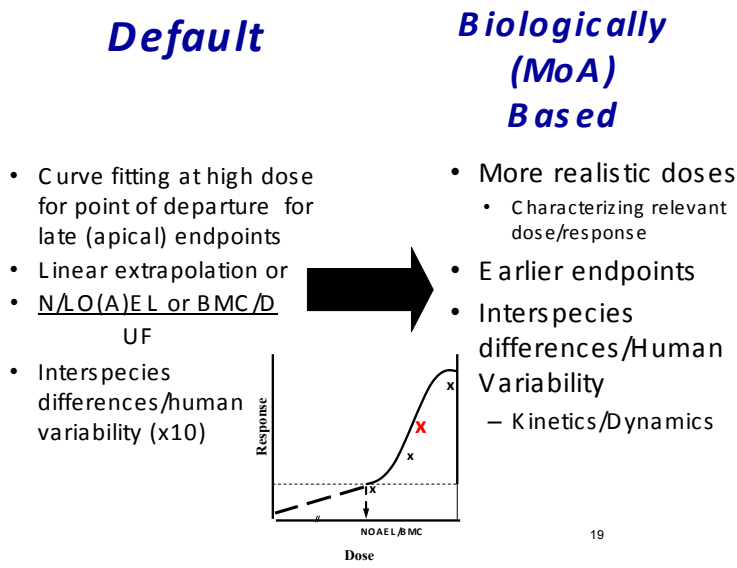

Figure 1. Moving from Default to More Mode of Action Based Approaches in Chemical Risk Assessment

Overview of this Thesis

This thesis is comprised of this Introduction, ten chapters representing individually published studies and a final chapter that provides a discussion of the key points. Also included in the final chapter are suggested next steps in increasing efficiency and effectiveness of chemical risk assessment through increased development and uptake of biological data.

Initially, the historical and evolving regulatory context of chemical risk assessment is addressed (Chapter 2) as a basis for understanding the need for considerable increase in efficiency and effectiveness and associated strategies for toxicity testing.

Chapter 3 addresses advances in process for preparation of chemical assessments in the area specifically of peer engagement. This is essential to consideration of increasingly biologically motivated vs. default approaches in hazard characterization, dose response analyses and risk characterization. The requirement under the Canadian Environmental Protection Act to set priorities from amongst thousands of existing chemicals has provided opportunity for incorporation of efficient and increasingly complex peer involvement in both assessments of individual or groups of substances. Specifically, this has involved more formal peer input at the earlier stages of development and greater complexity of peer input, consultation, and peer review for complex issues. This approach maximizes efficiency in acquiring necessary early multidisciplinary input while maintaining the defensibility of output.

In chapter 4, the continuing evolution of Mode of Action Human Relevance Frameworks (MOA/HR) as a basis to systematically consider the weight of evidence of hypothesized modes of action in animals and their potential human relevance for both

cancer and non-cancer effects is described. Chapter 5 outlines in detail the basis and nature of the expansion of previous frameworks considering the weight of evidence for modes of action in animals to relevance to humans. This is based on systematic evaluation of comparability between key events in animals and humans taking into account chemical specific data and more generic information on, for example, biology, physiology and human disease models. The chapter includes several case studies representing several different examples of application of the human relevance component of the framework.

While developed and refined initially to consider principally hazard characterization, MOA/HR frameworks have been extended recently to consider implications for dose-response analysis. Chapter 6 addresses the continuum of increasingly mode of action informed approaches in dose-response analyses from default ("presumed protective") to more predictive options. Chapter 7 outlines the contribution of the most highly evolved of these options, namely a biologically motivated computational model for formaldehyde. Chapter 8 described the objectives and nature of a less data intensive mode of action driven approach on this continuum, namely derivation of chemical specific adjustment factors as a basis to incorporate partial data on kinetics and/or dynamics to replace default. Chapter 9 provides an illustration of integration of the frameworks for human relevance analysis of mode of action and chemical specific adjustment factors.

Chapters 10 and 11 address the implications for risk characterization of MOA/HR analysis in hazard characterization and extension to dose-response analysis through provision of examples for two specific chemicals. For the first, namely chloroform, available data on mode of action indicate that non-cancer precursor effects (i.e., sustained cytotoxicity and regenerative cellular proliferation in the kidney and liver) are likely protective for cancer and for the second, namely formaldehyde, dose-response analysis takes into account both early interaction with DNA and cytotoxicity/regenerative cell proliferation in the development of nasal tumors in rats.

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Chapter 2

The Assessment and Management of Industrial Chemicals in Canada

Meek, M.E. and Armstrong, V.C. (2007)

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Van Leeuwen, K. and Vermeire, T.
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1 INTRODUCTION

The chemical industry is one of the largest manufacturing sectors in Canada and employs more than 90,000 people; nearly every major global chemical company in the world has production or research and development facilities. In 2003, more than two thousand companies, including 21 of the 25 world's largest manufacturers, had operations in this country; shipments of chemical products were worth $42 billion (~26.4 billion €). The industrial chemicals sub-sector, which includes companies manufacturing petrochemicals, industrial gases, pigments, other inorganic and organic chemicals, resins, and synthetic fibres, accounted for close to 50% of this amount [1].

In this Chapter, emphasis is placed on the progressive, legislated requirements for assessment and control of significant numbers of existing substances under the Canadian Environmental Protection Act (CEPA) [2], which include emissions and by-products associated with chemicals production. This has involved the in-depth assessment of 69 substances (including complex mixtures and groups of substances) identified as priorities under the first CEPA (CEPA-1988) [3] in two mandated five year timeframes. These assessments were followed by the implementation of risk management measures for a significant proportion that were deemed to present a risk to the environment or human health.

More recently, under CEPA–1999 [4], precedent-setting provisions to systematically identify, in a timely manner, priorities for assessment and management from among the approximately 23, 000 existing commercial substances have been introduced. This has necessitated the development of innovative methodology including evolution of the previously linear or sequential steps of risk assessment and risk management to a more iterative approach where the need for, and focus of, potential control options are identified at an early stage of assessment. It has also required development of assessment products that efficiently dedicate resources, investing no more effort than is necessary to set aside a substance as a non-priority or to provide necessary information to permit risk management. Similarities to, and variations from, approaches adopted or contemplated under US and European chemicals control legislation are also outlined.

It is to be stressed that the nature of the actions taken under CEPA-1999 and the associated methodological developments necessitated by the provisions of this Act continue to evolve. Therefore this Chapter can provide only an overview of the status of industrial chemicals management as it was at the time of its completion, that is, early in 2007. Additional detail and further developments are and will be available at website references listed in the bibliography.

2 LEGISLATIVE BACKGROUND

2.1 Federal-provincial regulatory structure

Canada is a federation of ten provinces and three territories, for which responsibilities for matters pertaining to the environment are shared. Indeed, the Supreme Court of Canada has ruled that environmental protection is of such importance that it requires action by governments at all levels. In January, 1998, the provinces, with the exception of Quebec, and the federal government signed an Accord on Environmental Harmonization, which sets the framework for collective goals and action to protect the environment [5, 6]. The Canadian Environmental Protection Act (CEPA), the cornerstone of federal environmental protection, is entirely consistent with the Harmonization Accord and is the tool for implementation of Harmonization Agreements.

International aspects of the assessment and control of toxic chemicals fall under the purview of the federal government; these include responsibilities related to international air and water pollution and participation in international initiatives of, for example, the International Programme on Chemical Safety, the United Nations Environment Programme and the Organization for Economic Cooperation and Development.

CEPA has been structured to avoid duplicating effective measures that have already taken or are proposed to be taken by other federal and provincial departments or ministries. Should an assessment conducted under CEPA indicate the need to take action to protect health or the environment and should such action not be undertaken under other Canadian statutes, the risk management provisions of CEPA can be invoked. Any actions that are to be taken under other legislation as a result of initiatives under CEPA must be deemed to be equivalent to those proposed under this Act. In order to coordinate work with the provinces and avoid duplication, especially with respect to the development of regulations, a National Advisory Committee has been established as required under CEPA-1999.

With respect to assessment and control of the environmental and public health impacts of new substances, (an area in which the provinces and territories do not have a mandate), actions taken under other federal statutes must be equivalent in terms of requiring notification prior to import into, or manufacture in, Canada and assessment of potential risks to both the environment and human health. These equivalency provisions have had an impact on the assessment and control of substances that fall under the purview of federal legislation such as the Food and Drugs Act [7], the Feeds Act [8] and the Fertilizers Act [9].

2.2 Evolution of the Canadian Environmental Protection Act

In contrast to some of its other health protection statutes such as the Food and Drugs Act which dates back to 1920, Canada's environmental protection laws have been developed relatively recently. The Department of the Environment was created in 1972 and the first federal environmental protection act, the Environmental Contaminants Act (ECA) [10] "An Act to Protect Human Health and the Environment from Substances that Contaminate the Environment." was promulgated in 1975. This legislation, like its successors, was administered jointly by the Department of Health and the Department of the Environment and was developed, in part, to provide a

means to respond domestically to international environmental initiatives such as those being undertaken by the Organization for Economic Cooperation and Development (OECD) to control polychlorinated biphenyls (PCBs). A number of other substances of concern at that time (e.g., polybrominated biphenyls, polychlorinated terphenyls, mirex, and lead from secondary lead smelters, asbestos and mercury), were subsequently banned or controlled under the ECA.

While the ECA required companies to identify chemicals not previously used as "new" there was no systematic testing or assessment of chemicals for toxic effects prior to their introduction. "New" was defined as previously unused by the company and while relevant quantities were also to be specified, notification was required only after introduction of the substances into commerce. Submission of information and testing by industry could be required only if the Ministers of Health and of the Environment had "reason to believe that a substance is entering/may enter the environment in amounts that are a danger to health or the environment" based on consideration of information that had already been generated or obtained from other sources. While information on a large number of new chemicals was examined, the administrative procedure for effecting control was complex and therefore none was undertaken through the short history of the program.

Proposals from an Environmental Contaminants Act Amendments Consultative Committee (ECAACC) [11, 12] were considered during the Parliamentary review of the ECA and the Canadian Environmental Protection Act (CEPA), "An Act Respecting the Protection of the Environment and of Human Life and Health", came into force in 1988. CEPA-1988, a much more comprehensive piece of environmental protection legislation, not only superseded the Environmental Contaminants Act, but also subsumed other Canadian environmental protection statutes (and their regulations) such as the Clean Air Act, the Ocean Dumping Control Act, part of the Canada Water Act and part of the Department of the Environment Act into a single piece of legislation. One of the salient new features of this Act was embodiment of the notion of pre-import/ pre-manufacture notification and assessment of new substances including biotechnology products with adoption of a minimum data set based on that developed under the Chemicals Programme of the OECD [16]. CEPA-1988 was first passed into law in June, 1988, amended in June, 1989 and was replaced with a new and further expanded Act which received royal assent in September, 1999 (CEPA-1999), following a review of its operation and implementation as required to be undertaken within 5 years of the promulgation of the Act.

2.3 The current Canadian Environmental Protection Act (CEPA-1999)

CEPA-1999 is entitled "An Act Respecting Pollution Prevention and Protection of the Environment and Health in order to Contribute to Sustainable Development" [4]. New principles to guide the application of this Act are spelled out in its preamble; thus in implementing the various provisions of CEPA, Environment Canada and Health Canada are expected to ensure:

- that consistency between federal government departments and collaboration with other jurisdictions results in effective and integrated approaches, policies and programs to manage the health and environmental risks of toxic substances;

- recognition that the risks from toxic substances is a matter of national concern that transcends geographic boundaries;
- that the importance of an ecosystem approach is recognised;
- that that there is a commitment to implement pollution prevention as a national goal and as the primary mechanism to promote environmental protection;
- that the Government of Canada is able to fulfil its international obligations in respect of the environment;
- implementation of the precautionary principle;
- implementation of the "polluter pay" principle; and
- that there are public participation, openness and transparency in decision-making and that there are mechanisms available for supporting these goals.

Prevention and management of risks posed by toxic and other harmful substances remain as the principal objectives of the revised CEPA. The provisions for implementing pollution prevention, investigating and assessing substances, controlling toxic substances, and those for fuels, international air and water pollution, motor emissions, nutrients, environmental emergencies, for regulating the effects of the federal government's own operations and waste disposal at sea and the import and export of wastes were added or expanded upon. Recognition of the growing importance of biotechnology led to the creation of a specific section with provisions that parallel those for chemical substances.

CEPA-1999 also provides for the gathering of information for research and the creation of inventories of data and for the development of environmental objectives, guidelines and codes of practice. In addition, new rights were bestowed on Canadians to participate in decisions on environmental matters, including the ability to compel investigation of an alleged contravention of the Act and the possibility of bringing civil action when the government is not enforcing the law. Aboriginal governments have the right to be represented on a National Advisory Committee which must be established as a way of "enabling national action to be carried out taking cooperative action in matters affecting the environment and for the purposes of avoiding duplication in regulatory activity among governments".

The new CEPA contains 343 operative sections and six schedules, and is divided into the parts shown in Table 1:

Table 1. The operative parts of CEPA-1999

Part	Title
1	Administration
2	Public Participation
3	Information Gathering, Objectives, Guidelines and Codes of Practice
4	Pollution Prevention
5	Controlling Toxic Substances
6	Animate Products of Biotechnology
7	Controlling Pollution and Managing Wastes
8	Environmental Matters Related to Emergencies
9	Government Operations and Federal and Aboriginal Land
10	Enforcement
11	Miscellaneous Matters

2.4 Administration of the CEPA

Responsibility for the administration of CEPA is shared between Canada's Department of the Environment and Department of Health. The Minister of the Environment has overall responsibility for the administrative aspects of the Act and for most of its other provisions, notably those for enforcement and compliance. Also in most of the instances where the Minister of Health is named, responsibilities must generally be carried out collaboratively. These joint responsibilities include assessing and controlling toxic substances and assessing the impacts of (international) air pollution, and (international) water pollution. The Minister of Health can act independently in conducting health-related research investigations and other studies, setting environmental objectives, guidelines and codes of practice to protect health, and in establishing advisory committees with respect to these responsibilities. It has also been customary for the Health Department to provide advice to Environment Canada on health related issues arising under parts of the Act in which the Health Minister is not explicitly named, (e.g., with respect to the potential health effects of fuels and vehicle, engine and equipment emissions).

2.5 The CEPA definition of "Environment"

The definition of "environment" in CEPA is sufficiently broad to encompass the occupational as well as the general environment; however, since the provinces and territories are generally responsible for the health and safety of their workers, assessments of the impacts on health of substances under CEPA have been confined to those on members of the general public.

Box 1. CEPA definition of "environment"

"Environment" means the components of the Earth and includes
a. air, land and water;
b. all layers of the atmosphere;
c. all organic and inorganic matter and living organisms; and
d. the interacting natural systems that include components referred to in paragraphs (a) to (c).

3 CEPA's PROVISIONS FOR TOXIC SUBSTANCES

Canada's environmental protection strategy is based on sustainable development; a key component of this is controlling substances that can be harmful to human health or the environment in order to ensure that the risks are prevented or reduced. CEPA-1999 requires the Minister of the Environment, in carrying responsibilities with respect to Toxic Substances, "…to the extent possible, to cooperate and develop procedures with jurisdictions other than the Government of Canada, (that is other governments in Canada or those of member states of the Organization for Economic Cooperation and Development), to exchange information respecting substances that are specifically prohibited or substantially restricted by, or under, the legislation of those jurisdictions for environmental or health reasons".

Controlling toxic substances is viewed as a two phase process, risk

assessment and risk management. The first of these entails a science-based evaluation to enable decision-making on whether a substance poses a risk to health or the environment; the second phase identifies the most suitable control measures [13]. The Act provides a framework for the identification and control of existing substances and management of those considered to pose a risk to human health and/or the environment. This framework is broad, transparent and evidence-based, taking into account aspects (i.e., exposure and effects) of a substance in relation the potential risk it may pose.

3.1 Definitions of "Toxic" and "Substance"

Box 2. CEPA definition of "substance"

"Substance" means, in part: "any distinguishable kind of organic or inorganic matter, whether animate or inanimate, and includes ....any mixture that is a combination of substances.....,....any complex mixtures of different molecules that are contained in effluents, emissions or wastes that result from any work, undertaking or activity."

The broad definition of "substance" under CEPA encompasses not only discrete (industrial) chemical compounds but also complex mixtures formed naturally or as a result of chemical reactions, emissions and effluents and products of biotechnology. All such substances are therefore candidates for assessment under the legislation. Animate biotechnology products can be whole organisms, or parts, or products of organisms, including those developed through genetic engineering. The definition of "substance" is somewhat more restrictive with respect to "new substances" in that articles, physical mixtures and effluents and emissions are excluded.

Box 3. CEPA definition of "toxic"

A substance is "toxic" if it is "...entering or may enter the environment in a quantity or concentration or under conditions that:

a have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
b constitute or may constitute a danger to the environment on which life depends; or
c constitute or may constitute a danger in Canada to human life or health.

The purpose of carrying out an assessment under CEPA is to determine whether a substance is or is not "toxic". The definition of "toxic" is a legal one and embodies the notion that the ability of a substance to harm the environment or human health is a function of its release into the environment, the intrinsic toxicity (i.e. toxicity in the traditional sense) and the concentration of the substance to which a person, (or other environmental receptor), is exposed. Also, inclusion of the word "may" in the definition with respect to both entry into the environment and the potential danger or harm (i.e., effects) allows the approach to designating "toxic" to be developed in a manner which takes into account uncertainties and is consistent with the generally accepted principles of health risk assessment. Thus, "risk" is considered more precisely as depending on the nature of the possible effects and the likelihood of their occurrence; the probability (that any given effect will occur) in turn is a function of the potency of the toxicant, the susceptibility of the exposed

individual, or species, and the level of exposure.

The existence of information that is consistent with the designation of a substance as "toxic" under the Act sets the stage for reviewing options for controlling risks to human health and/or to the environment and, hence, for adding the substance to Schedule I of CEPA (the "List of Toxic Substances").

3.2 Provisions for new substances and the Domestic Substances List

Under the New Substances Program, companies or individuals wishing to import or manufacture substances that are new to Canada must notify the government of that **intent** so that the substances can be assessed for possible effects on the environment and human health; certain information specified in regulations must also be provided [14,15]. The New Substances Notification Regulations for chemicals and polymers first came into force in July, 1994; in October, 2005, these were replaced with amended regulations.

The new substances provisions were a critical component in the introduction of CEPA-1988 since they allowed Canada to meet its obligation to honour the OECD Council Decision [16] concerning the requirement for a Minimum Pre-market Data Set for assessing new chemicals. CEPA allows for the control of a new substance before it is manufactured or imported whenever there is a "suspicion" that the substance is "toxic" under the Act.

In order to distinguish commercial substances that are new to Canada and those already in use, a Domestic Substances List (DSL) [17] was compiled under CEPA-1988; the DSL included some 22,400 substances nominated to Environment Canada that were, between January 1, 1984, and December 31, 1986:

- in Canadian commerce;
- used for commercial manufacturing purposes; or,
- manufactured in, or imported into, Canada in a quantity of 100 kg or more in any calendar year.

Substances on the DSL are referred to as "existing" substances. (Under CEPA, an existing substance can also be one that is released as a single substance, an effluent, a mixture or a contaminant in the environment.) A Non-domestic Substances List (N-DSL) [18] was also compiled for substances not on the DSL but believed to be in international commerce, though not in Canada, during the reference period. The N-DSL was based on the 1985 Toxic Substances Control Act Inventory (excluding DSL entries), published by the US EPA, chosen as representative of substances that were in commercial use in an "ecozone" similar to that of Canada over the 1984-1986 reporting period [12]. The N-DSL now comprises more than 58,000 substances and is updated bi-annually. Information requirements for substances which are listed on the N-DSL when notified as new to Canada are reduced. [For additional information, see http://www.ec.gc.ca/substances/nsb/eng/home_e.shtml]**.**

The DSL is amended from time to time to include new substances that have been assessed for their risks to human health and the environment and which are deemed not to require the imposition of conditions; substances for which a SNAc provisions (See Section 3.7) have been imposed can also be added.

Between January, 1987 and the coming into force of the New Substances Notification Regulations for chemicals and polymers (July, 1994), about 4,400

commercial chemicals were imported into, or manufactured in, Canada; CEPA-1988 included transitional provisions for post-market notification of these substances.

Substances that are not on the DSL or the N-DSL cannot be imported into, or manufactured in, Canada in quantities greater than those stated in the NSNR (Chemicals and Polymers) until prescribed information has been notified to Environment Canada. These regulations specify the information that must be provided to meet the notification obligations; the main features are [19]:

- establishment of categories of substances (e.g., chemicals, biochemicals, polymers, biopolymers, and organisms);
- identification of administrative and other information requirements;
- specification of conditions, test procedures and laboratory practices to be followed in developing test data;
- timing of notification before manufacture or import or activity outside the scope of a previously issued SNAc Notice; and
- assessment periods for the submitted information.

The establishment of different categories of substances enables different levels of notification requirements to be established depending on the characteristics of the substance and the quantities in which it is to be imported or manufactured.

Thus, substances are first generally categorized by type (i.e., chemicals, polymers, biopolymers or organisms) and, then, each substance type is further separated into notification groups based on factors such as use, volume of manufacture or import use and whether the substance is on the N-DSL Eight Schedules of information requirements are specified for chemicals and polymers and one for biochemicals and biopolymers under the NSNR (Chemicals and Polymers) [14]. There are reduced requirements for special category substances, those for research and development, contained site-limited intermediates and contained for export only. There are also reduced requirements for certain polymers that meet the "reduced regulatory requirement" criteria. Additional information may be required for chemicals and polymers released to the aquatic environment in high quantities or to which the public may be significantly exposed. The most comprehensive data package is required for substances that are not on the N-DSL and are to be imported or manufactured in a quantity greater than 10,000 kg/year.

Box 4. Possible outcomes of the assessment of information

The possible outcomes of the assessment of information submitted are:

- a determination that the substance is not toxic or capable of becoming toxic;
- a determination that the substance is toxic or capable of becoming toxic;
- a determination that the substance is not toxic or capable of becoming toxic, but a suspicion that a significant new activity in relation to the substance may result in the substance becoming toxic.

If the substance is not suspected to be toxic, the notifier may import or manufacture the substance after the assessment period has expired. Where the substance is suspected of being toxic, or becoming toxic, the government may take measures under the Act to ensure that the substance is handled in ways that will adequately manage these risks. These measures could include imposing conditions

under which the substance may be used, prohibiting import or manufacture of the substance or requesting additional Information or test results that would enable a determination of whether or not the substance is toxic. If the substance is not suspected to be toxic but could become so by means of a significant new activity, it can be subject to a re-notification through the issue of a Significant New Activity (SNAc) Notice (See Section 3.7).

The time periods that Health Canada and Environment Canada have to assess the notified information and to impose any controls prescribed within the NSNR (Chemicals and Polymers) and the NSNR (Organisms) vary depending on the notification requirements and range from 5 to 75 days for chemicals and polymers [14], and 30 to 120 days for organisms [15]. Failure to assess a new substance within the legislated time period automatically permits the manufacture or import of the substance in(to) Canada with no (environmental) restrictions on how it can be used. In such cases, CEPA still provides measures for addressing the substance, even though the time period for assessment has expired and the substance has been added to the DSL.

The New Substances Program is regarded as a first line of defence against the release of harmful substances into the Canadian environment; the notification regulations are seen as an integral part of the federal government's national pollution prevention strategy. Approximately 800 substances new to the Canadian marketplace are assessed annually [20].

3.3 Provisions for existing substances

Under Part II of CEPA-1988, a framework for systematically determining the toxicity of substances deemed to be of high priority was implicit in the legislation. Thus, the Ministers of Health and the Environment were required to establish a list of substances (the Priority Substances List) deemed to be of highest concern with respect to health or the environment and to assess the risks of these substance (whether CEPA "toxic"). Ministers were also required to respond (within 90 days) to public nominations for additions to the List. If a report of an assessment was not published within 5 years of the substance being added to the List, establishment of a Board of Review could be requested under the Act. A summary of each assessment was to be published in the *Canada Gazette* along with an indication of whether Ministers intended to recommend the development of regulations to control the substance.

Two lists of Priority Substances (PSL 1 and PSL 2) were generated prior to the introduction of CEPA-1999. The first Priority Substances List, published in February 1989, comprised 44 substances. A second list comprising 25 substances was published in December, 1995. Both lists included classes of substances and complex mixtures as well as discrete industrial chemicals. They were developed by Panels of experts (Ministers' Expert Advisory Panels) drawn from stakeholders and convened under the authority of the Act. Annexes 1 and 2 list these priority substances.

As described below (Section 4.1), assessment of the health and environmental risks of priority substances entailed a comprehensive and scientifically rigorous approach to decision making. Examination of the 69 listed priority "substances" resulted in assessment of far more than this number in terms of discrete chemical entities because of the complex nature of some of the entries (i.e.,

mixtures and classes). Nevertheless, public expectation to consider the potential health and the environment impacts of all 22,400 or so existing industrial chemicals in Canada was increasing, a trend evident also in other parts of the world. This expectation was reflected in the views of the Parliamentary Committee that reviewed CEPA-88 and by the Commissioner on Environment and Sustainable Development (CESD) [21]. As a result, significant changes were made to the provisions for existing substances in the renewed Act (CEPA-1999).

3.4 Categorization of the Domestic Substances List and screening assessments

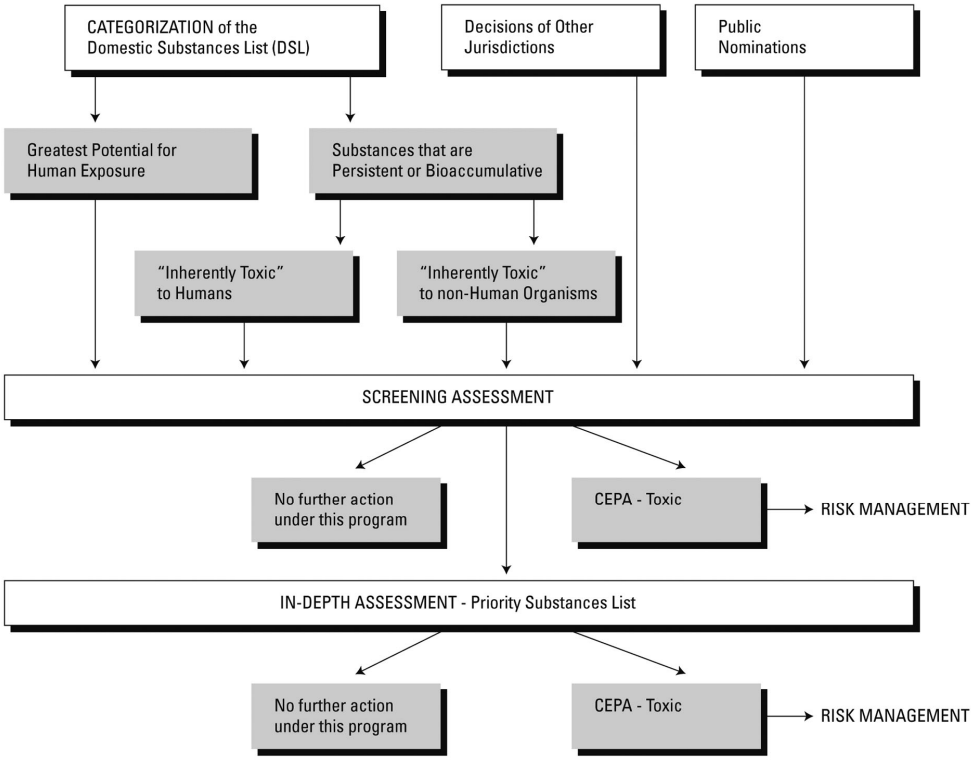


Figure 1. Existing Substances Program under CEPA-1999

CEPA-1999 incorporates a number of requirements to ensure that more existing substances are assessed for health and environmental risks in shorter timeframes, while at the same time retaining the PSL Assessment Program for substances, mixtures or effluents deemed to require a more in-depth assessment. Figure 1 depicts the processes for selecting and assessing existing substances. The

three principal phases of identification and assessment of priorities for risk management specified under CEPA 1999 are categorization, screening assessment and in-depth (Priority Substances List) assessment.

Box 5.Substances were identified for further work if they met the following criteria:

- may present, to individuals in Canada, the greatest potential for exposure; or
- are persistent and/or bio-accumulative in accordance with regulations (see Section 5.1), and
- are "inherently toxic" to human beings or non-human organisms. Note that in this context the meaning of toxic is that in the generally accepted scientific sense, as determined by laboratory or other studies.

Substances identified as priorities from categorization or other selection mechanisms must undergo screening risk assessments to determine whether they are "toxic" or capable of becoming "toxic". Another mechanism for triggering an assessment of toxicity under CEPA-99 is the requirement to review decisions made by other jurisdictions to prohibit or substantially restrict a substance for environmental or health reasons. The requirement to establish a list of Priority Substances, and the mechanism for doing so are retained under CEPA-1999.

Box 6. The possible outcomes of a screening level risk assessment, a risk assessment of a Priority Substance, or a review of a decision made by another jurisdiction are that:

- no further action be taken (typically if the substance is found not to be toxic);
- a recommendation be made (to the federal Cabinet) that the substance be added to the List of Toxic Substances with a view to developing to controls and, if applicable, be subject to virtual elimination in order to adequately manage the risks to the environment or to human health;
- the substance be added to the PSL for further review (if the substance is not already on the PSL).

The primary objective of screening and in-depth assessments is to determine whether a substance is "CEPA-toxic" as defined under the Act, which may then set the stage for addition of the substance to Schedule 1 (the List of Toxic Substances) of the Act and for reviewing options for controlling risks to human health and/or the environment.

3.5 Options for controlling existing substances

A wide range of regulatory instruments can be used under CEPA to control exposure to substances deemed to be toxic with respect to any aspect of their lifecycle, from the research and development stage to manufacture, use, storage and transport and, ultimately, disposal. Regulations can address, for example, the amounts released to the environment and where releases can occur, the conditions of release, quantities manufactured or offered for sale in Canada, quantities imported, countries from, or to, which a substances may be imported or exported, the manner in, and conditions under, which a substance is advertised or offered for sale, how it is to be handled, stored and transported.

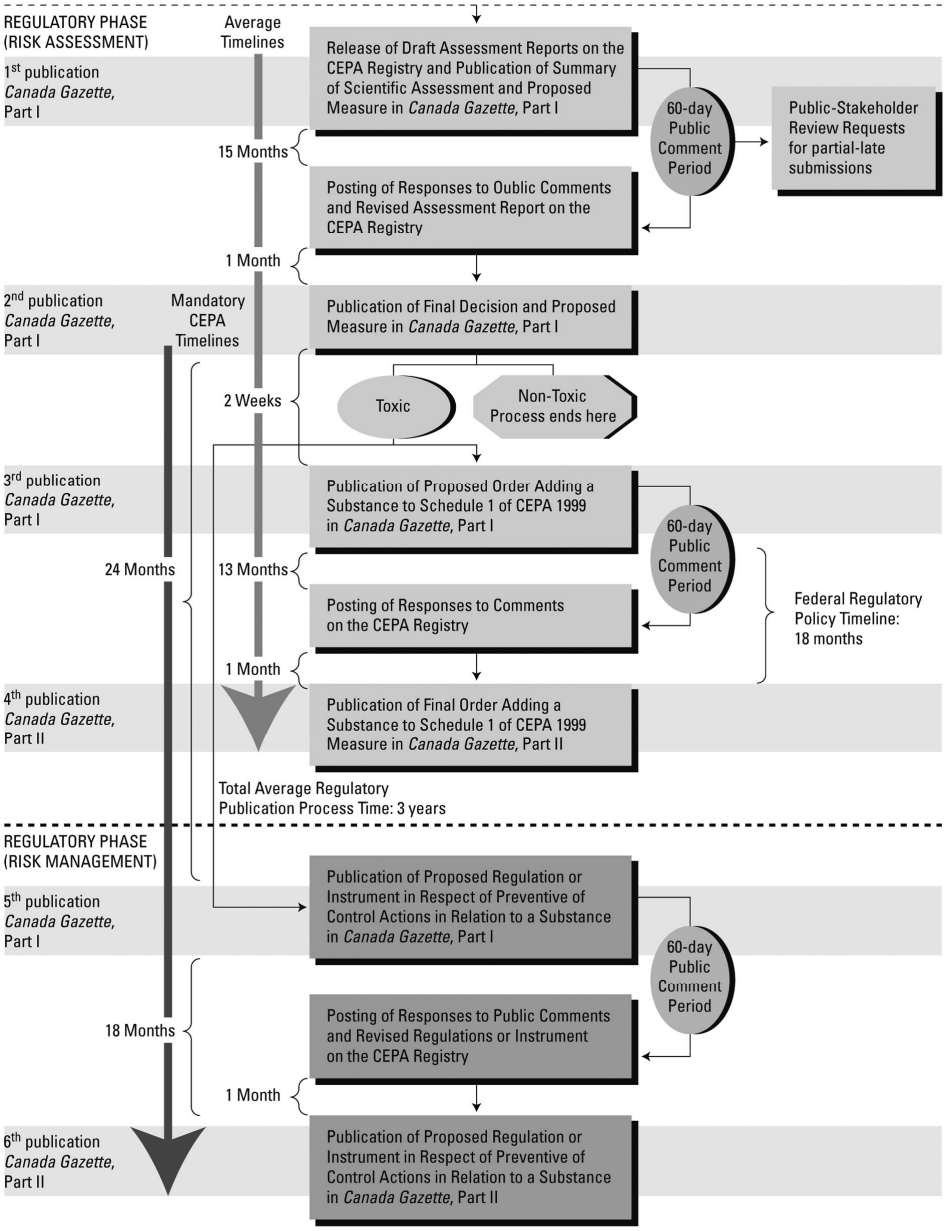


Figure 2. Regulatory Publication Process

Provisions also allow for the partial or total prohibition of manufacture, import or export, and for the submission of information on the substance, the conduct of analyses and monitoring and of tests, submission of samples to the government and the maintenance of records. Before any such regulations are made, it must be ascertained that a regulation does not address an aspect already effectively regulated under another Act (See Section 2.1).

Controls can also take the form of guidelines, standards, codes of practice, plans and voluntary or non-regulatory initiatives and may include any other measures deemed appropriate based on the known level of risk, available technology, and socio-economic considerations. The Act states that, in developing the regulations or other control options, priority is to be given to pollution prevention actions.

For substances that are "categorized in" and for which subsequent screening assessment indicates that they are "toxic" to human health and/or the environment, addition to the List of Toxic Substances requires that a proposed regulation or other control instrument respecting preventative or control actions in relation to the substances be published in the *Canada Gazette* within two years of the additions. Final regulations or instruments must normally be developed and published in the *Canada Gazette* within 18 months following the proposal**.** Figure 2 is a schematic representation of the steps involved in developing control measures for toxic substances.

Box 7. Risk management tools that can be considered in identifying options for managing toxic substances under CEPA, 1999 are [22]:

- Regulations, pollution prevention plans, environmental emergency plans, administrative agreements, codes of practice, environmental quality objectives or guidelines, release guidelines;
- Voluntary approaches - Environmental Performance Agreements, Memoranda of Understanding;
- Non-CEPA 1999 economic instruments - financial incentives and subsidies, environmental charges and taxes
- Joint federal/provincial/territorial initiatives - Canada-wide Standards, guidelines, codes of practice;
- Provincial/territorial Acts - regulations, permits, or other processes;
- Other federal Acts - e.g., Fisheries Act, Pest Control Products Act, Hazardous Products Act.

3.6 Virtual elimination (provisions for persistent, bioaccumulative, toxic (PBt) substances)

When a substance is deemed to be "toxic" under CEPA and also meets certain criteria for persistence and bioaccumulation, is not a naturally occurring radionuclide or naturally occurring inorganic substance, and its presence in the environment results primarily from human activity, ,the substance is then proposed for virtual elimination under the Act.

Box 8. Definition of virtual elimination

Virtual elimination is "the ultimate reduction of the quantity or concentration of the substance in the release below the level of quantitation specified by the Ministers in the (virtual elimination) List".

A *Virtual Elimination List* (the "List") specifies the level of quantitation for each substance included in the List. Virtual elimination would generally be achieved through a series of progressive release limits set by regulations and/or other risk management measures.

3.7 Significant New Activities

Provisions for dealing with significant new activities with respect to chemical and biotechnological substances were introduced in CEPA-1999; these provisions address any new activity that results in, or may result in, significantly greater quantities or concentrations of a substance in the environment, or a significantly different manner or circumstances of exposure to a substance. They are intended to provide additional flexibility and refinement in the application of both the new and existing substances provisions by triggering re-notification of the substance under certain circumstances.

The significant new activity (SNAc) provisions can be used to require a re-notification of a new substance. A SNAc Notice may be issued defining what constitutes a significant new activity in relation to the substance, by inclusion or exclusion. The criteria under which a notification is required and information requirements are also specified therein. This information is further assessed prior to the commencement of any significant new activity to allow the substance to be imported, manufactured, used or released in ways that would not pose a risk to the environment and/or human life or health.

Significant New Activity Notifications (SNANs) contain all prescribed information specified in the SNAc Notice and must be provided within the prescribed time and prior to a company undertaking the significant new activity. Assessment of the information must be completed within the prescribed assessment period [19].

A new substance subject to an SNAc Notice can be added to the DSL with a SNAc ("S") flag; this allows any individual to manufacture, import, use and release the substance in ways that are not defined as a "new activity" under the terms of the definition of 'significant new activity'.

For existing substances, if an activity can be reasonably anticipated which could substantially change the exposure and consequently the risk posed to the environment and/or human life or health, an amendment to the DSL can be published in the *Canada Gazette*. This amendment would include publishing a SNAc Notice and placing a SNAc ("S") flag on the substance. This again allows any individual to manufacture, import, use and release the substance in ways that are not defined as a "new activity" under the terms of the SNAc Notice.

3.8 Information gathering

Provisions for gathering and generating information required for the assessment or control of existing substances under the Toxic Substances provisions of CEPA include

ones to ascertain who is using the substance, and to furnish the government with any existing information (e.g., toxicological information, monitoring data, uses, quantities in use) or samples and to conduct toxicological or other tests. Powers to require industry to carry out testing or studies cannot be invoked unless there is "reason to suspect that the substance is toxic or capable of becoming toxic or it has been determined under this Act that the substance is toxic or capable of becoming toxic". Also a user, manufacturer or importer of a substance is required to provide to the government any information that supports the conclusion that the substance is toxic or capable of becoming toxic.

3.9 Consultation and communication

The results of an assessment of an existing substance (i.e., screening, PSL) or a review of a decision made by another jurisdiction must be made public by issuing a notice in the *Canada Gazette.* The notice must indicate whether no further action is to be taken or whether the substance is to be added to the List of Priority Substances (for further assessment) or to the List of Toxic Substances. A 60 day comment period follows the issuing of these proposals. Provisions exist for objections to be raised if no recommendations are made to add a Priority Substance to the List of Toxic Substances; establishment of a Board of Review may be requested to review the assessment conclusions. If it is proposed that a substance be added Schedule 1 (the Toxic Substances List), consultation with the public is required through the publication of a Notice in the *Canada Gazette*.

Control instruments are developed through consultations with stakeholders, including industry and industry associations, non-governmental organisations (e.g. environment, health and labour), provincial governments, economists, enforcement officials and legal services. Provincial and territorial governments may be involved in developing and implementing the options. All actions regarding toxic substances should be consistent with the Toxic Substances Management Policy [24] (see also section 6).

4 HEALTH ASSESSMENTS UNDER CEPA

This section includes a brief description of the approaches used to implement the key health-related components of the toxic substances provisions of CEPA-1999, with emphasis on novel methodologies developed to address progressive and precedent-setting requirements of the legislative mandate for Existing Substances (See references listed under Section 8 for information relevant to assessment of New Chemicals).

Central to the evaluation of Existing Substances are two types of assessments, namely screening and in depth (PSL). Differences and similarities between these two types of health assessments are presented in Annex 3.

The provisions of CEPA-1999 for selecting (categorization being the most significant), assessing (screening and in-depth) and managing the risks of existing chemical substances, as depicted in Figure 1, are consistent with the principles outlined in Health Canada's "Decision-Making Framework for Identifying, Assessing, and Managing Health Risks" [25].

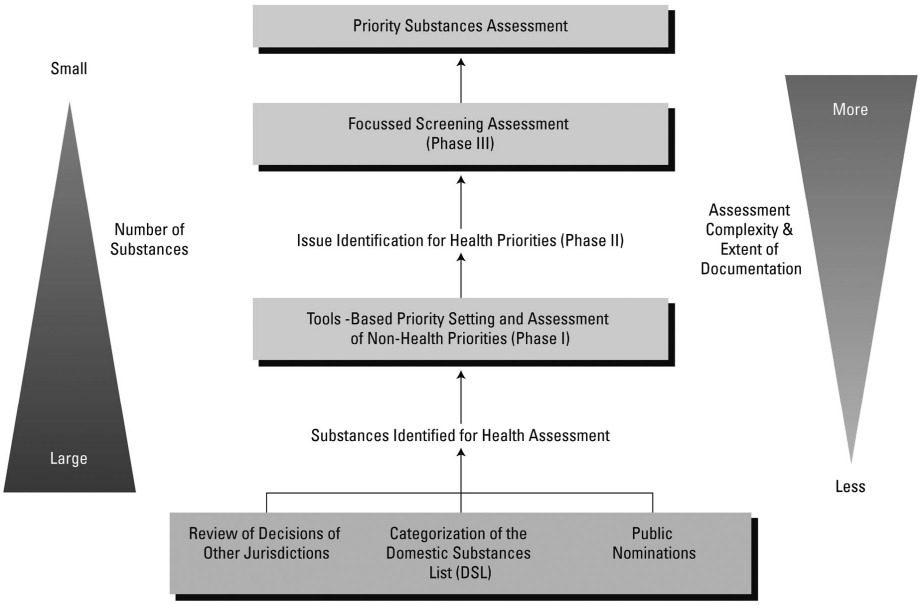


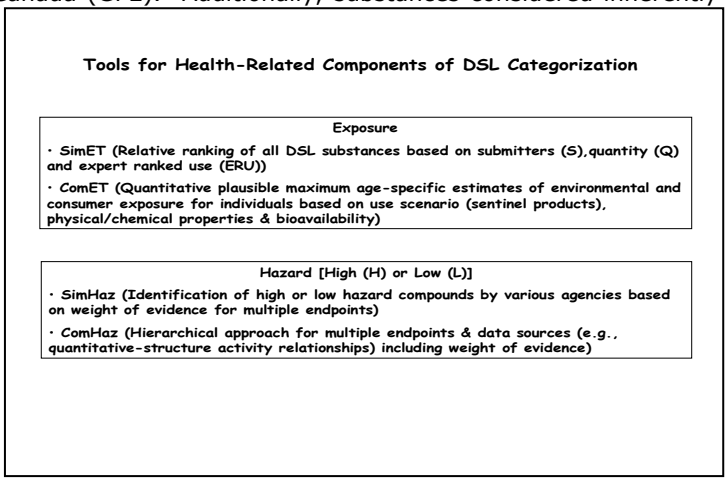
Figure 3. Phases in identifying and assessing health priorities

4.1 Comprehensive framework for health risk assessments under CEPA-1999

The objective of the health-related components of DSL categorization is the identification, for additional consideration in screening, of substances that are highest priorities in relation to their potential to cause adverse effects on the general population. Figure 3 illustrates the steps (Phases) involved in identifying and assessing health priorities in an integrated and iterative framework for priority setting and assessment. To maximize efficiency, the complexity of priority setting, the assessment and associated documentation is tailored to invest only that amount of effort required to identify non-priorities while, at the same time, ensuring that the assessment provides essential support for undertaking risk management of substances where this is deemed to be required.

Phase 1: Tools-based priority-setting (categorization) and assessment

An element of the categorization mandate relevant to human health was the identification of substances that present the greatest potential for the exposure of the general population of Canada (GPE). Additionally, substances considered inherently toxic to humans (iT-human) and persistent or bio-accumulative, (the criteria for which are specified in regulations under CEPA), were to be identified.



In order to identify true health priorities, however, a risk-based framework encompassing both exposure and hazard for all substances was developed, rather than restricting consideration of the criterion "inherently toxic to humans" to the subset of substances considered to be persistent or bioaccumulative. This required multiple stages of increasing complexity, involving development and application of simple and complex exposure and hazard tools.

The simple exposure tool (SimET) was developed to accommodate information submitted during the compilation of the DSL and has three lines of evidence:

- quantity in commerce in Canada;
- number of companies involved in commercial activities in Canada; and
- weighting by experts of the potential for human exposure based on consideration of various use codes.

Based on collective consideration of these three components, it was possible to relatively rank all substances in relation to their potential for exposure. Based on application of specific criteria for each of the components, all substances on the DSL were grouped into one of three categories, i.e., those presenting "greatest", "intermediate" or "lowest" potential for exposure (GPE, IPE or LPE). The results of relative ranking on this basis indicated that volume is not a good surrogate for exposure with many of the highest volume substances presenting "lowest potential for exposure".

Simple (SimHaz) and complex hazard tools (ComHaz) as well as a complex exposure tool (ComET) were and continue to be developed and implemented within an integrated framework for the health-related components of DSL categorization. The complex tools contribute considerably to predictive methodology for both exposure and hazard, including the development of significant numbers of additional

consumer exposure scenarios and a systematic weight of evidence approach to take into account data, results of a suite of quantitative structure activity analysis models and analogue approaches.(For additional information on the tools see http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/contaminants/existsub/framework-int-cadre_e.pdf)

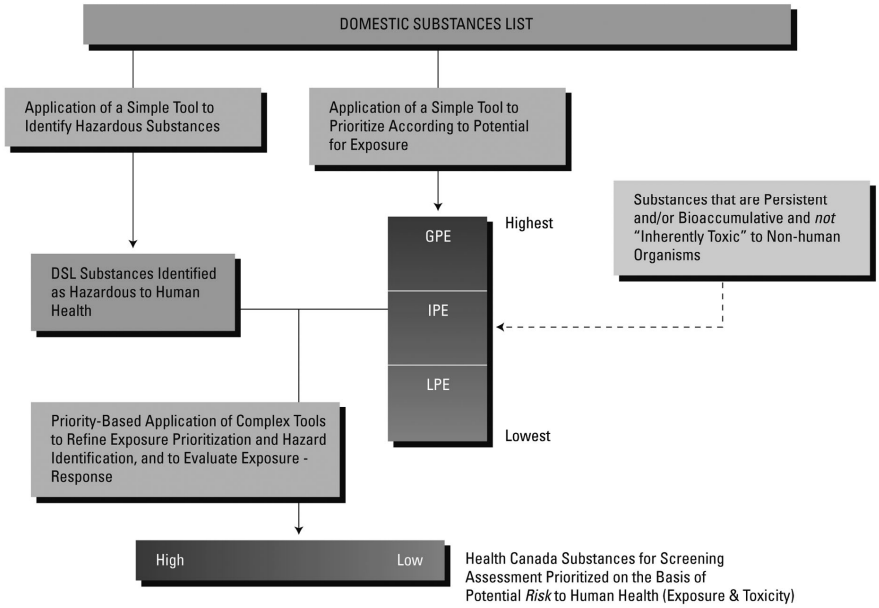


Figure 4. Tools-based approach for health-related components of DSL categorization

The simple exposure and hazard tools were applied to the entire DSL leading to a draft "maximal list" of health priorities, released in October, 2004 [26]**.** The potential for persistence or bioaccumulation to additionally contribute to exposure for certain subsets of substances, namely, those that are organic, was also taken into account (Figure 4).

This approach offered a number of advantages and exceeded the requirements of categorization, by:

- drawing maximally on work completed in other jurisdictions while avoiding continued focus on data-rich compounds;
- not only identifying substances for screening assessment on the basis of exposure, hazard, and/or risk, but also *prioritizing* them on the basis of potential exposure, hazard, and/or risk to human health;

- identifying true priorities for both assessment and data generation, since exposure and complex hazard components of the framework were unbiased in relation to data availability; and
- identifying not only those substances that were iT-human for a subset of substances, but all of the approximately 22 400 existing substances based on criteria for weight of evidence of hazard consistent with those for Priority Substances or screening health assessment.

Implementation of this framework and associated tools has application well beyond simply identifying substances for assessment. These tools enable the efficient prioritization and subsequent screening health assessment of *any substance* considered by the program. Health priorities from categorization have been prioritized by group, based on whether they are exposure, hazard, or risk based, and within groups, based on consideration of their relative potential for exposure. Continued application of the complex tools additionally focused the content of the results of categorization and will contribute to screening assessment for prioritized compounds.

The development of the tools and related products for categorization drew upon considerable prior program experience gained in developing the methodology for conducting in-depth, detailed human health risk assessments of the 69 "Priority Substances,"; most of these assessments were published in the peer-reviewed scientific literature and/or served as the basis for international criteria documents [27].

Substances considered as health priorities based on application of the simple tools are addressed in Phase II, Issue Identification.

Phase II: Issue identification

To increase the efficiency in assessment, it is envisaged that screening health assessments for Existing Substances will incorporate an early stage of Issue Identification. The objective is to ensure timely and maximum utilization of previously well documented peer reviewed assessments and adequate and accurate focus on more recent information and critical issues. While the process for input and content are still in development, robust senior internal technical review and external peer input would be critical to ensure integrity of the product. Formats for supporting use and toxicity profiles have been developed and draw maximally on available information, based on comprehensive and well documented search strategies and solicitation for submission of relevant information.

This stage provides risk managers and stakeholders with the opportunity to contribute information, for example, in the preparation of use and exposure profiles (i.e., identification of specific end uses and potential for exposure); it also provides early indication of potential focus of the assessment and (possible) subsequent risk management action.

Phase III: (Focussed) screening assessments

The objective of a screening health assessment is, to efficiently consider whether or not a substance poses a risk to human health. To increase efficiency, the focus of the assessment is limited principally to information which is considered most critical

with respect to exposure to, and health-related effects of, a substance, in particular the critical aspects identified during Issue Identification. Substances are assessed only to the extent necessary to deem them to be non-priorities, or to provide necessary guidance as a basis for risk management. Depending upon complexity of the issues, complexity of process for peer input may increase (e.g., more of the nature of that for Priority Substances). The objective is to maintain scientific rigour, depending, for example, on the priority for health effects evaluation (based on application of the simple tools) and on the extent of the database, but to vary the degree of detail (and hence the level of effort for the assessment).

Focussed screening health assessments result in the issue of a State of the Science Report which undergoes internal and external peer review and is posted on the web and/or sent to stakeholders. The state of the science report presents only the technical and scientific information on a substance or a group of substances and serves to provide an early indication of the basis for forthcoming conclusions and recommendations; the conclusion of whether or not a substance is "toxic" under the Act and any proposed Ministerial recommendations are published in the *Canada Gazette* which also serves to link the health and environmental assessments.

With respect to the health of the general public, it is the potential for adverse effects following long-term exposure to the, generally, low environmental levels that is often of importance as a basis for decision making (that is, to set a substance aside with no further action, add it to the Priority Substances List, or to recommend addition to the List of Toxic Substances). Hazard characterization for both screening and in-depth (PSL) health assessments entail an examination of the effects critical to adults' and children's health, such as potential organ-specific effects or more specialized hazards such as immunotoxicity, neurological/behavioural toxicity, reproductive toxicity, genotoxicity, cancer and developmental effects. Exposure analyses include consideration of all relevant media and are based on six different age groups (an example is provided in Table 2).

Decision-making for screening health assessments is based on analysis of a margin of exposure (MOE), that is, comparison of critical effect levels with estimates of exposure taking into account the confidence/uncertainties in the available exposure and toxicological databases and other relevant data (e.g., ancillary data on toxicokinetics and/or mode of action). This ensures maximal utilization of available data with several MOEs for potentially critical effects and studies being considered along with associated uncertainties. Delineation of the relative uncertainty and degree of confidence in the exposure and effects databases forms, therefore, a central component of the documentation for screening (and PSL) health assessments. For example, the adequacy of the margin for human health protection takes into account whether exposures are based upon only modelling, measured concentrations of a substance in media important to estimating human exposure (i.e., air, foodstuffs, drinking water, soil, consumer products) or human biomonitoring studies that provide a measure of actual human exposure. It also takes into account the extent of the database as the basis for characterization of hazard and dose-response for all effects particularly those considered critical, including degree of conservatism in the selection of the critical effect. Reliance on MOEs rather than TDIs in screening assessments contributes additionally to efficiency of the process by enabling assessment of larger numbers of substances, drawing maximally on the available database, while minimizing the need for development of exposure-based guidance values for substances that are not considered priorities for further action.

Table 2. Upper-bounding estimates of daily intake of 1,2-dibromoethane (From reference 28; citations in footnotes are as given therein)

Route of exposure	**Estimated intake (µg/kg-bw per day) of 1,2-dibromoethane by various age groups**						
	0–6 months[1,2,3]		**0.5–4 years[4]**	**5–11 years[5]**	**12–19 years[6]**	**20–59 years[7]**	**60+ years[8]**
	Formula fed	**not formula fed**					
Ambient air[9]	0.0050		0.011	0.0084	0.0048	0.0041	0.0036
Indoor air[10]	0.0044		0.0095	0.0074	0.0042	0.0036	0.0031
Drinking water[11]	0.0043	0.0016	0.0018	0.0014	8.0×10^{-4}	8.0×10^{-4}	9.0×10^{-4}
Food and beverages[12]		0.078	0.058	0.037	0.022	0.020	0.016
Soil[13]	1.6×10^{-5}		2.6×10^{-5}	8.4×10^{-6}	2.0×10^{-6}	1.7×10^{-6}	1.7×10^{-6}
Total intake	0.014	0.089	0.080	0.054	0.032	0.028	0.024

1 No data were identified on concentrations of 1,2-dibromoethane in breast milk.

2 Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (EHD, 1998).

3 For exclusively formula-fed infants, intake from water is synonymous with intake from food. The concentration of 1,2-dibromoethane in water used to reconstitute formula was based on data from City of Toronto (1990). No data on concentrations of 1,2-dibromoethane in formula were identified for Canada. Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age, and 90% by 6 months of age (NHW, 1990).

4 Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (EHD, 1998).

5 Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (EHD, 1998).

6 Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).

7 Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).

8 Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).

9 Based on the highest concentration (0.143 µg/m^3) detected for 1,2-dibromoethane in 6766 of 8275 samples of ambient air collected in a national survey across Canada between 1998 and 2002 (Environment Canada, 2002). This survey was selected due to its expansiveness and its currency, which will likely reflect declining use of 1,2-dibromoethane in Canada. Canadians are assumed to spend 3 h per day outdoors (EHD, 1998). Data from which the critical data were selected included Health Canada (2003), Environment Canada (1991, 1992, 1994, 1995 and 2001b), OMEE (1994) and CMHC (1989).

10 In the absence of measured data, the detection limit (0.018 µg/m^3) for a recent indoor air study of 75 homes in Ottawa, Ontario, was used (Health Canada, 2003b). Canadians are assumed to spend 21 h indoors every day (EHD, 1998). Data from which the critical data were selected included Otson (1986), Cal. EPA (1992) and Cohen et al. (1989).

11 In the absence of measured data, the detection limit (0.04 µg/L) from 7 bottled and 27 tap water samples in Toronto, Ontario, was used (City of Toronto, 1990). Data from which the critical data were selected included OME (1988), OMEE (1993) and Golder Associates (1987).

12 In the absence of Canadian monitoring data, detection limits were used in the calculations. A single 1,2-dibromoethane measurement of 13 µg/kg in sweet cucumber pickles in 1995 (U.S. FDA, 2003) was not considered, as the use of detection limits overcompensated its contribution to the overall intake of vegetables in the calculations. In addition, older studies (Gunderson, 1988) in which 1,2-dibromoethane was detected were not used to calculate intake levels, as pesticidal use of 1,2-dibromoethane at that time likely led to levels in food that would not be representative currently.

- Dairy products
- Fats
- Fruits
- Vegetables
- Cereal products
- Meat & poultry
- Fish
- Eggs
- Foods, primarily sugar
- Mixed dishes & soups
- Nuts & seeds
- Soft drinks & alcohol

Amounts of foods consumed on a daily basis by each age group are described by Health Canada (EHD, 1998).

[13] The method detection limit (4.0 ng/g) for soil measurements in urban (59 samples) and rural (102 samples) parklands in Ontario was used to represent the maximum exposure concentration of 1,2-dibromoethane (OMEE, 1993). Data from which the critical data were selected included Golder Associates (1987).

Where relevant and available, toxicokinetic data and weight of evidence for hypothesized modes of action and human relevance are taken into account in transparent analytical frameworks [29, 30]**.** An example of a margin of exposure analysis which appears in the State of the Science report on "perfluorooctane sulphonate (PFOS), its salts and precursors containing the $C_8F_{17}SO_3$ moiety" is presented in Table 3.

Decisions on the adequacy of margins take into account the experience gained through conducting screening assessment of large numbers of chemicals and considering the adequacy of various margins for human health protection for chemical substances with a wide variety of datasets. The approach by which these factors are considered in decision-making for screening health assessment has been built upon the experience gained, and are consistent with, decision-making in the health risk assessment of Priority Substances. Weight of evidence for effects for which available data on mode of action indicate that there is a probability of harm at all levels of exposure is also considered in decision-making.

Where it is ascertained from a screening assessment that a more comprehensive analysis of available data (e.g., a complex analysis of exposures from consumer products) and/or the generation of additional data (e.g., on mode of action) is warranted to more fully inform decision-making in order to reach a definitive conclusion, more detailed assessments are undertaken.

An option stipulated in CEPA-1999 is to recommend that a substance be added to the PSL. Any recommendation for this action would necessitate defining what needs to be done to further develop the assessment (e.g., request additional information from industry to be able to better assess exposure, examine mode of action) and ascertaining whether such a course would be more advantageous to the assessment outcome.

Table 3. Margins of exposure for PFOS (From reference 31; citations in footnotes are as given therein)

Critical study and effect	PFOS dose metric at critical effect	Metric(s) of human exposure to PFOS	Margin of exposure (critical effect/human exposure)
Microscopic changes in the liver of rats (m + f) receiving PFOS in the diet for 2 years[1]	**Serum PFOS level:** 13.9 µg/mL[2]	**Mean serum PFOS level in adults in Canada[3]:** 0.028 µg/mL	496
		95th percentile of human serum PFOS level in adults in Canada[3]: 0.0631 µg/mL	220
		Mean serum PFOS level in children in the United States[4]: 0.0375 µg/mL	371
		95th percentile of serum PFOS level in children in the United States[4]: 0.097 µg/mL	143
	Liver PFOS level: 40.8 µg/g[5]	**Mean[6] liver PFOS level:** 0.0188 µg/g	2170[7]
Thymic atrophy (f), reduced serum high-density lipoprotein (m), cholesterol (m), triiodothyronine (m) and total bilirubin (m) in monkeys administered PFOS for 26 weeks[1]	**Serum PFOS level:** 14.5 µg/mL[8]	**Mean serum PFOS level in adults in Canada[3]:** 0.028 µg/mL	518
		95th percentile of human serum PFOS level in adults in Canada[3]: 0.0631 µg/mL	230
		Mean serum PFOS level in children in the United States[4]: 0.0375 µg/mL	387
		95th percentile of serum PFOS level in children in the United States[4]: 0.097 µg/mL	149
	Liver PFOS level: 19.8 µg/g[9]	**Mean[10] liver PFOS level:** 0.0188 µg/g	1053[11]

1 Covenance Laboratories, Inc. (2002a)
2 Average of mean levels in males (7.6 µg/mL) and females (20.2 µg/mL).
3 Kubwabo et al. (2002)
4 3M Medical Department (2002)
5 Average of mean levels in males 26.4 (µg/g) and females (55.1 µg/g).
6 Mean level of PFOS in livers from 30 cadavers (Olsen et al., 2003).
7 Published data on 95th percentile not available; margin of exposure based upon highest level of PFOS in human liver from this study (0.057 µg/g) is 716.
8 Average of mean levels in males (15.8 µg/mL and females (13.2 µg/mL) (week 26).
9 Average of mean levels in males 17.3 (µg/g) and females (22.2 µg/g) (week 27).
10 Mean level of PFOS in livers from 30 cadavers
11 Published data on 95th percentile not available. Margin of exposure based upon highest level of PFOS in human liver from this study (0.057 µg/g) is 347.

PSL assessments

In view of the objective of CEPA1999 to assess much larger numbers of substances more efficiently, the comprehensive and process intensive approach adopted for Priority Substances will likely be confined to very limited numbers of compounds and/or specific aspects of assessments on specific substances, which warrant a complex process and content.

For substances on the first PSL (PSL 1), chemicals were classified formally into discrete groups with respect to both their potential carcinogenicity and mutagenicity in humans based on clearly defined criteria for weight of evidence which took into account the quantity, quality and nature of the results of available toxicological and epidemiological studies [32]. For the assessment of PSL 2 substances (and more recent screening assessments), descriptions of the weight of evidence for carcinogenicity are more narrative in nature, in the interest of accommodating increasing availability of data on mode of action. To provide guidance in setting priorities for managing the risks of substances considered to present a risk of cancer and/or heritable mutations, Exposure was compared with the dose associated with a specified (5%) increase in tumour incidence as a basis for development of a measure of dose-response (i.e., Exposure/Potency Indices).

For some Priority Substances, the critical effect for decision-making was considered to be associated with a mode of action for which where there is a dose or exposure concentration below which adverse health effects are not likely to be observed (i.e., organ specific toxicity and/or cancer associated with same). For these substances, Tolerable Intakes or Tolerable Concentrations (TI or TC) (i.e., the intake or concentration to which it is believed a person can be exposed daily over a lifetime without deleterious effect), were derived by dividing the critical effect level (e.g., Benchmark Dose or Concentration (BMD or BMC) or No- or Lowest-Observed-(Adverse)-Effect-Levels (NO(A)EL or LO(A)EL) by an uncertainty factor. The Benchmark dose/concentration is the effective dose/concentrations (or their lower confidence limits) that produce a specified increase in incidence above control levels. The basis for uncertainty factors is clearly delineated and, where available data permit, replaced by chemical-specific adjustment factors [32].

Details of the application of the above-mentioned approaches are available in the assessment reports for the Priority Substances, all of which are available at http://www.hc-sc.gc.ca/ewh-semt/contaminants/existsub/eval-prior/index e.html. (See also Annex 3 for information on differences and similarities between screening and PSL assessments).

Mixtures of substances

The approach for priority setting and/or assessing mixtures of chemicals depends on the nature of data available [32]. In some instances, the chemical composition of a mixture may be well characterized, levels of exposure of the population known, and detailed toxicological data on the mixture are available. More frequently, however, not all components of the mixture are known, exposure data are uncertain and the toxicological data are limited. Thus the approach that can be used will depend on whether data are available:

- for the mixture as a whole;

- only for components of mixture;
- for similar mixture(s).

4.5 Data requirements, information gathering and peer involvement.

Search strategies

Search strategies for all aspects of the program are comprehensive and documented in the reports of various stages of priority setting (categorization), and assessment (Issue identification, screening and PSL). For substances that are considered as health priorities in screening, in most cases, there is no legislated minimum dataset; however the Screening Information Dataset in the OECD High Production Volume Chemicals Program [33], or equivalent, is considered an appropriate basis to complete the assessments. Mechanisms for gathering information under CEPA are described in Section 3.8. Experience acquired in use-profiling from public sources for hundreds of chemicals based on hierarchical, evolving and comprehensive search strategies as part of input for the complex exposure tool provides a consistent and robust basis for understanding the use patterns of the vast majority of chemicals of interest.

Documentation

The conclusions and findings of an assessment, proposed and final regulations and other proposed or final actions under CEPA must ultimately be announced in the *Canada Gazette*. Reports of the outcome of screening assessments, assessments of priority substances and reviews of other jurisdictions' decisions can also be made available "in any other manner that the Minister (of the Environment) considers appropriate". These announcements must state whether the Ministers intend to take no further action respecting the substance, to add a substance to the PSL (unless it is already on the List), or to recommend that the substance be added to the List of Toxic Substances.

The nature and scope of the documentation for the health assessments has been modified with the change in emphasis from assessment of priority substances to categorizing and screening substances on the DSL. Available reports include State of the Science reports for screening assessments and their supporting documentation (including Issue Identifications and use and hazard profiles), PSL assessment reports and supporting documentation and briefer tabulations of output for non-priority tools based assessments, with much more extensive documentation available on the methodology. Considerable efficiency is gained in tailoring the level of documentation to the task at hand, involving no more effort than is necessary to set aside a substance as a non-priority or to provide necessary information to permit risk management (see Figure 3)**.**

Concise State of the Science reports of the screening health assessments constitute an essential basis for documenting and communicating to the public the scientific basis for the conclusions and decisions required under CEPA. The objective is to produce as concise a document as possible containing only the critical (relevant) information that supports the ultimate conclusion of whether a substance is "toxic", "suspected of being "toxic", or "not considered to be toxic", and the decision/recommendation for any further action; thus the initial focus is on the most

critical effects and conservative effect levels and upper-bounding estimates of exposure. State of the Science reports are issued without the Ministerial conclusions and recommendations as a means of alerting stakeholders to the scientific underpinning upon which any recommendations will be based; these conclusions appear subsequently in the *Canada Gazette* Notice along with a synopsis of the technical findings. The State of the Science report and Conclusions in the *Canada Gazette* represent the Screening Assessment Report under CEPA.

Critical information included in screening assessments comprises the identity, production and uses of the substances, sources and levels of human exposure, and health effects. The screening assessment report also outlines the objective of the screening assessment, and delineates the databases which serve as the basis for determining the critical effect levels and upper bounding exposure estimates. For brevity, both hazard (health effects) and exposure (intake) data are tabulated where possible. Screening assessment reports are made available following external peer review and Departmental management approval of their content and of the process followed in their preparation.

More detailed documentation supports the summarized technical data presented in the assessment reports. For the PSL assessment program, the supporting documentation comprised detailed text, multiple tables and a comprehensive reference list; this documentation was designed to present and describe in detail all relevant data needed to demonstrate how the critical exposure and effects were determined. The textual content of the supporting documentation was extensive and required investment of considerable time and resources.

In the interest of meeting objectives to more efficiently assess larger numbers of substances, supporting documentation for the screening assessments is much more issue-focussed and comprises a series of background documents and data tabulations prepared during the course of an assessment rather than integration into a comprehensive criteria document. The extent of this documentation is necessarily dependent upon the tools-based designated priority of the substance and complexity of the issues. For substances designated as priorities for assessment, it includes, as a minimum, Issue Identifications, exposure and hazard profiles. Tabular summaries of supporting information focussed in critical areas will also be included. These may include survey results, exposure scenarios, robust data summaries for critical studies, framework analyses for weight of evidence of specific endpoints (cancer/genotoxicity), and hypothesized modes of action and relevance to humans.

For "tools-based" assessments the results of which indicate non-priorities for additional work, supporting documentation is limited principally to the more extensive documentation on methodology (see http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/contaminants/existsub/framework-int-cadre_e.pdf) with some chemical-specific information available on request (e.g., weight of evidence for cancer/genotoxicity based on data, quantitative structure activity analyses and consideration of analogues). For such substances, State of the Science reports and Gazette Notices report conclusions on significant numbers of substances in tabular format.

Peer involvement

Because of CEPA's requirement to set priorities from thousands of existing chemicals and the associated need to develop novel methodologies and the expectation for rapid review of prioritized substances, the program provides opportunity for incorporation of increasingly complex peer involvement not, only in the assessments of individual or groups of substances, but also in the development of novel methodology for categorization.

Specifically, the program has incorporated to an increasing extent more formal peer input at the earlier stages of development for both methodology and assessments. In addition, the complexity of peer input, consultation, and peer review is greater for more robust assessments for substances of highest priority and complex issues such as methodology development. This approach maximizes efficiency while maintaining the defensibility of output of the three different levels of priority setting and assessments of increasing complexity within the program (categorization, screening assessment, and full assessment).

Table 4 Peer involvement for each stage of product development.

STAGE	Peer Input	Peer Consultation	Peer Review
Problem Formulation	√		
Draft Work Product		√	
Final Draft Work Product			√

The three types of peer involvement, the level and complexity of which increases with the stage of development of documentation and complexity of issues as discussed by Meek et al. [29] and their utilization in various stages of the program are presented in Table 4

Full assessments for Priority Substances generally include early peer input to identify relevant data followed by external panel peer reviews at the end of the process. On the other hand, for screening assessments, there is an early issue identification stage to solicit peer input on identification of relevant data and issues and confirming the focus of the assessment. Since screening assessments are less complex, at a later stage in their development, their peer review is generally restricted to written comments by several external experts. Panel meetings are convened only where there are subsequent outstanding issues. Development of methodology for priority setting and/or assessment of risk often entails all three stages of peer involvement (i.e., input, consultation and review).

5 ENVIRONMENTAL ASSESSMENT OF EXISTING SUBSTANCES

5.1 Categorization

As indicated in Section 3.4, in addition to categorizing all substances on the DSL to identify those that presented "Greatest Potential" for exposure and "inherently toxic" to humans, substances were to be identified for further work if they were persistent (P) and/or bio-accumulative (B) in accordance with regulations and "inherently toxic" (iT) to non-human organisms (i.e., if they were PiTs, BiTs, or PBiTs).

Persistence and bioaccumulation

The CEPA regulations for characterizing persistence and bioaccumulation came into force in March, 2000 [34]. Under these regulations, a substance is considered persistent if its transformation half-life, based on degradation through chemical, biochemical, and photochemical processes, satisfies the criterion in any one environmental medium [Table 5]. Alternatively, it is considered persistent if it is subject to long-range transport (e.g., transported to remote regions such as the Arctic).

Table 5 Criteria for persistence and bioaccumulation

Persistence		
Medium	Half-life	Bioaccumulation
Air	$\geq$2 days	BAF $\geq$ 5000
Water	$\geq$6 months	or
Sediment	$\geq$1 year	BCF $\geq$ 5000
Soil	$\geq$6 months	or
		log K_{ow} > 5

Criteria for bioaccumulation that were applied to organic substances are also presented in Table 5. Bioaccumulation factors (BAF) are preferred over bioconcentration factors (BCF); in the absence of BAF or BCF data, the octanol–water partition coefficient (log K_{ow}) may be used.

Inherently toxic to non-human organisms

Inherently toxic to non-human organisms is evaluated based on aquatic (including benthic) toxicity data. This choice reflects the comparative availability of test data in aquatic/pelagic versus terrestrial species and is consistent with required elements in international initiatives such as the Organisation for Economic Co-operation and Development's (OECD) Screening Information Data Set (SIDS) [33]. It also takes into account the absence of recognized standard tests/methods and paucity of data on inhalation toxicity for invertebrates, amphibians, reptiles, or birds. In addition, owing to the relative lack of experimental data, most of the values on which

categorization for inherently toxic to non-human organisms was based were modelled. Virtually all decisions were based on external effect concentrations, either median lethal (LC_{50}) or effective (EC_{50}) concentrations.

The criteria for inherently toxic to non-human organisms are presented in Table 6**.** The thresholds chosen are consistent with those in various European Union (EU) and U.S. Environmental Protection Agency (EPA) initiatives. Where both acute and chronic effects data are available, for reasons of consistency preference is for application of the acute toxicity values, since most available data are for acute endpoints.

Table 6 Criteria for acute and chronic toxicity to aquatic species (algae, invertebrates, fish)

Exposure duration	Criteria
Acute	LC50 (EC50) $\leq$1 mg/L
Chronic	NOEC* $\leq$0.1 mg/L

* NOEC = no-observed-effect concentration.

5.2 Ecological screening assessments

As with health assessments, the process for preparing ecological screening assessments continues to evolve. Approaches applied vary depending upon the nature of the substance (PBiT, PiT or BiT) and potential for exposure (based on quantities in Canadian commerce).

A typical screening assessment is similar to that for a substance on the PSL, involving consideration of entry, exposure and effects characterization, and risk analysis steps.

The objective of entry characterization is to identify the various uses and sources of the substance in Canada, the quantity of the substance released from each of these sources, and how the substance is released over time, to air, water or soil. Entry characterization includes all phases of the substance's life cycle, from manufacture or importation, through transportation and use, to final disposal. Information gathered during this phase is the first step in determining exposure. If the substance is found to be "toxic" under CEPA, this information is also used to guide the development of risk management options.

In exposure characterization, data from modelling and/or monitoring studies are used to describe the spatial and temporal trends in concentrations of the substance in various environmental media (air, water, sediment, soil) in Canada. At this stage the exposure relevant to each identified receptor organism is typically quantified, i.e., a Predicted Exposure Concentration (PEC), is calculated for each. A PEC is usually selected to reflect a high-end exposure value. However, in some cases exposure may be characterized as a distribution of exposure values.

The aim of effects characterization is to describe the types of impairment that can result when different classes of organisms (e.g., plants, aquatic and terrestrial invertebrates, fish, mammals) are exposed to the substance. Typically a Critical Toxicity Value (CTV), or the lowest concentration of a substance that will cause a certain adverse effect, is identified for each type of receptor organism. A CTV is usually calculated from the results of short-term (acute) and long-term (chronic)

laboratory toxicity tests and/or from modelling (e.g., QSAR) estimates. CTVs generally represent low or no effects toxicity values for sensitive organisms. However, in some cases effect levels may be represented by distribution of threshold effects values for an ecologically relevant range of species.

In risk characterization, a weight of evidence approach is used to determine the nature of, and where possible and appropriate, the likelihood of adverse effects. A first step usually involves deriving a Predicted No Effect Concentration (PNEC) for each assessment endpoint by dividing the CTV by an assessment factor. The magnitude of the assessment factor varies depending upon the quantity and quality of available effects data, and increases in proportion to uncertainties associated with extrapolating from effects in the laboratory to those anticipated in the field (e.g., the possibility that species found in the wild may be more sensitive than laboratory species; fluctuations in temperature in the field, which may increase susceptibility to effects).

Generally an important line of evidence when evaluating risk is the magnitude of the PEC/PNEC quotient. Quotients are estimated for each assessment endpoint, and values above 1 are typically interpreted to indicate risk. Risk may alternatively be quantified in a probabilistic or semi-probabilistic manner by comparison of the distributions of exposure and/or no effects values. However, decisions about ecological risks may be influenced by additional factors. These include information on the persistence and bioaccumulation potential of the substance, its inherent toxicity, and its distribution in Canadian environmental media. When available, evidence that environmental concentrations are changing with time or, evidence of effects in field situations is also be considered. Evidence that a substance is both persistent and bioaccumulative (as defined in the Persistence and Bioaccumulation regulations of CEPA-1999), when combined with evidence of toxicity and potential for release into the Canadian environment is interpreted to indicate potential to cause ecological harm. This is consistent with the requirements of CEPA-1999, which requires application of a weight of evidence approach and the precautionary principle in conducting and interpreting the results of assessments.

A number of substances that met the categorization criteria based on their PiT or BiT status, were deemed unlikely to pose an ecological risk because of the very small amounts anticipated to be in commerce in Canada. For these substances, a "rapid screening" assessment process was developed to determine whether they are indeed unlikely to cause harm.

Ecological screening assessments of existing substances under CEPA-1999 are *protective* in that they typically focus on high-end risks (i.e., actual cases where exposures and sensitivities of receptor organisms are expected to be highest). Plausibly *conservative* assumptions may be made in the face of *uncertainty* resulting from data gaps in such assessments. Stakeholders are provided opportunity to submit additional information before the assessment is finalized to reduce identified uncertainties; where no such information is received, the conclusion (based partly on conservative assumptions) may stand.

When combining several conservative assumptions, steps are generally taken to ensure that the overall amount of conservatism in an assessment is not extreme. However, in certain cases where the harm the substance could cause is judged to be serious or irreversible, and the uncertainties are especially large, a conclusion may incorporate a high amount of conservatism. In such cases, a conclusion that a substance may cause harm could be called *precautionary* in that

consequent risk management actions could be viewed as being consistent with the Precautionary Principle [35].

5.3 PSL assessments

From a methodological perspective Priority Substance and screening ecological assessments are quite similar. PSL assessments, however, typically involve a more in depth analysis and reporting of available data, and can take up to 5 years to complete. In addition for PSL assessments there is greater opportunity to fill data gaps and increase the realism of the assessment, especially when where is a possibility of concluding that ecological harm is occurring. Thus for PSL assessments, the amount of conservatism associated with a finding that a substance may be causing ecological harm is typically lower.

Environment Canada convenes an Environmental Resource Group (ERG) for each Priority Substance assessed. The ERG can include experts from industry, academia and members of other government departments or other levels of government who have particular expertise with the substance. The ERG participates in the assessment process by performing tasks ranging from the review of draft documents and assistance in data collection to the development or writing of sections of supporting documents or assessment reports.

6. MANAGEMENT OF TOXIC SUBSTANCES

6.1 Priority Substances and other early measures

Annexes 1 and 2 show the chemical entities on the Priority Substances Lists that have been deemed to be toxic with respect to health and/or the environment. By December, 2006, more than 40 had been added to Schedule 1 and, for nearly all of these, measures to control their presence in, or entry into, the environment had been put in place or were under development. The actions taken with respect to the Priority Substances make up only part of those taken to control the entry of toxic substances into the environment under CEPA. Thus, as a result of this and initiatives taken under other provisions of the Act, some 80 existing substances including classes of substances, complex mixtures, effluents and emissions had been placed on Schedule 1as of early 2007 [36].

Box 10. CEPA-1999 instruments that can be used to satisfy the requirement for establishing **preventive** or **control** actions 37:

- Regulations
- Environmental codes of practice
- Pollution prevention plans
- Environmental emergency plans
- Environmental release guidelines

For substances deemed to be "toxic" as a result of a Priority Substances List assessment, a screening assessment, or a review of a decision by another jurisdiction, it is required that a proposed regulation or instrument be published that will lead to **preventive** or **control** actions for managing the substances and hence reductions in, or elimination of, their risks to the environment or human health. As described in Section 3.5, there are time limitations for issuing and finalizing these

control instruments.

The "Toxics Management Process" has been set up to ensure that risk management tools are developed with due input from affected parties within timelines set out in the CEPA-1999 [37]. A key aspect of this Management Process is a Risk Management Strategy document which is prepared by Environment Canada and Health Canada in consultation with the CEPA National Advisory Committee [22] and affected stakeholders. This document sets out how the risks to health and the environment posed by release of each toxic substance are to be addressed.

Management strategies are not necessarily specific to each "toxic" (priority) substance. Thus, if control of several substances in one industrial sector is required, a sector-specific strategy could be developed. An example, of a broader strategy is that for acrolein and other aldehydes through the following set of initiatives [38]:

- Environmental Emergency Regulations
- Off-Road Compression-Ignition Engine Emission Regulations
- On-Road Vehicle and Engine Emission Regulations
- Off-Road Small Spark-Ignition Engine Emission Regulation

The rationale for these engine regulations was that several aldehydes including ones on the second Priority Substances List are emitted from vehicle exhausts thereby leading to efficiencies by regulating them together under the same instrument.

6.2 The Chemicals Management Plan

In December 2006 the Government of Canada announced its Chemicals Management Plan [39] in which it was sought not just to deal with the industrial chemicals identified as high priorities from categorization but also to strengthen CEPA's integration with other federal chemical regimes administered under statutes such as the *Hazardous Products Act,* the *Food and Drugs Act*, and the *Pest Control Products Act.* With respect to industrial chemicals, the Plan consolidated a number of actions already underway and, significantly, sought, by way of a "Challenge", to increase Industry's role in proactively identifying and managing the risks associated with the those it produces and/or uses. Ultimately, appropriate actions to deal with identified priorities would be taken by 2020.

Box 11. The Chemicals Management Plan included:

- a "challenge" to industry;
- prohibitions and virtual elimination;
- rapid screening of lower risk chemicals;
- restrictions on re-introduction and new uses;
- integrating government activities (e.g., relating to pesticides,
- monitoring, surveillance and research; and
- industry stewardship of chemical substances.

The Challenge to industry

Several hundred chemicals were identified through categorization as being priorities for action; these substances were identified as meeting:

- each of the ecological categorization criteria for persistence (P), bioaccumulation (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada and/or;
- the criteria for greatest or intermediate potential for exposure (GPE or IPE) and were identified as posing a high hazard to human health (that is showed evidence of carcinogenicity, mutagenicity, developmental toxicity or reproductive toxicity).

Inherent to taking action on these substances under the Chemicals Management Plan is reliance on strong stewardship on the part of Canadian manufacturers, importers and users who were challenged to provide new information on these chemicals in order to:

- improve, where possible, information for risk assessment (e.g., P, B and iT data);
- identify industrial best practices in order to set benchmarks for risk management and product stewardship; and
- collect environmental release, exposure, substance and/or product use information.

Industry was also required to provide new information about how it is manages specified chemicals, their use patterns, release and exposure pathways, potential substitution options, analytical methods and the financial implication of eliminating the specified substances.

The absence of information does not preclude action being taken to ensure that human health and the environment are safeguarded; thus if the requisite information is not provided by stakeholders, the federal government will implement controls, as appropriate.

Additional aspects pertaining to industrial chemicals announced under the Plan included the prohibition of two substances, perfluorooctane sulphonate and its salts and polybrominated diphenyl ethers that had been subject to screening assessments and the placement of restrictions on some substances under the SNAc provisions of CEPA (see Section 3.7)

In December 2006, Canada began issuing requirements under the Significant New Activity provisions of CEPA-1999 that affect those high-hazard chemical substances not currently in use in Canada. In accordance with these provisions, industry must provide data to the government under the New Substances provisions of CEPA before any of the subject chemicals can be re-introduced into Canada. Significant New Activity provisions are also to be applied to an additional 150 chemical substances that were found to be hazardous to humans. While the current uses of these substances may be adequately managed, this measure is designed to ensure that any new or increased use does not occur without the conduct of an informed assessment and implementation of appropriate controls.

7 CONCLUDING REMARKS

Developments in Canada have included the integration of sectoral legislation such as the Environmental Contaminants Act, the Clean Air Act and the Canada Water Act into a single piece of environmental protection legislation, the Canadian Environmental Protection Act. The definitions of "environment" and "toxic" therein require that all aspects of the environment (multi-sectoral) be addressed in assessing the environmental and health risks. Assessment approaches are risk based and management of identified risks can be taken on a "cradle-to-grave" basis. Moreover, the safety net provisions of CEPA require a multi-media approach to health risk assessment and management even though some aspects of management may ultimately be taken under other federal of provincial legislation. While Canada's early environmental legislation addressed both new and existing chemicals, subsequent developments in both of these areas have resulted in increasingly more prescriptive approaches in the legislation and the need for greater transparency and accountability for the actions taken.

Specifically, since its introduction in the late 1980s, chemicals control legislation in Canada has imposed time limited mandates for multimedia assessment of considerable numbers of existing chemicals and subsequent risk management of those considered to pose a risk to the health of the general population and/or environment. In the mid 1990s, Notification Regulations for New Substances (chemicals and polymers) were introduced which required companies or individuals wishing to import or manufacture substances new to Canada to provide certain information specified in regulations.

More recently, precedent-setting provisions introduced to systematically identify in a timely manner, priorities for assessment and management from among the approximately 22,400 existing substances has necessitated the development of innovative methodology including evolution of the previously linear or sequential steps of risk assessment and risk management to a more iterative approach where the need for and focus of potential control options are identified at an early stage of assessment. It has also required development of assessment products that efficiently dedicate resources, investing no more effort than is necessary to set aside a substance as a non-priority or to provide necessary information to permit risk management.

These provisions lead in many respects similar developments in other countries. Since the 1960s, the scope of the European Community's chemicals control legislation has expanded with respect to the media of interest (air, water, products) and in terms of consideration of environmental as well as human health effects. These developments have been accompanied by a change in approach from a hazard-based to a risk based one. This has required the development and refinement of approaches to risk assessment and, in particular, the development of exposure assessment methodologies and risk assessment models. The need to adopt a life cycle approach to effectively manage the harmful effects of chemicals was also recognised. Recently existing chemicals are increasingly being emphasized since their presence in the environment was viewed as cause for greater concern than for new chemicals.

Canada's approach to assessing new chemicals is in line with that followed in the EU in that it requires the up-front provision of prescribed data to permit a meaningful first assessment of the health and environmental risks. The data

requirements are based on the data-set developed within the OECD Chemicals Programme but can vary depending on the quantities involved and the nature of the substance. In the US system, initial reporting of test data is not required; rather results from quantitative structure activity relations can be used to require the generation of experimental data on new chemicals.

One of the most far reaching provisions of the current CEPA-1999 was the categorization of the 22,400 chemicals on Canada's Domestic Substances List. Canada became the first country to have carried out such an analysis of its list of existing commercial chemicals and, by doing so, attempted to respond to the concerns expressed that, under earlier legislation, too few chemicals were being addressed. Similar concerns about the slow rate of progress in managing industrial chemicals are evident in the EU. Initiatives such as those dealing with High Production Volume Chemicals have been undertaken by the US and internationally through the OECD. The results of categorization have identified other types of priorities thereby providing Canada an opportunity to contribute to international efforts to control toxic substances by identifying its own unique strategy for managing industrial chemicals.

In Canada, the responsibility for assessing and developing strategies for managing industrial chemicals has rested primarily on the shoulders of the environment and health departments of the Federal Government. Under the proposed REACH legislation in Europe, it is industry's responsibility to assess the risks of existing substances and to develop the means to adequately control any identified risks. Based, in part, on this European approach, fundamental to Canada's Chemicals Management Plan announced in December, 2006 is the "Challenge" made to industry to strength its role in proactively identifying and managing risks associated with the chemicals it produces.

A significant difference between CEPA and REACH is the considerable emphasis placed in the latter on assessment and control of occupational exposures to existing substances; occupational exposures are not addressed under CEPA because of the responsibility that the provinces have for occupational health and safety.

The number of substances managed to date as a result of the PSL assessment program compares favourably with actions taken by other jurisdictions. Many of the 69 priority substances on Lists 1 and 2 were deemed to pose a risk to health or the environment and measures to control the entry into the environment of most of these toxic substances have either been implemented or are under development. The challenging provisions of CEPA-1999 to determine and address priorities from among all substances on the DSL are only starting to be implemented (as summarized in Section 6.2) and are expected to result in a significant increase in the rate at which existing substances are assessed and, where toxic, managed.

Based on the progressive nature of the legislation which has necessitated increased efficiency in assessment and management of industrial chemicals, Canada continues also to contribute extensively to developments in international programs. This includes development of formats and processes for priority setting and assessments and assurance of their integrity through, for example, contribution to development of a robust peer review process.

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Annex 1
Standing of Priority List 1 Substances (PSL 1)

Substance	Conclusion-Health[a]	Conclusion-Environment[a]	Scheduled
Aniline	Proposed T	NT	No
Arsenic and its compounds	T for inorganic arsenic compounds	T for dissolved and soluble forms of inorganic arsenic	Yes[d]
Benzene	T	NT	Yes
Benzidine	T	NT	Yes
Bis (2-chloroethyl) ether	No conclusion[b]	NT	No
Bis (chloromethyl) ether	T	NT	Yes
Bis (2-ethylhexyl) phthalate	T	No conclusion	Yes
Cadmium and its compounds	T for inorganic cadmium compounds	T for dissolved and soluble forms of inorganic cadmium compounds	Yes[d]
Chlorinated paraffin (CP) waxes	T for short and proposed T for medium and long chain CPs	Proposed T for short, medium and some long chain CPs	No
Chlorinated wastewater effluents	No conclusion	T	Yes
Chlorobenzene	NT	NT	No
Chloromethyl methyl ether	T	NT	Yes
Chromium and its compounds	T for hexavalent chromium compounds; NT for trivalent chromium compounds	T for hexavalent chromium compounds; no conclusion for trivalent compounds	Yes[d]
Creosote-impregnated waste materials	No conclusion	T for creosote waste products and creosote-contaminated sites	Yes[d]
Dibutyl phthalate	NT	NT	No
1,2-Dichlorobenzene	NT	NT	No
1,4-Dichlorobenzene	NT:	NT	No

Substance	Conclusion-Health[a]	Conclusion-Environment[a]	Scheduled
3,3'-Dichlorobenzidine	T	NT	Yes
1,2-Dichloroethane	T	NT	Yes
Dichloromethane	T	T	Yes
3,5-Dimethylaniline	No conclusion[b]	NT	No
Di-n-octyl phthalate	Proposed NT	NT	No
Effluents from pulp mills using bleaching	No conclusion	T	Yes
Hexachlorobenzene	T	T	Yes
Inorganic fluorides	NT	T	Yes
Methyl methacrylate	NT	NT	No
Methyl tertiary butyl ether	NT	NT	No
Mineral fibres	T for refractory ceramic fibres; NT for other vitreous fibres	No conclusion	Yes[d]
Nickel and its compounds	T for oxidic, sulphidic and soluble inorganic nickel compounds; NT for nickel metal	T for dissolved and soluble inorganic nickel compounds	Yes[d]
Organotin compounds (non-pesticidal uses)	Proposed NT	NT	No
Pentachlorobenzene	NT	T	Yes
Polychlorinated dibenzodioxins	T	T	Yes
Polychlorinated dibenzofurans	T	T	Yes
Polycyclic aromatic hydrocarbons	T for benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, and indeno[*1,2,3-cd*]	T	Yes
Styrene	NT	NT	No
Tetrachlorobenzenes	NT	T	Yes

Substance	Conclusion-Health[a]	Conclusion-Environment[a]	Scheduled
1,1,2,2-Tetrachloroethane	Proposed NT	NT	No
Tetrachloroethylene	NT	T	Yes
Toluene	NT	NT	No
Trichlorobenzenes	NT	NT	No
1,1,1-Trichloroethane	Not assessed (See note c)	Not assessed (See note c)	Yes
Trichloroethylene	T	T	Yes
Waste crankcase oils	No conclusion	Proposed T for Use Crankcase oils	No
Xylenes	NT	NT	No

a Conclusions relate to those reached under paragraphs 11a (environment) or 11c (health) of CEPA-1988, or under 64a (environment) or 64c (health) of CEPA-1999. Generally, findings under 11b or 64b were NT unless otherwise specified. NT – not toxic; T – toxic.
b Not used or imported into Canada; therefore proposed deferring further action pending a submission under CEPA New Substances Notification Regulations
c Following addition of methyl chloroform (1,1,1-trichloroethans) in 1990 to the list of ozone-depleting substances under the Montreal Protocol, this substance was added to the List of Toxic Substances under CEPA; further efforts to assess methyl chloroform as a priority substance was therefore considered to be unwarranted.
d The forms found to be toxic are placed on Schedule 1

Annex 2
Standing of Priority List 2 Substances (PSL 2)

Substance	Conclusion-Health[a]	Conclusion-Environment[a]	Scheduled
Acetaldehyde	T	NT (toxic under 64b)[b]	Yes
Acrolein	T	NT	Yes
Acrylonitrile	T	NT	Yes
Aluminum chloride, aluminum nitrate, aluminum sulphate	Assessment period extended pending new data		No
Ammonia in the aquatic environment	No health conclusion included	T	Yes
1,3-Butadiene	T	T	Yes
Butylbenzylphthalate	NT	NT	No
Carbon disulfide	No health conclusion included	NT	No
Chloramines	No health conclusion	T for inorganic chloramines	Yes[c]
Chloroform	NT	NT	No
N,N-Dimethylformamide	NT	NT	No
Ethylene glycol	Assessment period extended pending new data		No
Ethylene oxide	T	NT	Yes
Formaldehyde	T	NT (toxic under 64b)[b]	Yes
Hexachlorobutadiene	NT	T	Yes[g]
2-Methoxy ethanol, 2-ethoxy ethanol, 2-butoxy ethanol	T for 2-Methoxy ethanol and butoxy ethanol; NT for 2-ethoxyethanol	NT	Yes[c]
N-Nitrosodimethylamine	T	NT	Yes
Nonylphenol and its ethoxylates	(See footnote d)	T	Yes
Phenol	NT	NT	N o

Substance	Conclusion-Health[a]	Conclusion-Environment[a]	Scheduled
Releases from primary and secondary copper smelters and copper refineries	T (see footnote e)	T	Yes
Releases from primary and secondary zinc smelters and zinc refineries	T (see footnote e)	T	Yes
Releases of radionuclides from nuclear facilities (impacts on non-human species)	No assessment	T (See footnote h)	No[i]
Respirable particulate matter less than or equal to 10 microns	T (for PM_{10} and, especially, for $PM_{2.5}$)	No assessment	Yes
Road salts	No assessment	T (see footnote f)	Addition proposed
Textile mill effluents	No conclusion	T	No

a Conclusions relate to those reached under paragraphs 11a (environment) or 11c (health) of CEPA-1988, or under 64a (environment) or 64c (health) of CEPA-1999. Generally, findings under 11b or 64b were NT unless otherwise specified. NT – not toxic; T – toxic.
b Deemed to "..constitute or may constitute a danger to the environment on which life depends….' Paragraph 64 b of CEPA-1999
c The species found to be toxic are placed on Schedule 1
d Based on consideration of the MoE between effect levels and reasonable worst-case or bounding estimates of intake by the general population from environmental media, NP and NPEs are not considered a priority for investigation of options to reduce human exposure through control of sources that are addressed under CEPA 1999. However, the relatively low MoE estimated for some products indicates that there is an important need for refinement of this assessment to determine the need for measures to reduce public exposure to these substances in products through other Acts under which they are regulated.
e Emissions of metals (largely in the form of particulates) and of sulphur dioxide from copper smelters and refineries and zinc plants are deemed toxic under paragraph 64a of CEPA-1999, and emissions of PM_{10}, of metals (largely in the form of particulates) and sulphur dioxide from copper smelters and refineries and from zinc plants of PM_{10} are deemed toxic under paragraph 64c of CEPA-1999.
f Road salts that contain inorganic chloride salts with or without ferrocyanide salts are toxic under paragraphs
g Added to the virtual elimination (VE) list.
h Releases of uranium and uranium compounds from uranium mines and mills are deemed to be toxic under paragraph 64a of CEPA-1999.
i Controls to be imposed under other federal legislation.

Annex 3
Comparison of Screening and Priority Substances List Health Assessments

Issue	Screening Assessment	Priority Substances List Assessment
Concept	Initial assessment of whether a substance poses a risk to human health.	A critical and comprehensive analysis of the risks to human health.
Possible Outcomes	There could be no further action on the substance, it could be considered for risk management or it could be considered for more in-depth PSL risk assessment.	There could be no further action on the substance or it could be considered for risk management.
Information Gathering	Comprehensive information search strategies employed, similar to those for PSL assessments. Greater reliance on other peer-reviewed assessments for identification and assessment of previously reviewed data.	Comprehensive information search strategies employed. The search strategies are noted in the PSL assessment reports.
Evaluation of Exposure	Focus on upper-bounding estimates of exposure, after consideration of all identified information.	Detailed analysis (e.g., probabilistic) of exposure, after consideration of all identified relevant information.
Evaluation of Effects	Focus directly on health-related effects, which occur at lowest concentration or dose.	Detailed review of all relevant health-related data and full weight of evidence analysis for hazard characterization. This includes weighting of all relevant data, taking into account factors such as consistency, plausibility of observed effects.
Hazard Characterization	Initial focus directly on the most conservative effect level associated with the critical health-related effect and/or identification of substances with high intrinsic toxicity to human health	Weight of evidence approach with in-depth evaluation of mode of action (i.e., how a substance induces its toxic effects), toxicokinetics (how the substance is absorbed and distributed within the body), metabolism and exposure–response (e.g., benchmark dose) relationships, where data permit.
Approach to Dose–Response Assessment	Margin of exposure approach, i.e., magnitude of the ratio between conservative effect level for effect considered critical and upper-bound estimated (or measured) level of human exposure.	Development of tolerable daily intakes/concentrations, employing default or compound-specific adjustment factors where data permit. Consideration/incorporation of physiologically based pharmacokinetic models or biologically motivated case-specific models, where data permit.
Confidence/ Uncertainties in the Assessment	Deals principally with characterization of the extent of the available database that serves as basis for the delineation of the critical data on exposure and effects. Specified in the screening assessment report and supporting working documentation.	Deals with characterization of the extent of the available database that serves as basis for the delineation of the critical data on exposure and effects, but primarily with the characterization of specific aspects of dose–response. Specified in the PSL assessment report.

Issue	Screening Assessment	Priority Substances List Assessment
Documentation Prepared	Screening health assessment report (published). Supporting working documentation (unpublished). Short amalgamated summary of health and environmental screening assessments published in the *Canada Gazette.*	Amalgamated health and environmental risk evaluations published in a PSL assessment report. Supporting documentation (unpublished) for the health components (exposure and effects) assessment. Synopsis of amalgamated health and environmental assessments published in the *Canada Gazette.*
Delineation of Follow-up Actions	When the recommendation is to add the substance to the PSL for more in-depth assessment, the additional work required is clearly delineated in the screening assessment report. When the recommendation is to consider the substance "toxic" under Paragraph 64(c) of CEPA 1999, the appropriate considerations for follow-up and guidance on the priority for the development of options to reduce exposure are provided to risk managers.	When the recommendation is to consider the substance ‚toxic^ under Paragraph 64(c) of CEPA 1999, the appropriate considerations for follow-up and guidance on the priority for the development of options to reduce exposure are delineated in the PSL assessment report.
Scientific Review — Internal	Internal review meetings by senior technical staff to consider critical issues and the conclusion of the assessment.	Review by senior technical staff.
Scientific Review — External	External review by small number of experts primarily to address adequacy of data coverage and defensibility of the conclusion or to address specific questions on identified critical issues. All reviewers must have declared non–conflict of interest.	External review often by convened panels of experts for adequacy of data coverage, defensibility of selection of the critical data, dose–response analysis and exposure assessment. All reviewers must have declared non–conflict of interest.
Public Comment	Sixty-day public comment period mandated under CEPA 1999.	Sixty-day public comment period mandated under CEPA 1999.

Chapter 3

Engaging expert peers in the development of risk assessments

Meek, M.E., Patterson, J., Strawson, J. and Liteplo, R. (2007)

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Abstract

The participation of external technical experts in the development of risk assessment documents and methodologies has expanded and evolved in recent years. Many government agencies and authoritative organizations have experts peer review important works to evaluate the scientific and technical defensibility and judge the strength of the assumptions and conclusions (OMB, 2004; IPCS, 2005; IARC, 2006; Health Canada, 2006; US EPA, 2006). Expert advice has been solicited in other forms of peer involvement, including peer consultation in, for example, the US EPA's Voluntary Children's Chemical Evaluation Program (VCCEP). This paper discusses how the principles and practices of peer review can be extended to other types of peer involvement activities (i.e. peer input and peer consultation) to develop high quality risk assessment work products. A comprehensive process for incorporating peer input, peer consultation, and peer review into risk assessment science is outlined. Four key principles for peer involvement – independence, inclusion of appropriate experts, transparency, and a robust scientific process – are discussed. Recent examples of peer involvement in the development of Health Canada's Priority Substances and Domestic Substance List (DSL) programs under the Canadian Environmental Protection Act (CEPA) serve to highlight the concepts.

1. Introduction

In recent years those both inside and outside of government have called for expert peer review of the scientific basis for government environmental regulations. The call has been for more involvement by external scientists to judge the scientific and technical merit, as well as the quality of documentation, which underlies the basis for regulations and government policies, including policies on risk assessment. In the United States, the federal government now requires external peer review of influential scientific information (OMB, 2004; US EPA, 2006). In Canada, peer review has been an integral component of health assessments for Existing Substances conducted under the Canadian Environmental Protection Act (CEPA) (see: http://www.hc-sc.gc.ca/exsd). International bodies, such as the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) have also designed their risk assessment programs to include expert review (IPCS, 2005; IARC, 2006). Engagement of peers is an important component of the US Environmental Protection Agency (EPA) Voluntary Children's Chemical Evaluation Program (VCCEP) in which chemical manufacturers prepare risk assessments on chemicals to which children are exposed (see http://www.epa.gov/chemrtk/vccep/index.htm).

This paper discusses how the principles and practices of peer review can be extended to other types of peer involvement activities to develop high quality risk assessment products. Based on the authors' experience conducting expert peer review and consultation for risk assessment documentation in Canada and the US, a comprehensive process for incorporating peer involvement into risk assessment science is proposed. Key principles for peer involvement are identified and discussed. Recent examples of peer involvement in the development of Health Canada's Priority Substances and Domestic Substance List (DSL) programs under the Canadian Environmental Protection Act (CEPA) serve to highlight the concepts. Perceived benefits of peer engagement in the development of specific products are highlighted in the interest of contributing to additional development of more objective criteria for

judging the nature and extent of improvement of the quality of risk assessment products resulting from peer involvement.

2. Background and Definition of Terms

Peer review has traditionally been understood by scientists in the context of deciding if a submitted manuscript is worthy of publication or a grant proposal should be funded. However, in the public arena, governments have engaged outside scientific experts to review formally the technical basis of policies since the 1800s (Guston, 2000). Recently, there have been increasing calls for peer review of documents and analyses that support regulation. The calls for peer review are based on a belief that external peer review will promote good science, which will lead to good policy, and that better science increases legitimacy of decisions (Guston, 2002). Expert panels have been commonly convened for review of complex health assessments of Existing Substances under CEPA, and in the US, the EPA has several standing scientific advisory committees (e.g., Science Advisory Board) to provide expert review.

Soliciting advice from scientific peers need not be limited to formal peer review. Scientists have long sought colleagues' opinions on ideas, hypotheses, and works-in-progress. Gathering advice and review from peers can be broadly termed "peer involvement" and can be useful to the development of sound risk assessment work products and decisions. Peer involvement can be formal or informal and engage those both within and outside the authoring organization. The US EPA defines peer involvement broadly as the "active outreach to and participation by the broad scientific, engineering, public health, economics and other social science communities beyond the Agency (external), as well as with in the Agency (internal)" (US EPA, 2006, page 12).

In this paper, the term "peer" is defined as experts who collectively are of equal standing, that is, those who have at least the same level of training and experience as the authors of the report. Therefore, they are qualified to evaluate and judge the adequacy of the authors' science and conclusions. Non-technical interested persons are not included in the definition of "peer". While it may also be desirable and appropriate to involve non-technical participants to gain insights into public opinion and priorities, this type of "public" involvement is not the subject of this paper.

Three types of peer involvement are addressed in this paper: peer input, peer consultation, and peer review. In this context, these key terms are defined as follows:

- **Peer input** – soliciting information, data, or opinion from scientific peers, generally at an early stage of a work product's development. For peer input, the emphasis is on appropriate focus, data acquisition and identification of issues. The process may be formal or informal. The experts may be internal or external and may or may not be independent of the authors or of the subject. For example, while not "peers" *per se*, scientists from stakeholder groups may provide input at this early stage in specific areas.
- **Peer consultation** – a formal or informal process to gather independent expert peer opinion and advice on a work product during its development. Peer consultation is most helpful when the document is complete enough to benefit from a review, but the analysis may still be in flux, allowing the experts' comments to be readily considered and influence future direction. Peer consultations may be conducted on an entire work product or on specific issues or analyses. The emphasis is on scientific expert opinion and advice, rather than data acquisition.

- **Peer review** – a formal, external, and independent review of an intended final work product. The intent of a peer review is to gain agreement from a group of external expert peers regarding a document's conclusions and the scientific basis for those conclusions. The emphasis is on agreement by the experts or agreement on the approach and conclusions, with consensus amongst the experts providing additional support and defensibility of the results.

The next section demonstrates how these three types of peer involvement can be incorporated into a comprehensive process that maximizes benefit from peer involvement in risk assessment science.

3. Comprehensive Process for Peer Involvement in Risk Assessment Science

While formal peer review of draft final work products by external scientists is now common practice for risk assessment projects in many parts of the world (OMB, 2004; IPCS, 2005; IARC, 2006; Patton and Olin, 2006; US EPA, 2006), additional peer involvement at earlier stages of product development may improve quality and efficiency. Most risk assessment documents involve three primary stages of development: Problem Formulation (including issue identification and data gathering); Draft Work Product; and Final Work Product. Different types of peer involvement can enhance quality and efficiency at each of these stages and improve the technical robustness of risk methodologies, assessments, and results. A comprehensive process for incorporating this additional peer involvement into all stages of risk assessment development includes three steps: identifying missing data and scientific issues early in product development (Problem Formulation or Issue Identification Stage); seeking advice from peers (consultation) on interim drafts (Draft Work Product Stage); and, formal peer review of the final draft product (Final Draft Work Product Stage). Table I identifies the key types of peer involvement activity at each stage of development, with key peer involvement features for each stage.

The scope of the work product and other factors dictate the desirability and usefulness of peer involvement at each stage. Complex work products or those that have significant impact (e.g., methodological approaches that impact entire programs or large numbers of assessments) might benefit from incorporating peer involvement into all stages, while less complex products may be sufficiently addressed with a simple written peer review of the final draft. To determine what level and type of peer involvement would be beneficial at the earlier stages of product development, a number of factors should be considered including scientific complexity and uncertainty, the level of risk involved and magnitude of potential risk, and standard practice within the organization. The greater the significance of these factors, the more peer involvement may serve to enhance the quality and credibility of the final work product.

The Canadian government's efforts to assess human health risks for large numbers of Existing Chemicals under the Canadian Environmental Protection Act (CEPA) serve to illustrate the nature of a comprehensive model including different stages of peer involvement in risk assessment. Canada is the first country to introduce a legislative requirement for systematic priority setting of all chemicals in commerce (Existing Substances). CEPA required that the Ministers of Health and Environment complete categorization (priority setting) of all of the approximately 23,000 substances on the Domestic Substances List (DSL) by September 2006, with subsequent screening and full risk assessment, where warranted.

Table I. Summary of the three stages of risk assessment development, the types of peer involvement techniques that can be used and the issues and questions appropriate for that stage.

Development Stage	Type of Peer Involvement	Questions and Issues to be Addressed
Problem Formulation, Issue Identification, and Data Gathering	**Peer Input** Data requests Workshops Meetings , informal or formal Informal discussions Expert Elicitation to fill data gaps or address uncertainties	Is there an accepted standard approach available? Are there previous relevant examples to follow? Are there data or analytical tools to suggest? Do outside parties have additional data/information? Are there outstanding science or science policy issues that must be resolved or addressed? Should additional studies be conducted or data collected? What is the available budget and timeline?
Draft Work Product	**Peer Consultation** Requests for written comments or review Panel meetings or conference calls On single issues or entire work product	Were all the appropriate data identified? Were the data interpreted correctly and presented in sufficient detail? Are there alternative approaches that should be considered? How can the work be strengthened and improved?
Final Draft Work Product	**Peer Review** Written or letter review Panel meetings or conference calls On near final work product	Focused and formal charge questions covering: The completeness and strength of the data presented The defensibility of the assumptions The use of appropriate analyses and methods The strength and defensibility of the conclusions The strength and scientific defensibility of the rationales provided for choice of: study, effect, level, models, uncertainty factors, etc. More specific questions regarding key chemical or document specific issues

CEPA has required, therefore, the development of novel predictive methodology to set priorities based on potential exposure and hazard from amongst thousands of existing chemicals. As a result, the program provides opportunity for incorporation of increasingly complex and robust models of peer involvement not only for assessments of individual substances or groups thereof (i.e., screening to Priority Substances) but also for development of complex predictive novel methodology (for categorization).

Specifically, then, the program increasingly includes more formal peer input at earlier stages of development for both methodology and assessments. In addition, the complexity of peer input, consultation, and peer review is greater for more robust assessments for substances of highest priority (see Figure I). This approach maximizes efficiency while maintaining the defensibility of output of the three different levels of priority setting and assessments of increasing complexity within the program (categorization, screening assessment, and full assessment).

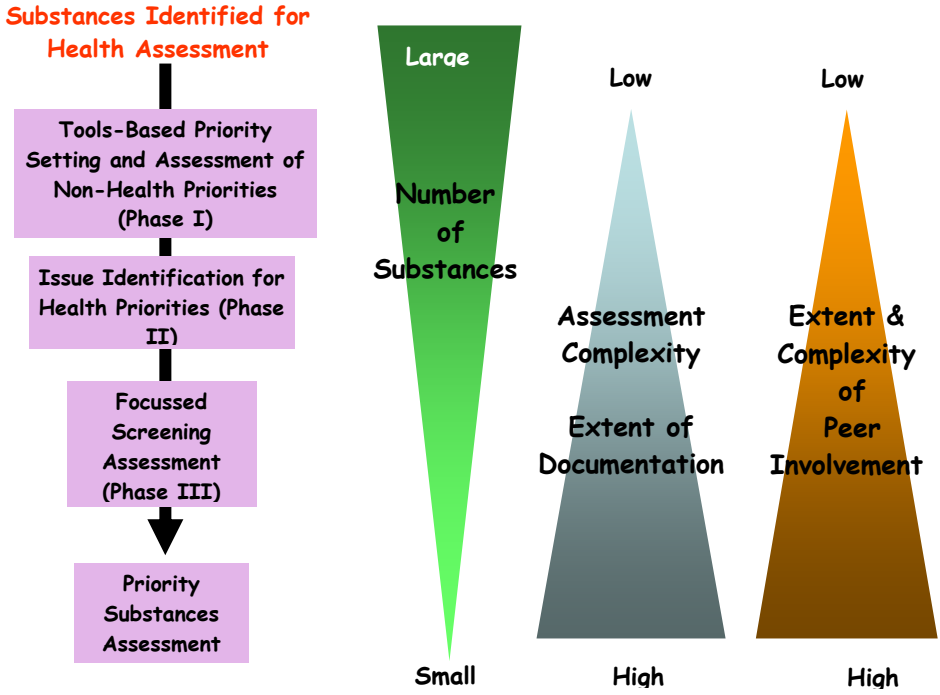

Figure I. Health assessment complexity for peer involvement. As chemicals are screened for more detailed assessments, the complexity of the assessments increase along with the extent and complexity of the peer involvement activities.

Full assessments for Priority Substances generally include early peer input to identify relevant data, followed by external panel peer reviews at the end of the process. On the other hand, for screening assessments, an early issue identification stage is envisaged to solicit peer input on identification of relevant data and issues and confirmation of the focus of the assessment. Since screening assessments are less complex, at a later stage in their development, peer review for these assessments is generally restricted to written comments by several external experts (i.e., a letter review). Panel meetings will be convened only where there are subsequent outstanding issues.

Development of methodology for priority setting and assessment of risk often entails all three stages of peer involvement (i.e., input, consultation, and review). Examples from the Existing Chemicals health assessment program under CEPA are included in each section below to illustrate use and potential benefits of peer involvement.

3.1 Peer Input in Problem Formulation Stage (Including Data Gathering and Issue Identification)

In the initial planning or problem formulation stage of development of a risk assessment, the project lead, authors, and decision makers must clearly define the problem or issue to be addressed by the work product. From these efforts, a work plan is developed. This may be fairly straightforward if there are previous similar efforts or if the usual approach is commonly accepted (e.g., a simple site assessment within a program with explicit guidance, or a chemical assessment document). However, in other situations involving novel ideas, development of methodologies, analytical approaches, or complex assessments, the problem formulation stage is a substantial and important effort. In these situations, incorporating peer involvement to help develop the approach can improve efficiency of production and content of the overall work product. In addition, peer input can identify the need for additional data, bring to light unpublished data and studies, and identify key scientific issues that will need to be addressed.

To increase the efficiency of assessment of larger numbers of substances, it is envisaged that screening health assessments for Existing Substances under CEPA will incorporate an early, formal stage of Issue Identification. The objective is to ensure maximum utilization of previously well-documented, peer-reviewed assessments, and adequately and accurately focus on more recent information and critical issues. While the process for input is still in development, it is envisaged to incorporate robust senior internal technical review and external peer input.

Peer input is the most common type of peer involvement at the problem formulation and issue identification stage. Peer input focuses on gathering data, information and input on issues and proposed focus. It is most useful in early stages of the development of a risk assessment to define the project scope, identify and explore issues, identify needed data, and potential alternative approaches. One of the most common types of peer input is a request for written comments in order to identify relevant data that may have been missed. Other techniques may also include formal and informal meetings, workshops, data calls, teleconferences, and web-based information solicitation and collection. The primary purpose of peer input is to gain information from experts about data, issues, or methodologies that will assist the authors in developing the approach for conducting the work. Peer input may be formal and structured or more informal. It may involve one-way solicitation of input, or a dialog between parties. While not "peers," non-technical participants (i.e., stakeholders) from political, business, public interest and the general public may also be queried to gain insights into their opinions and priorities.

There can be significant benefit from obtaining input from experts in the problem formulation and issue identification stage. Early identification of issues allows the organization to best target resources and increase efficiency. Peers from both within and outside of the authoring organization can help to identify issues or provide missing and or/essential data. Authors can seek opinions and suggestions from peers in areas that may affect the scoping of the project, including possible options and feasibility, availability of data, and outstanding scientific or science policy issues. Peer input at this early stage leads to a better understanding of the scope, identifies issues to be resolved where resources should be focused, and facilitates collection of data. As a result, the draft document is strengthened by considering complete data sets and targeting resources to focus on, address, and resolve key issues early in the process.

The Existing Substances Division of Health Canada recently invited peer input to assist in guiding the development of the Complex Exposure Tool (ComET), one of several of the simple and complex tools designed to identify, in increasingly discriminating and iterative fashion, priorities for additional consideration from amongst all 23,000 substances on the DSL. This development was necessitated to meet the "categorization" requirement by the mandated deadline under CEPA of September 2006 (Health Canada, 2005).

A one-day workshop was held in the fall of 2004 as part of a larger effort to solicit input and data from scientific peers and others on the proposed construct and information base for ComET. For this workshop, Health Canada was also interested in communicating with and soliciting specific input from stakeholder groups (in addition to input from scientific peers). Therefore, the benefit of the input received from stakeholders during the workshop is mentioned here, while recognizing that non-scientific stakeholders would not be considered "peers". ComET assimilates information as a basis for quantitative plausible maximum estimates of exposure of individuals in the general population by age group based on use scenario (sentinel products and emissions), physical/chemical properties and bioavailability. The tool encompasses estimation of both environmental (far-field) and consumer (near-field) exposure, the latter being based on "sentinel products" — that is, those products yielding the highest estimates of exposure for individuals in different age groups. The tool draws maximally on generic (i.e., non-substance-specific), publicly available information and transparently delineates assumptions and uncertainties. It is designed to be health protective, with conservative choices being made in the absence of data.

ComET is relevant to priority setting for both categorization and screening and constitutes the basis for identification of substances for which exposures of the general population are expected to be minimal and that as a result, deserve little additional risk-based regulatory attention in assessment. There were three goals for the peer input meeting:

- To increase understanding amongst peers and stakeholders of how the "tool" will contribute not only to the CEPA program but also potentially to other government and industry efforts.
- To solicit input, information, and comment on both the architecture of the data base and the supporting available data.
- To encourage collaboration with others to avoid duplication of effort and maximize the impact of resources invested to contribute to development of iterative approaches to efficient priority setting and assessment.

Significant effort was made to engage stakeholders, including the CEPA Industry Coordinating Group and Canadian Environmental Network in the peer involvement efforts. The meeting was announced widely to exposure experts, scientific societies, consumer and public interest organizations, government agencies, academics, companies and industry groups. Specific invitations were targeted to reach exposure assessment experts who could comment on the architecture, as well as industry stakeholders and others who may have useful data to populate ComET. The meeting was open to the public and participants attended in person, by webcast, or by conference call. Health Canada and their contractors described the goals and architecture of ComET and explained what data were currently available. This was followed by a number of breakout sessions on specific areas to solicit input on the

proposed construct of the tool and to identify additional sources of data. Over 100 people participated, either in-person or via the conference telephone call or Internet webcast.

By offering interested persons multiple ways to attend the session, participation was maximized in order to gain essential information from a broader group. Use of the live webcast and conference lines allowed additional participation by those who could not attend in person, including Europeans who were considering similar types of information needs for the REACH (Registration, Evaluation and Authorisation of Chemicals) initiative. Those off-site submitted questions via email, and their questions were answered during the meeting itself in real time. In addition, a recorded version of the webcast was made available after the meeting.

As a result of the peer input workshop, Health Canada obtained data that had not previously been available to them. The data were provided, principally as a result of increased understanding by potentially impacted stakeholders and/or partners of the value in this context of making publicly available, relevant but previously unreleased information, or additionally mining available data to increase its utility for ComET in the interest of leveraging resources to collaborate. In addition, by including key exposure experts, the peer input efforts ensured that the ComET architecture incorporated more inclusively, considerations based on the most recent developments in methodology for exposure assessment.

3.2 Peer Consultation in Draft Work Product Stage

At different times during development, a draft work product may benefit from the advice of peers to determine completeness of data coverage, to identify gaps in data or understanding, to gain comment on selection and application of approaches and analyses, or to evaluate the scientific strength of conclusions. Peer consultation is of most value at this draft stage of development because it allows for interaction between the authors, sponsors, and the expert peers and solicitation of advice from the individual experts, which can be considered in the analyses and documentation.

Peer consultation is an evolving concept in the field of risk assessment that contributes in the development of draft work products. It involves the formal solicitation of review and advice from experts, usually at an early stage of development when approaches and analyses may still be evolving prior to establishing a position or deciding on a preferred approach. Peer consultations do not usually seek to provide consensus approval (as would be the case for peer review). Rather, the output of a peer consultation is opinions, advice, and recommendations of the individual experts or group that may be used to improve the document or guide further efforts. A peer consultation may be held to review an entire document, or be focused on data and approaches to a specific issue or analysis.

Commonly, peer consultations are conducted either through a written (letter) review process involving one or more individuals, or by a group in a face-to-face meeting or teleconference. The format of the peer consultation will depend upon the complexity of the issues and the goals of the authors and sponsors. When soliciting written comment from experts, the request may be targeted to specific individuals and groups, or a broader net might be cast to invite comment from any interested expert. Convening an expert panel to meet in person with the authors allows for maximum interaction amongst participants, which can ensure better communication and understanding of the issues and recommendations. For a complex project, several

rounds of written reviews or meetings may be held, in an iterative fashion for a single issue, or on different issues. The choice of peer consultation technique and timing depends on the complexity and controversy of the scientific issues and the need for expert advice.

Conducting a peer consultation for a draft risk assessment product can potentially, provide many benefits. In a peer consultation, experts can evaluate the strength, appropriateness, and defensibility of the work product's analyses and conclusions, and provide recommendations for improvements. A peer consultation meeting provides the opportunity for interaction among the experts, authors, and sponsors. The interaction of a meeting allows for complex concepts to be more fully expressed by all participants and a common understanding or new ideas may emerge from the discussions. The advice and recommendations of expert peers can assist the authors in identifying scientific weaknesses and addressing them, as well as selecting the best data or decisions or modifying focus of next steps in methodology development. Incorporating peer consultation prior to a peer review can help avoid the "rough draft syndrome" at a late stage wherein the authors may submit a less than complete work, expecting the peer reviewers to provide guidance on how to proceed, which is not the intended objective of peer review. Peer consultation also increases awareness of the program content and expands familiarity with sources of expertise for increased opportunities for future collaboration.

Peer consultation has been incorporated into various aspects of the Health Canada Existing Substances program to garner expert advice about a variety of chemical specific risk issues. For example, in relation to aluminum salts which were included on the second Priority Substances List under CEPA, it was determined that the available data were inadequate as a basis for completion of the mandated risk assessment (Health Canada, 2000) and peer consultation has contributed considerably to the design of relevant studies.

This peer consultation involved a committee of scientists from universities, government, laboratories, industry, and consultants who assisted Health Canada by providing expert advice on the design of research to address data gaps for aluminum. This maximized access to a broader range of specialized expertise than would have been available within governments, or to a single group of stakeholders, and facilitated collaborative interface with potential partners internationally in the conduct of the research. Preparation and posting of the report of the meeting maximized transparency as a basis for additional input from interested parties.

In another example, iterative peer consultations were helpful in the development of complex methodology related to the systematic identification of priorities for assessment (i.e., categorization) from amongst the thousands of chemicals on the Domestic Substances List (Health Canada, 2005). The complex hazard tool (ComHaz) is another of several of the simple and complex tools designed to iteratively identify in increasingly discriminating fashion, priorities for additional consideration from amongst all 23,000 substances on the DSL. It involves hierarchical consideration of various sources of information (including reviews, empirical data, [quantitative] structure–activity analysis, and comparison with analogues) for a range of endpoints of toxicity, which are also considered in stepwise fashion.

Genotoxicity is a critically important component of the tool, due to the availability of data for comparatively large numbers of substances, for this endpoint. The objective of an initial peer consultation held in March 2002, was to review a scoring system for this component developed by genetic toxicologists within Health Canada

(ILSI, 2002). This system differentiated between mutagenicity, clastogenicity and indicator assays in *in vivo* tests in mammals and *in vitro* tests, and incorporated, as well, consideration of predictions generated by (quantitative) structure activity relationship [(Q) SAR] models. Specifically, the panel at the peer consultation responded to a request to assess the degree of confidence/uncertainty in a number of variants of the scoring system in the context of priority setting for existing chemicals to be taken into consideration in further development of the tool.

The construct of the ComHaz tool is hierarchical, not only in consideration of sources of data but also complexity of consideration. The first stage is based on a conservative "first hit" approach for data and endpoints based on specified criteria; the next stage involves consideration of weight of evidence for qualitative endpoints of capture (e.g., cancer, genotoxicity). This latter aspect was the subject of an additional consultation (*TERA*, 2005a), which built on the output of the prior genotoxicity consultation. In the second peer consultation, a group of experts in both genotoxicity and quantitative structure activity was convened to evaluate Health Canada's draft *Complex Hazard Tool (ComHaz) Preliminary Weight of Evidence Framework for Genotoxic Carcinogenicity*. The framework was a work in progress and the purpose of the peer consultation was to access advice from leading experts on how it might be further developed as one element of an approach to efficiently set priorities based on hazard for large numbers of chemicals. Through written review and conference calls, experts considered the relative weighting of empirical data versus (Q)SAR predictions, the relative weighting of predictive power of the underlying assays for (Q)SAR output within a line of evidence, the appropriate integration of a (Q)SAR battery, simple indicators of model robustness, and application of analogue/surrogate approaches. The panel made several recommendations concerning weighting of analogue/surrogate approaches as a separate line of evidence, and resulted in the framework giving equivalent weighting to SAR and QSAR.

In the first consultation, experts were principally endpoint specialists; in the second, modelling expertise was critical to ensure familiarity with the available (Q)SAR software and approaches. Interactions amongst the experts and Health Canada developers during these consultations allowed for greater understanding of the tool and framework's purpose and how the models and result could be used. Experts were screened for independence, to insure they had not done prior work for Health Canada on developing the QSAR weight of evidence framework; and also for conflict of interest, in particular to exclude those who may be directly paid by or actively affiliated with (Q)SAR modeling software companies (both those whose models served as the basis for Health Canada's proposed framework and competing commercial models).

Another significant benefit of these peer consultations is increasing the familiarity of the broader scientific community with current challenges in risk assessment in a regulatory context to stimulate relevant additional work. Consideration of complex and novel questions in peer consultation to address progressive regulatory mandates often presents considerable challenges in communication of the requirement for input. This is often related to the variation in context of the objectives from traditional applications with which the experts are most familiar. That investment in this area is warranted, however, is underscored by the considerable potential benefit in improvement of the final product, based on robust consideration of a wide range of expertise and creative input.

3.3 Peer Review of the Final Draft Work Product

In many organizations, near final risk assessment documents are routinely peer reviewed by one or more independent experts, that is, those outside of the authoring organization who were not involved in the development of the document and have no conflicts of interest with the work product or sponsors. The Presidential/Congressional Commission on Risk Assessment and Risk Management in its 1997 report notes the critical importance of peer review in regulatory decision-making to "enhance the credibility of agency decision and position and to improve their technical quality" (Presidential Commission, 1997, p. 103). For risk assessment products, peer review involves an in-depth assessment of the inclusiveness (i.e., comprehensive coverage), assumptions, calculations, alternate interpretations, methodology, and conclusions. Peer review panels often seek to reach consensus or common agreement regarding the adequacy of the product reviewed.

The authors should be fairly certain that their work product is nearly final, is well documented and transparent, that the data are complete, that the analyses and techniques used are scientifically defensible, and that the conclusions are well supported. The peer review can take place through the individual letter reviews by one or more experts, or may involve one or more meetings of experts to discuss the document and reach group conclusions.

Expert peer review assists in explicitly delineating the uncertainties inherent in any scientific process, and perhaps the risk assessment process in particular. For example, experimental methods and analytical techniques adopted in environmental and human health risk assessment often are controversial and may not have wide scientific consensus. Improvements in methodology are continually introduced. Because risk assessments rely upon significant scientific judgment and assumptions, these assumptions must be critically evaluated. Testing the assumptions and communicating and understanding the uncertainties is important to be able to provide decision makers with a fuller understanding of the strength of the assessment and conclusions. In risk assessments, strength-of-evidence or weight-of-evidence approaches are commonly adopted in which the body of knowledge is assessed to reach conclusions regarding a chemical or agent's potential to cause human health risk.

The Health Canada Priority Substances program involves both peer input and two stages of peer review for more complex health assessments. Early in the process, draft supporting text is sent to technical experts within stakeholder groups for input on the adequacy of coverage – i.e., whether any key data or studies are missing or issues unidentified. Later, in the first stage of peer review, the supporting documentation, draft hazard characterization, and dose-response analysis are sent to selected experts for written review. Finally, for the more complex and controversial assessments, a panel of experts is convened to review the draft final supporting documentation, hazard characterization, and dose-response analysis. For more recently introduced screening assessments, a stage of peer input ("Issue Identification") and written peer review is envisaged. Consideration is being given, currently, to development of a continuing series of meetings for peer review of multiple screening assessments.

This routine incorporation of extensive peer input and review in the assessment of Existing and Priority Substances contributes considerably to the defensibility and integrity of output. It has also contributed considerably in the communication of program objectives and resulting engagement and access to a wide range of expertise.

Beneficial impact for individual substances has included, for example, maximal utilization of available data in drawing conclusions (e.g., quantitation based on epidemiological data for acrylonitrile and ethylene oxide to bound estimates of dose-response from animal studies). On occasion, it has verified the need for additional analyses of the output of key studies forming the basis of conclusions in previous assessments based on consideration of their adequacy by Health Canada authors. This has led to the conduct of more appropriate studies to inform risk assessment.

For example, in 2000, the supporting documentation, draft hazard characterization and dose-response assessment on ethylene glycol were reviewed by a panel of experts convened by Toxicology Excellence for Risk Assessment (*TERA*) (*TERA*, 2000). At the conclusion of the peer review meeting, Health Canada authors had continuing unanswered questions regarding the pathological findings of a key study. Additional input was solicited from one of the authors of this study and Health Canada's revised analysis was submitted to the same peer review panel for additional review. A conference call was held with the panel and at this meeting the study author participated to answer questions regarding the study and kidney pathology. An additional expert in pathology was brought in by *TERA* to serve as an advisor to the panel and to provide independent opinion on the histopathological analysis for this and another critical study. The content of the draft documentation prepared by Health Canada and subsequent input from peer review resulted in suspension of the assessment and conduct of an additional chronic study by industrial stakeholders (Environment Canada and Health Canada, 2000). More recently, the Existing Substances program has collaborated with a consortium of industrial stakeholders to sponsor the ethylene glycol category for consideration in the Organization for Economic Co-operation and Development (OECD) High Production Volume Chemicals program. This peer review illustrates the benefits of the need for flexible process to engage the appropriate experts sufficiently (in multiple meetings if necessary) to resolve technical issues.

4. Application of Peer Review Principles to Peer Involvement

A successful peer involvement program requires active support of management. Management responsibilities in scientific peer review were discussed in a recent report by the International Life Sciences Institute (ILSI, 2005; Patton and Olin, 2006). The authors describe nine areas of responsibilities to agency managers on shaping peer review programs, including: creating a peer review culture; determining the need for and form of the peer review; defining and distinguishing the review processes; planning and allocating resources; assessing the readiness of the work product for review; developing the "charge," selecting reviewers and matching expertise; and, use of peer review comments in completing the report. When management addresses well these responsibilities, the organization builds a solid foundation for a peer involvement program.

Working from this foundation of management responsibilities, four broad principles are identified that are important for consideration and organization of peer involvement activities for each stage of product development. Adherence to these principles contributes to the quality of work products and integrity of peer involvement in risk assessment. These principles are independence, inclusion of appropriate expertise, transparency, and robust scientific process.

4.1 Principle 1: Independence

Independence is defined as both distance from the development of the work product and freedom from institutional or ideological bias and conflicts of interest. At early stages of project scoping, data and issue identification, independence is not as critical. Input from those who work closely in the same organization as the authors or from individuals with strong biases or even conflicts of interest may be permitted in order to acquire important knowledge and insight. In these situations, however, it is wise to ask participants to disclose any potential basis for perceived lack of independence to ensure that all are informed and aware. At later stages, independence becomes more important.

Independence of the peers and the review process is critical in the last stage of peer review to ensure the scientific adequacy and strength of a risk assessment. At this point, a lack of impartiality on the part of the peers may result in the peers not challenging weak data, analyses, or conclusions, and may damage the credibility of the review. Peer reviewers must also be independent in that they should not work in the same agency or organization as the authors, nor work closely with the authors. They should also not have contributed significantly in the development of the work product. However, in some situations with complex assessments, it may be beneficial to involve the same peers in a series of peer consultations or reviews, covering distinct issues or portions of the assessment. The conclusions and recommendations from earlier consultations and reviews may contribute to the work product at later stages.

At the later stages, the reviewers must also be free of conflict of interest with the authors, sponsors and affected parties to ensure independence and establish credibility. Conflict of interest is defined by the US National Academy of Sciences (NAS) as:

> "any financial or other interest which conflicts with the service of the individual because it (1) could significantly impair the individual's objectivity or (2) could create an unfair competitive advantage for any person or organization.…The term 'conflict of interest' means something more than individual bias. There must be an interest, ordinarily financial, that could be directly affected by the work of the committee." (NAS, 2003)

Potential conflict of interest needs to be evaluated for each reviewer prior to selection; and a clear and unambiguous conflict of interest policy applied.

Those organizing a peer review should also be independent of the work product so as to be objective when selecting the reviewers, developing the charge questions, conducting the review, and reporting the results.

4.2 Principle 2: Inclusion of Appropriate Expertise

The success of a peer involvement hinges on the participation of highly qualified "peers" -- those who are qualified through training and experience to offer scientific opinions on the questions and issues at hand. Those responsible for organizing peer reviews agree that appropriate expertise is the primary factor in selecting reviewers (NAS, 2003; OMB, 2004; ILSI, 2005; SOT, 2003; and *TERA*, 2005b). For risk assessment products, it is essential to identify and involve experts from fields such as toxicology (including sub disciplines such as pathology), epidemiology, biochemistry, statistics, and modeling. The peer review of

ethylene glycol discussed above highlighted the importance of having essential expertise available to resolve key issues and questions.

There are a number of considerations in selecting experts for peer involvement. It is helpful to have the peers come from diverse backgrounds and affiliations (e.g., government, academia, industry, environmental or public interest groups, consulting) to provide a range of scientific perspectives. Peers should not "represent" a particular interest or group (e.g., industry, government, or environmental) rather, as the NAS points out a wide range of perspectives is "often vital to achieving an informed, comprehensive, and authoritative understanding and analysis of the specific problems and potential solutions to be considered by the committee." (NAS, 2003, page 2). In addition to perspective, if there are clear opposing views on key issues, those different views should be included. Organizers should ask questions to evaluate how strongly panel candidates hold to these opposing views, since a panel could be ineffective if members hold views so strongly that they are not able to consider new data and work toward consensus. Organizers should document their panel selection and note situations where clearly opposing views on key issues have been addressed in the panel selection.

For panel meetings on more significant and complex risk assessments, it is important that a sufficient number of peers are included to ensure that all important aspects are addressed with duplication in critical areas so that the multiple qualified experts can meaningfully exchange ideas and opinions. In addition, a balance of three types of expertise is essential: scientists knowledgeable about the subject chemical, those with experience in the disciplines relevant to assessment of animal and human health effects, and experts in risk assessment methodologies and practices.

In addition to selecting the right types of expertise, an experienced chairperson will make sure that all views are heard, keep the discussion on subject, and be able to summarize and synthesize the diverse statements and discussions in a meaningful way.

Selecting the right peers also involves ensuring that they are independent of the authors and sponsors and can provide objective opinions (see Principle 1 above). It also entails that they are prepared to dedicate sufficient time and effort to familiarize themselves with relevant background materials. For this reason, it is often helpful to solicit submission of written comments prior to discussion at any review meetings. As discussed above, independence of peers is essential for peer review, but may not be as important for other peer involvement activities.

4.3 Principle 3: Transparency

Peer involvement activities should be transparent, so that those both within and external to the process can evaluate how the activity was organized and conducted, and judge for themselves the adequacy and credibility of the process and the results. In the context of peer involvement, the word "transparency" encompasses more than just the need to reveal the names of the reviewers. Rather, transparency refers to a philosophy that encourages open communication about the basis for and nature of the important decisions made during the process of conducting a review, to enable judgment of its credibility. Particularly critical in this context is the basis for selection of the reviewers and sufficiently detailed record of the panel members' deliberations and basis for conclusions and recommendations. Transparency is most significant and important for peer review of a near final product, where expert judgments are being made on the adequacy of the product; but transparency is also a good practice for other peer

involvement activities. Efforts to be transparent can range from the simple -- making public the list of peers consulted on an issue and reports on the use of their input – to more involved, open meetings with the public in attendance. When panel meetings are held, interested parties and the public may be invited to observe the proceedings and examine the same materials as the reviewers. Those responsible for peer involvement activities should be careful to insure that all relevant peers have access and the opportunity to input, to avoid an actual or perception of favoritism to one party over another.

Transparency is enhanced with good documentation of the process and results. If real-time observation of a meeting is not planned, then at a minimum the process and results should be fully documented so that the interested parties have a clear picture of how the peer review was organized and the results obtained. For example, a report of a peer review would include identification of the reviewers and their credentials, how the reviewers were selected; the questions the reviewers were asked to address, the materials that they reviewed; and, the reviewers' major comments and conclusions

4.4 Principle 4: Robust Scientific Process

To insure high quality peer involvement, it is important for the focus to be on the science – the robustness of the available data, the analyses, and the defensibility of the conclusions. The focus of the peer involvement should be on the scientific evidence and conclusions, and not policy or implementation issues (these need be addressed in equally transparent fashion but in separate efforts).

Robustness is dependent on a number of key factors. Appropriate experts must be involved, the experts must be asked the proper questions to address critical areas, the materials should be complete enough to facilitate a high quality process, and the results of the peer involvement should be well documented. A robust scientific process that addresses the first three principles will contribute to robust scientific results and work products.

An essential element of a robust peer involvement process is involvement of scientists who have the requisite expertise to provide input or evaluate the work product. For peer input, the scientists may be self-selected and volunteer their information and opinions. However, for peer consultations and reviews, the person or organization selecting the peers need to have a sufficient understanding of the key scientific issues and methods in order to identify the necessary expertise to address those issues and to insure that the right types of expertise are brought to the process.

Robustness also implies that the scientific completeness of the questions being asked the peers. Where there is peer involvement in early stages, critical scientific issues will be identified by all participants, contributing to robustness. The broader the expertise of the peers involved, the greater the input and perspectives. However, for a peer review, it is preferable to have a third party, independent of the work product, develop the charge to peer reviewers. The charge asks reviewers focused questions regarding direction and scope of the document to guide their review. It should focus on the critical issues and questions to be efficient, but allow the reviewers to raise issues that might be important, but not initially identified or anticipated. The authors and sponsors may contribute the questions and issues they wish to see addressed, but an independent party should make sure the charge is complete and the questions objectively presented. Experts involved in peer reviews and consultations should be

given the opportunity to raise issues for discussion independently to ensure that no key issues or critical questions are missed.

Robustness also involves insuring that the materials are complete and transparent so that the peers can provide meaningful input or opinion. Those organizing peer involvement need to ensure that the materials are sufficiently robust so as to maximize the value of the review by peers.

Finally, those preparing reports on the peer involvement activities (e.g., peer review report) should sufficiently comprehend the review materials, so that the report accurately characterizes the discussions. This requires the engagement of experienced risk assessment scientists who understand the issues under discussion. For peer reviews, the report should document how the panel reached its conclusions and contain unambiguous recommendations that are presented in a way that is useful to the authors.

5. Conclusions

Increased efficiency in completion of defensible assessments, including incorporation of tailored approaches to peer input, consultation, and review is essential to meeting expanding mandates worldwide to consider health impact of exposure to much larger numbers of existing chemicals, which were introduced into commerce with little or no pre-market assessment. In this paper, developments in the nature of a process for various stages of peer involvement have been described and illustrated through consideration of specific examples highlighting perceived benefits in scientific robustness and quality of the end products.

It is hoped that these examples are helpful in increasing understanding of the potential contribution of expert peers in the identification of additional data, alternate analytical approaches, and weaknesses in logic and reasoning. Experts' participation can strengthen the risk assessment and enhance the credibility and public confidence of the results. Peer involvement contributes to communication within the scientific community and beyond. Further to the evolution of distinct stages of peer involvement described here and their perceived benefits, additional development of measurable criteria that would permit a more objective analysis of the impact of peer involvement on the quality of risk assessment products would be a logical next step in additionally strengthening the basis for peer review and other peer involvement's expanded role within the US government (OMB, 2004; US EPA, 2006), as well as international governments and health agencies (see for example http://www.hc-sc.gc.ca/exsd; IPCS, 2005; IARC, 2006).

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Chapter 4

Recent Developments in Frameworks to Consider Human Relevance of Hypothesized Modes of Action for Tumours in Animals

Meek, M.E. (2008)

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Abstract

This paper summarizes recent developments in the continuing evolution of Human Relevance Frameworks to systematically consider the weight of evidence of hypothesized modes of action in animals and their potential human relevance for both cancer and non-cancer effects. These frameworks have been developed in initiatives of the International Life Sciences Institute Risk Sciences Institute and the International Programme on Chemical Safety engaging large numbers of scientists internationally. They are analytical tools designed to organize information in hazard characterization as a basis to clarify the extent of the weight of evidence for mode of action in animals and human relevance and subsequent implications for dose-response analysis in risk characterization. They are also extremely helpful in identifying critical data gaps. These frameworks which are illustrated by an increasing number of case studies, have been widely adopted into international and national guidance and assessments and continue to evolve, as experience increases in their application.

Background

A postulated mode of action (MOA) is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological, genetic and biochemical events – i.e., those that are both measurable and necessary to the observed effect. MOA contrasts with mechanism of action, which generally involves a much greater understanding of the molecular basis for an effect.

In 2001, as part of its efforts to harmonize risk assessment practices, the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP) published a framework for assessment of MOA for carcinogenesis in laboratory animals (animal MOA). This was based on consideration of specific aspects of data analysis developed much earlier by Bradford Hill as a basis for considering causality of observed associations in epidemiological studies. Relevant factors include dose response and temporal concordance, consistency, biological plausibility and coherence (Sonich-Mullin et al., 2001). More recently, the IPCS framework has been expanded to address human relevance (HRF) (Boobis et al, 2006), based on previous work of the International Life Sciences Risk Sciences Institute (ILSI RSI) (Meek et al., 2003). In each of these publications, a number of case studies is presented, as a basis to illustrate application of the framework.

Development of this framework for mode of action/human relevance (HRF) has involved engagement of more than 150 scientists internationally. The framework has been widely incorporated into international and supra-national guidance and is being applied in this context (EFSA, 2006; European Commission, 2003; IPCS, 2006; JMPR, 2006; OECD, 2002; UNECE, 2007). It has also been extensively adopted in risk assessments by the U.S. Environmental Protection Agency (USEPA, 2005; SAB 1999, 2007; SAP, 2000; Dellarco and Baetcke, 2005), the UK (COC, 2004), Health Canada (see, for example, Liteplo and Meek, 2003) and other governmental organizations. Contribution of the framework has also been recognized by the Society of Toxicology in its 2006 awards for Best Paper in *Fundamental and Applied Toxicology* and *Toxicological Sciences* (Green et al., 2005 and Pastoor et al., 2005). Since December 2006, the ILSI Research Foundation, in collaboration with scientists

from the US EPA and Health Canada, has also been offering training workshops on the framework.

In this framework, the weight of evidence of an hypothesized MOA observed in experimental animals is considered in the context of key events along the causal pathway. Once established in experimental animals, the human relevance framework (HRF) provides an analytical tool to enable the systematic evaluation of the data in order to consider its human relevance based often on consideration of more generic information, such as anatomical, physiological and biochemical variations among species. In this manner, the framework encourages maximum use of both chemical-specific and more generic information. While the framework was originally developed for cancer, it has now been extended to non-cancer effects (Seed et al., 2005; Boobis et al , in press). It is envisaged to be of value to risk assessors, both within and outside of regulatory agencies and also to the research community. For the former, the framework provides an analytical tool which assists in structuring consideration of the weight of evidence for a specific mode of action in animals and its relevance to humans. For the latter, it contributes to efficient identification of critical data gaps.

Use/Application of the Framework

Envisaged objectives and use of the framework are:

- Over the long term, to provide an internationally harmonized approach to the establishment of an MOA in experimental animals and its relevance to humans.
- To facilitate systematic consideration of available data and explicit documentation for the conclusions drawn. This includes delineation of a series of key events in a postulated mode of action that are measurable and critical to the induction of the toxicological response. Subsequently, the weight of evidence for these key events is assessed in animals, taking into account the factors delineated by Bradford Hill. Finally, relevance to humans of each of the key events is considered, explicitly taking into account variations between experimental animals and humans.
- To facilitate peer input and review through consistency of presentation and explicit consideration of key aspects.
- To generate criteria for the MOA against which subsequent cases can be considered, i.e. to show whether a compound shares an established MOA.
- To enable clarification of key information relating to the human relevance of the MOA, to inform the assessment of other chemicals that share the MOA.
- To identify critical data deficiencies and research needs. This derives from the requirement in each step of an HRF analysis to explicitly consider strengths and weaknesses and assess confidence in the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database, and the nature and extent of concordance between animals and humans.
- To inform subsequent quantitative aspects of the assessment, specifically dose-response analysis in risk characterization. Where the proposed mode of action is considered relevant to humans, the kinetic and dynamic information taken into account in both the qualitative and quantitative consideration of human relevance in the MOA/HRF framework is relevant to the subsequent dose-response analysis in risk characterization. This information is critical in

considering the adequacy, for example, of available information as a basis for replacement of default uncertainty factors in the development of chemical specific adjustment factors (CSAF) (IPCS, 2005).

Use of the HRF framework also promotes harmonization of approaches to risk assessment for all endpoints, bridging previously distinct approaches on, for example, cancer and non-cancer effects. Harmonization in this context refers to a biologically consistent approach to risk assessment for all endpoints, for which exploration of biological linkages is critical to ensuring maximal use of relevant information. Often, for example, organ toxicity is a critical key event in postulated modes of action for induction for tumours at the same site. The HRF, then, sets the stage for identification of critical pre-cursor non-cancer key events for which subsequent quantitation of interspecies differences and interindividual variability in dose response analysis is relevant (See, for example, the case study on chloroform included in Meek et al., 2003). In other cases, a postulated MOA may lead to toxic effects in multiple organs and these would be considered in the same non-cancer HRF analysis.

In addition, consideration in the framework may identify factors which, whilst not key in themselves, may modulate key events and resultantly, contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology.

Such an analysis may also provide an indication of those components of a proposed MOA which may only operate over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in a MOA, then the relevance to human risk becomes a matter of exposure. Thus, the exposure assessment step of the subsequent risk characterization is critical to a comprehensive evaluation.

Importantly, then, application of the HRF contributes to identification of any specific sub-populations (e.g., those with genetic predisposition) who are at increased risk and provides information relevant to consideration of relative risks at various life stages. In many cases, this is not based on chemical-specific information but rather inference, based on knowledge of the MOA as to whether or not specific age groups may be at increased or decreased risk. This requires explicit consideration of comparative developmental and ageing processes and events in humans and animal models. These considerations are critical to determination of focus in the remaining stages of risk assessment such as dose-response analysis.

Iterative application of the HRF, even before all of the data are available, to the analysis of a postulated mode of action and/or its relevance to humans is beneficial as a basis for developing and refining research strategies as additional information becomes available. In this context, the framework should prove helpful in facilitating discussion between risk assessors and research scientists in jointly understanding the nature of data that would support human relevance analysis of a postulated mode of action in animals and defining next steps in data acquisition. Iterative consideration of mode of action in designing research strategies is also expected to increase efficiency by focussing resources in critical areas in more tiered and targeted approaches.

As knowledge advances, it is expected that MOAs will become less chemical specific and based more on the key biological processes involved, allowing greater generalization of human relevance from one compound to another.

Content of the Framework

An outline of the framework is presented in Figure 1 and described in more detail below, based on its component elements.

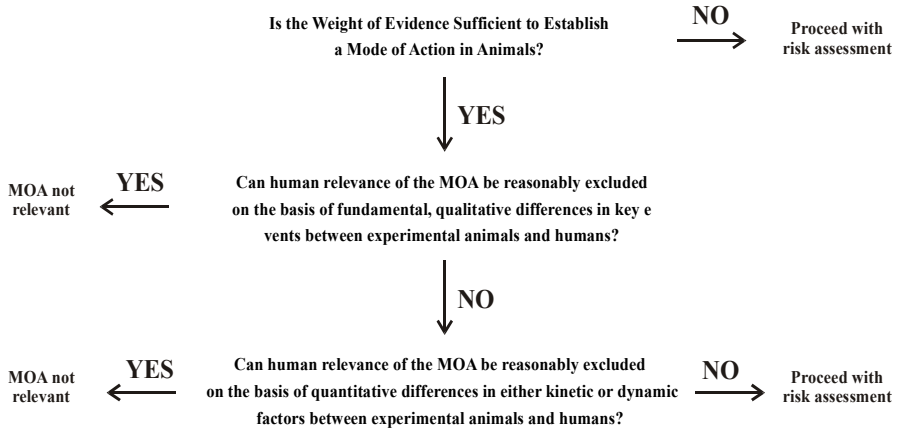

Figure 1. Determining human relevance of an MOA for toxicity observed in experimental animals.

1. Is the weight of evidence sufficient to establish a mode of action in animals?

This comprises consideration of the weight of evidence of a hypothesized mode of action in animals based on identification and consideration of a series of key events using an approach based on factors developed by Bradford Hill originally for application in consideration of causality in epidemiological investigations (Hill,1965). It involves delineation of the postulated mode of action and key events and subsequent consideration of dose response relationships, concordance, temporal associations, strength, consistency and specificity of the association of these key events with the response. Biological plausibility of the hypothesized mode of action with the information in the broader database of scientific research is also considered. Evaluation of the weight of evidence for possible alternative MOAs for which there is some meaningful support, and an evaluation of the overall strength of evidence supporting the MOA under consideration are also included. Ultimately, a decision concerning the weight of evidence supporting the MOA and the level of confidence therein is made. Critically important data gaps which would increase confidence in potential modes of action are also identified.

For a given chemical, often the primary source of information for evaluating an MOA in animals is data generated for that specific chemical in the animal model.

Obviously, data from other sources can and should also be used, as appropriate, along with data on chemicals with similar chemical structures, similar modes of action, or both. [If the mode of action for a chemical is novel, considerably more data will be required to support the conclusion that it is related to the carcinogenic process of the tumors induced by that chemical than subsequent examples of chemicals acting by the same mode of action.]

2. Can human relevance of the MoA be reasonably excluded on the basis of fundamental, qualitative differences in key events between animals and humans?

This step involves a qualitative assessment of the relevance of the MOA to humans. It entails listing of the critical key events that occur in the animal MOA and directly evaluating whether or not each of the key events might occur in humans, taking into account (for example) physiological, anatomical and biochemical differences. Presentation in tabular form, indicating the nature and extent of the evidence for each key event in both animals and humans referred to as a concordance table, can be particularly useful.

The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical-specific. Later events will be more generic to the MOA. Whilst information for evaluating the key events in humans originate from *in vitro* and *in vivo* studies on the substance itself, basic information on anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans can be of value. These include but are not limited to information on, for example, lack of relevant proteins for binding, variations in human/rodent ageing and reproductive and senescence cycles, anatomical variations and human disease states.

3. Can human relevance of the MoA be reasonably excluded on the basis of qualitative differences in either kinetic or dynamic factors between animals and humans?

If the MOA in experimental animals cannot be judged to be qualitatively irrelevant to humans (NO to the question above), a more quantitative assessment is undertaken, taking into account any kinetic and dynamic information that is available from experimental animals and humans. Kinetic considerations include the rate and extent of absorption, tissue distribution, metabolism and excretion. Dynamic considerations include the consequences of the interaction of the chemical with cells, tissues and organs. Only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. Again, tabular comparison of the data from experimental animals and humans is often helpful in the evaluation. Information from studies of other compounds acting by the same or a similar MOA can also contribute. As understanding of the basis for differences in responses between experimental animals and humans improves, differences in key events thought to be qualitative may be shown to be due to specific quantitative differences.

An example of a concordance table is presented in Table 1. Other examples are presented in Meek et al. (2003), Seed et al. (2005) and Boobis et al.. (2006).

Table 1. Concordance analysis - key events - animals and humans - liver - chloroform (Meek et al., 2003)

Key Event	Animals- Liver	Humans – Liver	Weight of Evidence
Metabolism by cyp2E1	Incidence/severity of toxicity correlate with covalent binding of metabolites in rats and mice, more prevalent in necrotic lesions	Irreversible binding to macromolecules in human liver microsomes requires prior metabolism	considerable in animals, limited in humans
Sustained cytotoxicity	In all cases where examined, sustained cytotoxicity (as measured by histopathological effects and release of hepatic enzymes) in the liver of mice at doses that induce tumours	Liver also a target organ in humans based on reports of effects associated with occupational exposure	considerable in animals, limited in humans
Persistent, regenerative proliferation	In all cases where examined, persistent regenerative proliferation (as measured by labelling indices) in the liver of mice at doses that induce tumours	No data but plausible	considerable in animals, none in humans
Liver Tumours	Mice	Inadequate epidemiological data	considerable in animals, inadequate in humans

4. Statement of confidence; analysis; implications

Following application of the HRF and answering the three questions, a statement of confidence should be provided that addresses the quality and quantity of data underlying the analysis, the consistency of the analysis within the framework, the consistency of the database and the nature and extent of the animal to human concordance analysis. Alternative modes of action should have been evaluated, when appropriate, with the same rigour. A critical outcome is the identification of specific data gaps that could be addressed experimentally to increase confidence in the analysis.

The output of the HRF provides information that is useful for more than just determining whether or not the MOA for toxicity in experimental animals is relevant to humans. It can also provide much information that is critically important in subsequent steps in the risk characterization for effects deemed relevant to humans. For example, it may be possible to develop chemical specific adjustment factors on the basis of the information provided. Application of the framework can also provide information on relevant modulating factors which are likely to affect risk. In addition, it can identify those elements of a proposed MOA which operate only over a certain dose range. Where an obligatory step in a MOA occurs only following a high experimental dose of a compound, then the relevance of the MOA to human risk is determined by the exposure.

The analysis also contributes to the identification of any specific sub-populations (*e.g.* those with genetic predisposition) who may be at increased risk and often provides information useful in considering relative risk at various life stages. This may not always be based on chemical-specific information but rather on inference, on the basis of knowledge of the MOA, as to whether or not the risk in specific age groups might be expected to differ.

The objective is that data and their analysis using the HRF are reported in a clear and comprehensive manner, so that others can determine the basis of the conclusions reached. It is anticipated that this will lead in the short term to increased understanding and over the longer term, to greater consistency in assessing weight of evidence for hypothesized modes of action.

Application of the IPCS HRF to DNA-reactive carcinogens

A fundamental question to be addressed in considering the mode of action for cancer is whether or not a chemical interacts directly and mutates DNA as a key event. Because of similarities in the carcinogenic process between rodents and humans and the comparable initial interactions with DNA by DNA-reactive carcinogens, it would be expected that, in general, DNA-reactive carcinogens would be assessed as progressing to the step of "NO", to the step in the IPCS HRF which asks the question "can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans". However, considering human relevance of rodent tumours in the context of a set of key events that clearly describe the cancer process for any particular DNA-reactive carcinogen has potential to more meaningfully inform dose-response for key events and permits development of more integrated test strategies (e.g., consideration of key events in identified target tissues).

In a recent paper, Preston and Williams (2005) presented a set of key events for tumour development that provided a guide for the use of the HRF with DNA-reactive carcinogens. This guide supported the view that for most DNA-reactive chemicals, the animal MOA would be predicted to occur in humans. However, it was also argued that there could be exceptions and that in addition to being a valuable tool for identifying these, use of the HRF can also assist in quantifying differences in key events between rodents and humans that may be of value in extrapolating risk to humans. For example, human-rodent differences in tumour response for DNA-reactive carcinogens might be a function of the generally more proficient DNA repair processes that occur in humans than in rodents (Hanawalt, 2001; Cortopassi and Wang, 1996) or a unique pathway of bioactivation in rodents could result in there being "YES" answers to the steps in the IPCS HRF that address the queries "Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?" and/or "Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?". Alternatively, the IPCS HRF could provide quantitative information on these processes for use later in the risk characterization step.

Specifically, consideration in a mode of action context would facilitate more targeted investigation consistent with the hypothesized mode of induction of specific tumours. For example, consideration of information on dose response for key events in *in vivo* studies such as reaction with DNA in target cells to produce DNA damage, misreplication or misrepair of DNA damage, and formation of mutations in critical genes in an HRF weight of evidence approach has considerable potential to additionally inform estimates of risk. This provides an attractive alternative to consideration of the results of batteries of tests inherently prone to yield positive findings and difficult to interpret in a MOA context.

Conclusions

In conclusion, the Human Relevance Framework provides a systematic approach for judging whether data support a postulated mode of carcinogenic action for a chemical and for evaluating its relevance for humans**.** The framework is of value both to the risk assessment and research communities in furthering our understanding of carcinogenic processes, in identifying data gaps and informing the design of studies related to MOAs. There has been additional work on extending the framework to non-cancer endpoints (Seed et al., 2005; Boobis et al. b, in press), and in this context, the HRF should be an invaluable tool for harmonization across endpoints. The preparation of a unified HRF framework that is applicable to all toxicological end-points, including cancer and elaboration of the integration of framework approaches into the risk assessment process with illustrative examples is envisaged. For example, identification of key events in the MOA can provide insight into the sources and magnitude of inter-species and interindividual differences.

Training materials relevant to the application and interpretation of the outputs of the HRF continue to be developed. Training is facilitated by the availability of a number of suitable case studies including those published to date for both DNA-reactive and DNA-non-reactive modes of action (Table 2).

Table 2. Tumour Types and Test Compounds Considered in the ILSI RSI and IPCS Human Relevance Frameworks (Meek et al., 2005; Boobis et al.a, in press)

- Multiple: (ethylene oxide, 4-aminobiphenyl)
  - Direct alkylation of DNA
- Brain: (acrylonitrile)
  - Data inadequate to support hypothesized MOA
- Kidney: (d-limonene)
  - Chemical-induced species and sex specific protein
- Mammary: (atrazine)
  - Suppression of luteinizing hormone
- Thyroid (Phenobarbital, thioazopyr)
  - Increased hepatic clearance of throxin
- Bladder (melamine)
  - Urinary tract calculi
- Nasal (formaldehyde)
  - Sustained cytotoxicity and regenerative proliferation
- Liver/kidney (chloroform)
  - Sustained cytotoxicity and regenerative proliferation

Consideration is also being given to maintenance of a database of generally accepted MOAs together with informative case studies. Such a database would be of particular importance as experience continues to evolve in the development of modes of action, and in determining whether the MOA for a compound is novel or has been described previously for other compounds.

Acknowledgements

This paper summarizes the work of a large number of scientists who participated in the development and application of the HRF of the International Life Sciences Institute Risk Sciences Institute and the International Programme on Chemical Safety. While the content is based on a presentation made by the author at the Environmental Mutagen Society meeting held in Vancouver, in September, 2006, the work represents the product of a significant number of contributing authors and collaborators, as outlined in Meek et al. (2003), Seed et al. (2005), Boobis et al. (2006) and Boobis et al. (in press).

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Chapter 5

A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action

Meek, M. E., Bucher, J. R., Cohen, S. M., Dellarco, V., Hill, R. N., Lehman-McKeeman, L. D., Longfellow, D. G., Pastoor, T., Seed, J., Patton, D. E. (2003)

Critical Reviews in Toxicology, 33(6): 591-602; 606-611; 635 - 632

PREFACE

Two critical assumptions have governed cancer risk assessment for many years. In the absence of information to the contrary, risk assessors generally assume that tumors observed in laboratory animals are predictive of human cancer and that the mode of action (MOA) defined in laboratory animals applies also to humans. In June 2001, the Risk Science Institute of the International Life Sciences Institute (ILSI RSI) formed a Workgroup to examine these issues, with a focus on using MOA information to determine the human relevance of animal tumors. The Workgroup divided into two Subgroups, one developing and using a Famework for MOA relevance analysis and the other studying Peroxisome Proliferation Activated Receptor (PPAR)α-agonist activation as the MOA for certain rodent chemical carcinogens.

To develop useful new perspectives for risk assessment guidance and practice, the Framework Subgroup studied several different MOAs, each illustrated by a different chemical carcinogen. The experience and insights gained are the subject of this report, which emphasizes the importance of transparent, weight-of-evidence principles and methods for assessing the human relevance of MOA information from animal and human sources. This report presents the ILSI RSI Framework and related case studies.

This report serves several purposes. For risk assessors in and out of government, it offers guidance and models for using MOA data, related animal tumor data, and available human information in evaluating the hazard potential for humans. The approaches developed should become mainstay tools in the scientific community's overall effort to enhance the predictive power, reliability and transparency of cancer risk assessment.

Although guidance proposed by the U.S. Environmental Protection Agency (EPA) and International Programme for Chemical Safety (IPCS) served as a springboard for this activity (see text), the work reported here was conducted independently of the ongoing efforts of both organizations. This project is supported by funding from several offices of the U.S. EPA and the Existing Substances Division of Health Canada. This report reflects the comments and recommendations of an 18-person peer review panel, convened by ILSI RSI, which met in Washington D.C. on December 4-5, 2002.

A FRAMEWORK FOR HUMAN RELEVANCE ANALYSIS OF INFORMATION ON CARCINOGEN MODES OF ACTION

M.E. Meek, John R. Bucher, Samuel M. Cohen, Vicki Dellarco, Richard N. Hill, Lois D. Lehman-McKeeman, David Longfellow, Timothy Pastoor, Jennifer Seed, and Dorothy E. Patton

There are two sections in the Framework for Human Relevance Analysis. Part A provides background information on components of the Human Relevance Framework for using MOA information to assess the human relevance of animal tumors. (Selected) case studies comprising Part B illustrate application of the Framework to the postulated MOAs for several chemical carcinogens that differ as to available MOA data and related risk assessment requirements.

PART A

BACKGROUND

Introduction. The National Research Council "red book" paradigm for chemical risk assessment describes four fields of analysis, each with closely related but distinct information inputs and analytical results: hazard identification, assessment of dose-response relationships, exposure assessment for populations at risk, and risk characterization (NRC, 1983, 1994). Hazard identification is based on data and information from laboratory animal studies and, if available, human studies. In hazard identification and subsequent hazard characterization, the primary question is whether the effects observed in animal study populations might be expected in humans, a question that depends at least in part on extrapolating hazard and dose-response data from laboratory animals to humans. (Definitions and additional details on the NRC paradigm appear in the attached Appendix.)

The MOA of the chemical under study is a fundamental aspect of these extrapolations (Vainio, et al., 1992; IARC, 1992; NRC, 1994; NTP, 1998). To address this issue, the EPA and the IPCS have proposed generally comparable guidance for evaluating animal MOA information to assess the relevance of animal tumors for human risk assessment (U.S. EPA, 1999; Sonich-Mullin et al., 2001; U.S. EPA 2003). Both proposals focus on identifying "key events," generally described "as measurable effects that are critical to the induction of tumors, as hypothesized in the postulated mode of action." Similarly, both proposals offer specific guidance on developing and analyzing MOA information from *animal* studies, with considerably less guidance on applying this information to assess human relevance.

ILSI RSI Project. The Framework for using MOA information to assess the human relevance of animal tumors (MOA/Human Relevance Framework) expands the EPA and IPCS frameworks into a new four-part analysis (Table 1). The resulting system for MOA relevance analysis lies mainly in the hazard identification phase of the risk assessment. That is, where MOA information is available from both animals and humans, the MOA/Human Relevance Framework calls for a weight-of-evidence analysis and informed characterization of the available tumor data as to potential human relevance. This characterization should be fully transparent as to data sources (both chemical-specific and generic), data gaps, assumptions about the applicability of generic data, and extrapolations within the MOA analysis. At the same time, the Framework is non-prescriptive, offering a simple structure for making and articulating critical scientific judgments.

The MOA/Human Relevance Framework adopts the customary presumption that animal tumors are relevant for human hazard or risk assessment (IRLG, 1979; OSTP, 1985; U.S. EPA, 1986, 1999, 2003). Similarly, the animal MOA is presumed to describe processes in humans as well as in animals. Although the presumption of relevance applies alike to DNA- reactive and non-DNA-reactive carcinogens, presumptive judgments of human relevance for non-DNA-reactive carcinogens often generate controversy and stimulate calls for MOA data to rebut the presumption for individual chemicals. To augment guidance currently available on this contentious issue and since data are most abundant in this area, this report focuses mainly on non-DNA reactive carcinogens.

Employing an iterative approach, the Framework Subgroup combined case study methodology and current EPA and IPCS guidance proposals to assess the human relevance of chemical carcinogens with generally well-studied postulated animal MOAs. The Subgroup had several expectations: gaining experience in using the proposed guidance, identifying and isolating useful principles and approaches, and identifying case studies that could serve as models for practitioners. As work progressed, completing the case studies required new approaches for using MOA analysis to determine whether or not related animal tumors provide data appropriate for human risk assessment. In this regard, three observations were pivotal points leading to the new four-part framework.

Limited utility of animal MOA information. No matter how well-defined and fully analyzed, MOA information derived *solely* from animal studies does not permit definitive conclusions about human relevance or lack of relevance. Specifically, although an absence of human data permits an assumption of human relevance, conclusions about *lack of* human relevance depend in part on consideration of the potential applicability of the animal MOA to humans.

This observation is important because the literature on the MOA for chemical carcinogens in laboratory animals is expanding in several different ways. Information is available for increasingly more chemicals, and the new data are generally more reliable and more detailed. The result is enhanced understanding and confidence in methods for developing such MOA information. Moreover, completing an MOA relevance analysis generates new questions and requires additional information, with the result that a well-defined animal MOA can stimulate research on the potential for human hazard and risk. Nonetheless, animal MOA data alone cannot answer the human relevance question.

***Necessity for human information*.** To understand and describe the potential MOA in humans, information about humans that permits evaluating the applicability of the animal MOA is needed. Ideally, epidemiological studies would provide information such as biomarkers of exposure and of effect for use in MOA analysis. However, these studies are rarely designed to provide data to address MOA issues. Rather, pertinent information is available from other sources, generic and specific, with the most directly useful information coming from work involving the agent under study. Sources for such chemical-specific information include *in vivo* and *in vitro* studies and exposures at all levels of organization, ranging from population studies to cultured cells. Other sources include data from pathology specimens or diagnostic and other clinical tests, and from people with pertinent disease.

These human information sources may provide data derived from studies involving *other* chemicals (structurally or functionally related to the chemical under review) or *various* disease states. Such generic data are useful in addition to or in lieu of chemical-specific data, especially because data for specific chemicals may not be available or attainable. For example, MOA data on key events (or associated features) for other chemicals involved in the same disease process or the same or different chemicals in still other species may be used for this analysis. Information on comparative biochemistry, endocrinology or physiology may be used. The essential question is biological plausibility in the sense of overall consistency between weight-of-evidence conclusions about the MOA in humans and established scientific information and principles. In this regard, consultation and collaboration to foster

coordinated approaches to the conduct of epidemiological studies could enhance the database for MOA relevance analyses.

***Utility of comparative analysis*.** Side-by-side comparisons of evidence relating to key events or associated features in animals and in humans promote information-based assessment of potential MOA comparability. Such MOA concordance analysis, which should not be confused with tumor site concordance, requires a systematic look at the key events identified for the animal MOA and determining if there are or may be comparable events in the human. As a minimum, a narrative description is necessary, and a "concordance table" may aid developing and presenting the analysis.

Scope. Several limitations on the scope of the proposed Framework require attention. At the outset, it is important to distinguish between *mode* of action, the primary focus of this report, and *mechanism* of action, an issue not addressed in this report. The MOA refers to a plausible hypothesis supported by observations and experimental data regarding events leading to a toxic endpoint. It describes the way a chemical interacts with cellular components to produce toxic effects. Discerning the MOA involves developing information about key events -- cellular or biochemical events identified in terms of measurable parameters (see below) – considered to be in the pathway leading to adverse effects, including carcinogenicity or other changes. Mechanism of action generally implies a detailed description and sufficient understanding of the molecular basis of cancer (or other effects) to establish causation in molecular terms. Such mechanisms are seldom, if ever, fully known.

As noted above, the framework focuses mainly on the animal to human extrapolation in the hazard characterization phase of risk assessment. The corresponding question in the dose-response analysis may require applying the generally higher dose levels used in animal studies to estimate the hazard potential at the much lower exposure levels generally expected for human populations, especially for environmental chemicals. This extrapolation does not apply, however, to pharmaceuticals or even to some components of food where animal and human exposure may be similar. Because quantitative considerations from the dose response and exposure analyses are critical for some aspects of the mode of action analysis, this report points to these factors where appropriate, but does not address at length the use of mode of action information in discerning hazard potential.

Similarly, the report recognizes the interface between dose-response and exposure assessment in risk estimation and touches on these issues where appropriate. However, the report does not specifically address these issues, focusing instead on the applicability of animal modes of action to human cancer hazard. That is, the MOA framework addresses the hazard potential, but does not reach later stages of the risk assessment in which risk is quantified and characterized. Such conclusions require a complete risk assessment, including dose-response and exposure assessment, in line with the NRC risk assessment paradigm. Rather, an MOA analysis based on available data and analyzed in line with the framework contributes to confidence in conclusions about the carcinogenic potential for humans and the likelihood and magnitude of human risk.

ELEMENTS OF THE MOA RELEVANCE FRAMEWORK: APPLICATION TO CASE STUDIES

The Framework analysis moves from an initial focus on key events (and associated features) in the animal MOA and other factors to a weight-of-evidence conclusion about the relevance to humans of the animal tumors under study. The elements of the framework are posed as questions which when addressed provide direction on how to proceed with the overall evaluation of relevance to humans (Table 1).

Table I. MOA in Assessing the Human Relevance of Animal Tumors

1.	***Is the weight of evidence sufficient to establish the MOA in animals?***
a.	Postulated MOA
b.	Identification of key events
c.	Animal evidence
d.	Application of EPA/IPCS animal MOA guidance (Table 2)
2.	***Are key events in the animal MOA plausible in humans?***
a.	Concordance analysis of animal and human responses
b.	Statement of confidence
3.	***Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?***
a.	Concordance analysis of animal and human responses
b.	Statement of confidence
4.	***Statement of confidence; analysis; implications***

To develop and test the MOA framework, the Framework Subgroup selected animal carcinogens for which substantial animal MOA data and information are available in the published literature. Chemicals were selected and analyzed as representing a specific MOA for the sole purpose of testing the framework rather than to summarize or re-evaluate the chemical. That is, chemicals were selected because they represented fairly well-recognized MOAs, and in no case were data reanalyzed to confirm, modify or refute existing animal MOA analyses. Developing the case studies provided an iterative assessment of the framework and helped to uncover critical issues and problems that ultimately led to new approaches, principles, tools, and formats for MOA and relevance analyses. A discussion of the framework with a brief summary of how case studies contributed to its refinement follows.

1. Is the weight of evidence sufficient to establish the MOA in animals?

Given a finding of tumors in animals, current EPA and IPCS guidance spells out topics for organizing and presenting the information at hand (*Table 2*) (U.S.EPA, 1999; Sonich-Mullin, 2001). This approach employs the criteria used by epidemiologists to assess causality. In adopting this approach, the IPCS emphasized that it "is not a checklist of criteria but rather presents an analytical approach to considering the weight-of-evidence for a MOA" (Sonich-Mullin, 2001). As such, it is an important aid

in identifying key events, uncertainties and data gaps, and in determining whether the MOA is demonstrated.

The process begins with a statement of the proposed MOA and an enumeration of key events. The analysis continues with summaries of dose response and temporal relationships, along with analyses of the strength, consistency and specificity of key events, tumor responses, and biological plausibility and coherence. After considering other potential MOAs that may account for the tumors, the animal MOA analysis ends with an overall conclusion about the weight of evidence as to the MOA and the level of confidence in that decision. This MOA information is also essential for the evaluation of human relevance. The presentation identifies inconsistencies and data gaps to explain the weight of evidence and the level of confidence. It also provides a basis for identifying additional research needs.

Table 2. Framework for Evaluation of an Animal MOA*

a. Postulated MOA
Brief description of the sequence of measured effects starting with chemical administration to cancer formation at a given site.

b. Key events
Clear description of each of the key events (measurable parameters) that are thought to underlie the MOA.

c. Dose response relationships
Dose response relationships presented for each key event, and comparisons presented of dose response relationships among key events and with cancer.

d. Temporal association
Sequence of key events over time that lead to tumor formation.

e. Strength, consistency and specificity of association of key events and tumor response
Complete assessment and presentation of the relationships among the key events, precursor lesions and tumors. Portrayal of the consistency of observations across studies of different designs.

f. Biological plausibility and coherence
Determination whether key events and sequence of events are consistent with current biological thinking, both regarding carcinogenesis in general and for the specific chemical under review.

g. Other MOAs
Alternative MOAs that may be applicable for the chemical under review. Comparison of their likelihood vis-à-vis the proposed MOA.

h. Conclusion about the MOA
Overall indication of the level of confidence in the postulated MOA.

i. Uncertainties, inconsistencies and data gaps
Identification of information deficiencies in the case; description of inconsistent findings in the data at large; evaluation of uncertainties; proposal of pointed research that could significantly inform the case.

*Adapted from U.S.EPA (1999), Sonich-Mullin (2001)

The animal MOA analyses in the case studies in this report are organized in line with Table 2, which the Framework Subgroup found to be appropriate, helpful

and generally complete. Redundancies exist in the animal MOA analysis, but case study authors modified the presentations to minimize such repetition. The adequacy of the MOA data set is a fundamental component of this analysis. Data needs for determining how chemicals induce cancer in animals differ for different MOAs. In view of a myriad of different modes, developing criteria for determining what is required and whether enough information exists to establish a particular MOA is difficult. However, once a MOA has been well delineated for one chemical, data needs for verifying this mode for subsequent chemicals working through the same MOA will usually be significantly reduced.

However, if the MOA in animals is not well defined, then the questions of plausibility in humans and/or issues of quantitative differences between animals and humans are not useful for the evaluation of the carcinogenic potential. An example of an inadequate data set for delineating MOA is provided in the selected case studies (Case Study 2, acrylonitrile). Although the available data suggest that oxidative damage may be responsible for acrylonitrile-related tumors in laboratory animals, the data do not adequately define the MOA. In addition, an alternative MOA involving DNA reactivity was explored, and it also was judged to be inadequate to account for the tumors. As a result, given only preliminary MOA information, the assessor must assume that animal tumors indicate a cancer potential for humans; a default risk assessment proceeds based on the potential hazard and risk to humans. This scenario applies to many chemicals for which preliminary information suggests, but falls short of establishing a hypothesized MOA. In these cases, a full risk assessment for this endpoint is required

2. Are key events in the animal MOA plausible in humans?

This question represents a *qualitative* assessment of the relevance of the MOA to human cancer potential. Generally, the default assumes that animal tumors and the accompanying MOAs are relevant to humans. The animal MOA describes the pathway toward tumor development in terms of key events and associated features that describe or aid understanding of tumor formation. Corresponding information on humans needs to be assembled and compared to that in animals. For example, one format (Table 3) aligns each key event (or other factor) in the postulated MOA with relevant information regarding both animal and human responses. Such a table can be useful and informative to assessing the strength of evidence supporting each event and facilitates the analysis of the qualitative relevance of the animal observations to humans.

Table 3. Concordance of Animal and Human Key Events (and Associated Processes)

Key event	Animal effect	Human and/or Non-human Primate	
		Source of information	Effect
1.			
2.			
3.			

Human information is typically of two types dealing either with data on the specific chemical under evaluation (or of chemically- or functionally-related agents) or generic information that is not related to the chemical but is pertinent to the MOA. Both can contribute significantly to the analysis; however there is merit in marking each key event as to whether the data arise from the chemical under test (or analogues) or from other sources. Where such data are available, the greater the degree of concordance between animals and humans for the assessed factors, the greater the confidence that the animal MOA is applicable to humans. In addition to the key events in the animal MOA, other useful information includes such factors as:

1. Cancer incidence at the anatomical site and cell type of interest, including age, sex, ethnic differences and risk factors including chemicals and other environmental agents.
2. Knowledge of the nature and function of the target site including development, structure (gross and histological), control mechanisms at the physiological, cellular and biochemical levels.
3. Human and animal disease states that provide insight concerning target organ regulation.
4. Human and animal responses to the chemical under review or analogs following short, intermediate and long term exposure, including target organs and effects.

It should be emphasized that data must be significant and convincing to deviate from the default and conclude that the MOA in animals is not relevant to humans. This requires substantial qualitative information indicating that humans are not expected to respond, as do animals. If the data strongly support a species-specific MOA that is not relevant to humans, chemicals producing animal tumors by that MOA would not pose a cancer hazard to humans and no further risk assessment steps are necessary. Conversely, the default is retained and the animal MOA is presumed relevant for humans unless the animal MOA can confidently be rejected as not pertinent to human hazard potential.

To date, only a few MOAs for animal cancers have cleared the high hurdle to be generally accepted as not relevant to humans. Case studies are provided (though not presented here) for α2u-globulin-associated male rat kidney tumors (*d*-limonene; Case Study 3) and inhibition of LH surge-related rat mammary tumors (atrazine; Case Study 4). These examples feature comprehensive data sets providing evidence of molecular or physiological characteristics that are unique to rats and that do not operate in humans. In the case of *d*-limonene, binding of the chemical metabolite to α2u-globulin in the kidney is the initial step in the carcinogenic process, resulting in renal tubule protein overload, cytotoxicity, reparative cell proliferation and tumor development. There is a large body of data demonstrating that the presence of α2u-globulin is prerequisite to the development of this renal syndrome. Although this protein is present in the male rat, it is absent from female rats, mice (who show no evidence of renal tumorigenicity) and humans. No structurally-related protein present in any other species, including humans, binds to a broad group of xenobiotics and elicits a renal syndrome in a manner similar to α2u-globulin. Thus, male rat kidney tumors by this MOA are not considered applicable to human hazard.

The second case study where qualitative differences dictate the evaluation of human relevance of animal tumors is atrazine, which affects the hypothalamus of female Sprague-Dawley (SD) rats leading to an inhibition of the LH surge during the

estrous cycle. This sets into motion persistent secretion of estrogen and prolactin, leading to the development of mammary tumors. The hormonal changes and tumor response elicited by atrazine are not seen in Fisher rats or CD1 mice. Physiological responses to sustained hormone secretion in humans are so different from the SD rat that, even if the human hypothalamus were affected in a manner similar to the SD rat, a totally different syndrome, namely a hypoestrogenic state would be expected. Such an effect in humans would not lead to the induction of breast cancer.

3. Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?

If the animal MOA is judged to be *qualitatively* applicable to humans, an analysis of potential *quantitative* differences in sensitivity between animals and humans is necessary. Without such information, assessors usually assume that humans respond, as do animals. Factors of interest include: differences in the biotransformation and effects of the chemical under investigation, that is, toxicokinetics (i.e., time course of chemical uptake, distribution, metabolism, excretion), and in toxicodynamics (i.e., consequences of the interaction of the agent and tissues) that may increase or decrease susceptibility of humans relative to animals. Likewise, physiological, cellular and biochemical differences between species regarding endogenous chemicals and control systems may require consideration.

As with the concordance analysis, a tabular display may provide a useful summary of the analysis of kinetic and dynamic factors relative to a MOA. Generally, the output of the analysis is a set of quantitative comparisons between animal and human responses, resulting in numerical differences in responsiveness. The remaining case studies (cases 5-7, respectively, phenobarbital, chloroform, and melamine of which chloroform, only, is presented) provide examples of quantitative differences that inform the evaluation in different ways. These cases illustrate a spectrum of quantitative differences and demonstrate how these differences can be applied to addressing human relevance. For example with phenobarbital, multiple distinct differences exist that collectively suggest lack of relevance to humans. In contrast, the chloroform example, presents a case in which processes leading to tumor formation are quite comparable across species including humans. Melamine represents an intermediate case, where the differences between species impact the decision regarding the overall applicability of the animal MOA to human hazard.

Although hormonal axes are qualitatively similar across species, the disruption of the thyroid-pituitary status by phenobarbital (Case Study 5, Phenobarbital, not presented) includes a number of quantitative factors that determine relevance to humans. Phenobarbital enhances the hepatic clearance of thyroid hormone in rats by inducing the Phase II enzymes involved in thyroid hormone metabolism. This results in reduced circulating levels of thyroid hormone and triggers an increased output of TSH. Sustained stimulation of the thyroid gland by TSH increases cell proliferation and leads to tumor formation. Rats are distinguished qualitatively from humans because they lack thyroxine-binding globulin that transports thyroid hormone in the serum of humans. As a result, rats differ quantitatively from humans in that the half-life of thyroid hormone is much shorter, and TSH levels are higher than in humans. Consequently, humans are less likely to show such change in TSH levels. Like phenobarbital, none of the liver enzyme

inducers is known to increase TSH in humans. In addition, whereas changes in hormone levels readily result in tumor development in rodents, especially the rat, this is not the experience in humans. These kinetic and dynamic factors argue persuasively that the MOA for phenobarbital related to the thyroid is not relevant to humans and no further cancer risk assessment is necessary.

The remaining case studies present examples in which animals and humans are qualitatively and quantitatively relevant regarding the animal MOA. In a case involving multiple tumors, separate analyses for the liver and kidney showed that cytotoxicity from chloroform (Case Study 6) depends on formation of a highly reactive metabolite, phosgene. This biotransformation step occurs in both rodents and humans, and exposed humans have shown toxicity in the same organs developing tumors in animals. Thus, the MOA in animals is relevant to humans. Toxicokinetic comparisons in both rats and humans show that the key to the MOA is the requirement for a dose sufficient to produce cytotoxicity and subsequent cellular regeneration.

In the case of urinary bladder stones (melamine; Case Study 7 not presented), several qualitative and quantitative factors contribute to the overall MOA analysis. At high doses in animals, melamine precipitates in the urine to form calculi that accumulate in the urinary bladder. Stones in the bladder damage the urothelium, stimulating compensatory cell proliferation and tumors. In humans, calculi (formed from other substances) are more likely to be retained in the renal pelvis or ureters rather than in the bladder. Whereas bladder stones tend to be retained in the rodent bladder they are more likely to be passed in the urine in humans or removed (surgically or by lithotripsy) because of urinary obstruction and pain. Overall, the relationship between urinary calculi and the development of bladder cancer in humans is not totally clear, but epidemiological data suggest a small relative cancer risk associated with the presence of urinary calculi in humans. Therefore, the framework analysis identifies this MOA as a potential human hazard requiring further risk assessment. For melamine, human exposure is the critical element that ultimately defines the potential for human risk. For both chemical cytotoxicity (chloroform) and urinary tract calculi (melamine) MOAs, further risk assessment is necessary, including evaluation of the dose response and human exposure. In performing this risk assessment, it is presumed that the knowledge regarding MOA will be incorporated, providing scientific guidance for the evaluation.

An important consideration regarding the case studies concerns the issues of multiple tumor sites. For the chloroform case, liver and kidney tumors are generally considered to arise from the same MOA involving metabolic activation. In contrast, in the case of phenobarbital, it is widely recognized that this compound is also a mouse liver carcinogen, but these data are not included in the case study. Overall, the merits of the MOA for each tumor type must be considered separately. Although the issue of multiple sites is a very important consideration in the overall risk assessment for a particular compound, inclusion of a case in which two tumor types arise from different MOAs was not included in the current case studies.

The decision analysis in Figure 1 summarizes the Framework Subgroup's conclusions regarding human relevance for the six case studies analyzed for this report.

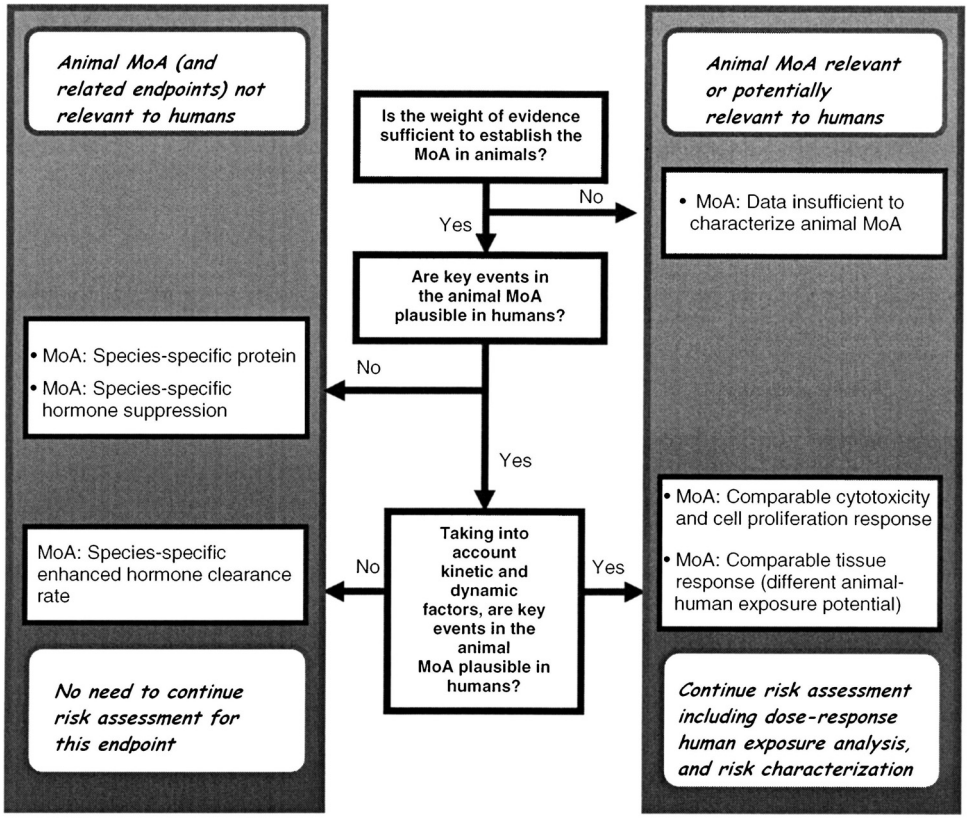

FIGURE 1. Schematic illustrating human relevance results for six case studies representing several different MOAs (and one example of data insufficient to characterize an animal MOA). The left side depicts data-based findings that the animal tumors are irrelevant because the MOA is unlikely to have a human counterpart due to a tumor-related protein specific to test animals (Case Study 3, *d*-limonene), the tumor consequences of hormones suppression typical of laboratory animals but not humans (Case Study 4, atrazine), and chemical-related enhanced hormone clearance rates in animals relative to humans (Case Study 5, phenobarbital). In cases where the animal tumor MOA is not expected in humans, the tumors are considered irrelevant and there is little reason to continue dose-response, exposure, and risk characterization for this endpoint. The cases involving relevant tumors on the right side portray one example of insufficient data (Case Study 2, acrylonitrile) and two examples that have human counterparts for the animal key events and associated processes. Full risk assessments are necessary for the insufficient data case, the MOA involving cytotoxicity and cell proliferation in animals and humans (Case Study 6, chloroform) and formation of urinary-tract calculi (Case Study 7, melamine). Risk estimates based on relevant tumors associated with different chemicals will vary with quantitative differences in exposure, toxicokinetics, and toxicodynamics.

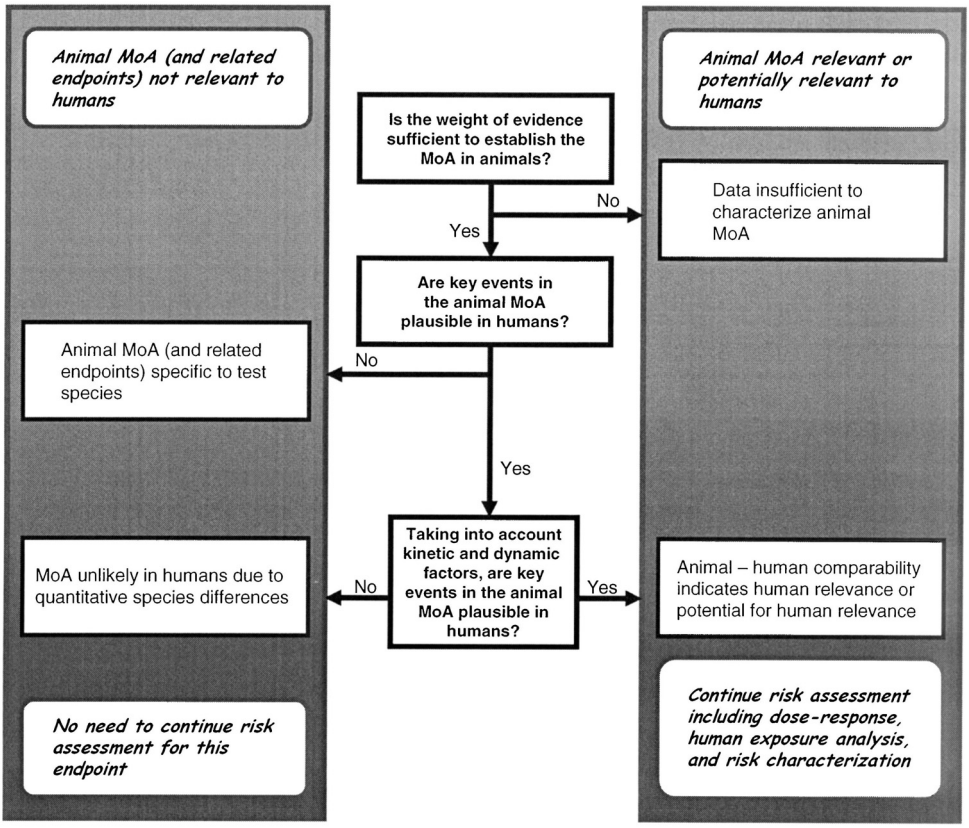

FIGURE 2. General schematic illustrating divergent outcomes for different MOAs analyzed in line with the four-part human relevance framework. The left side depicts data-based findings that animal tumors are irrelevant for human risk assessment because a tumor-related animal MOA is unlikely to have a human counterpart for the endpoint under study. When qualitative considerations identify MOAs specific to the test species or quantitative considerations indicate that the animal MOA is unlikely to occur in humans, such hazard findings are conclusive and there is little reason to continue risk assessment for that endpoint. The right side portrays two outcomes leading to complete risk assessments. One is the default: When data are insufficient to confidently characterize an MOA for test animals, the animal tumor data are presumed to be relevant to humans and a complete risk assessment is necessary. The other is the product of data-based findings that the animal MOA is relevant to humans: Risk assessment is required when the human relevance analysis shows qualitative or quantitative comparability between the test species and humans as to key events in the postulated mode of action (and related processes) for the endpoint under study.

4. Statement of Confidence, Analysis and Implications

As appropriate, the statement of confidence should address such issues as the quality and quantity of data underlying the analysis, consistency of the analysis with the Framework set forth in Table 1, consistency of the database with the criteria set forth in Table 2, nature and extent of the concordance analysis, and likelihood of alternative modes of action.

GENERAL AND FUTURE APPLICABILITY OF THE FRAMEWORK

The MOAs analyzed for human relevance for this report produced the specific results presented in Figure 1. Figure 2 is a general schematic illustrating the framework's capacity to accommodate other MOAs.

MOA analysis is receiving increasing attention in risk assessments for health effects other than cancer. For example, MOA analyses are beginning to have in role in U.S. EPA assessments for non-cancer effects (U.S. EPA, 2000, atrazine; U.S. EPA 2002a, vinclozolin; U.S. EPA, 2002b, mesotrione). Similarly, Health Canada's Existing Substances Division has posted assessments for 2-BE and carbon disulfide (Health Canada, 2002). Based on these examples, the concepts, analytical tools, and format guidance presented here for carcinogens merit consideration in MOA analyses for health effects other than cancer. Because several of the MOA case studies considered by the Framework Subgroup involve non-cancer endpoints, the applicability of the proposed framework for endpoints other than cancer is apparent.

Similarly, the ILSI RSI Framework may be useful for addressing questions regarding the relevance of animal tumors for assessing human risk at different life stages. A case study approach would test applicability of the framework and identify appropriate refinements.

With these examples in mind, ILSI RSI is organizing a follow-on study to assess the value added of using the framework to evaluate the human relevance of other carcinogenic and non-carcinogenic endpoints.

SUMMARY

Although current EPA and IPCS guidance offers excellent starting points for animal tumor MOA analysis, completing the case studies revealed several issues requiring special attention and new methods. In different ways, each case is instructive regarding information needed to reliably assess the relevance, or lack of relevance, of animal tumors to humans. The cases make clear that defining a postulated MOA in laboratory animals is a necessary first step in a multi-part analysis and that conclusions about the human relevance of animal tumors depend on comparing key events in the animal MOA with comparable information from human data sources. In addition, the cases demonstrate the importance of transparency -- an explicit analysis of both qualitative and quantitative factors for evaluating the likelihood that the MOA postulated for animals can occur in humans.

The cases as a whole illustrate some of the issues and analyses that practitioners, scholars, and policy-makers will encounter as the scientific and regulatory communities give increased attention to MOA information from animal and human studies to evaluate the applicability of animal tumors to humans. As such,

they also suggest new approaches to informed and consistent use of MOA information for health effects other than cancer and for different life stages.

PART B

CASE STUDY 2

MOA: Data Inadequate to Suppport Hypothesized Mode of Action

Brain Tumours Associated with Acrylonitrile Exposure

M.E. Meek, Health Canada

Introduction

A range of tumours in rats[1] — including those of the central nervous system (brain and/or spinal cord), ear canal, gastrointestinal tract and mammary glands— has been consistently observed following exposure to acrylonitrile by both ingestion and inhalation. In almost all adequate bioassays in rats, there have been reported increases in astrocytomas of the brain and spinal cord, which are the focus of this case study.

In this case, limited available data do not provide convincing or consistent support for the hypothesized mode of induction of the relevant tumour in animals (i.e., astrocytomas of the brain). Hence, the response to the first question in the framework analysis - i.e., Does available data reasonably support a hypothesized mode of action for tumours - is no and it is not possible to address the subsequent questions of whether the mode of action is qualitatively or quantitatively applicable to humans. However, this does not preclude consideration of, for example, quantitative toxicokinetic variations between animals and humans in subsequent dose-response analyses.

I. Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

A. Postulated Mode of Action/Key Events:

There is considerable evidence of the carcinogenicity of acrylonitrile, based on the results of primarily early unpublished investigations, which have been restricted to one species (rats).[1] In the most sensitive bioassays, a range of tumours (both benign and malignant) has been consistently observed following both ingestion and inhalation, including those of the central nervous system (brain and/or spinal cord), ear canal, gastrointestinal tract and mammary glands. In almost all adequate bioassays, there have been reported increases in astrocytomas of the brain and spinal cord following inhalation (Quast et al., 1980b), ingestion in drinking water (Quast et al., 1980a; Bio/Dynamics Inc., 1980a, 1980b) and by gavage (Bio/Dynamics Inc., 1980c). These tumours, for which dose–response trends are clear and which occur consistently at highest incidence across studies, are rarely observed spontaneously in experimental animals. Astrocytomas of the brain and spinal cord have sometimes been reported at non-toxic doses or concentrations and

[1] Preliminary results of an NTP carcinogenesis bioassay indicate that acrylonitrile is also carcinogenic in mice exposed by gavage (NTP, 1998).

at periods as early as 7–12 months following the onset of exposure. Tumours have also been observed in exposed offspring of a multigeneration reproductive study at 45 weeks (Litton Bionetics Inc., 1980).

Acrylonitrile is metabolized primarily by two pathways: conjugation with glutathione to form N-acetyl-S-(2-cyanoethyl)cysteine and oxidation by cytochrome P-4502E1 (Sumner et al., 1999) to form remaining urinary metabolites (Fennell et al., 1991; Kedderis et al., 1993a). Oxidative metabolism of acrylonitrile leads to the formation of 2-cyanoethylene oxide, which is either conjugated with glutathione (Fennell and Summer, 1994; Kedderis et al., 1995) to form a series of metabolites including cyanide and thiocyanate or directly hydrolysed by epoxide hydrolase (Kim et al., 1993; Borak, 1992).

Available data[2] are consistent with conjugation with glutathione being the major detoxification pathway of acrylonitrile, while the oxidation of acrylonitrile to 2-cyanoethylene oxide can be viewed as an activation pathway, producing a greater proportion of the total metabolites in mice than in rats.

Based on studies in which 2-cyanoethylene oxide has been administered, there is no indication of preferential uptake or retention in specific organs, including the brain (Kedderis et al., 1993b).

It has been hypothesized that acrylonitrile induces brain tumours through metabolically generated reactive oxygen species which cause damage to DNA. The hypothesized mode of action is not well additionally defined, though oxidation to 2-cyanoethylene oxide by cytochrome P-4502E1 is a likely requisite initial step. Available data indicate that the events preceding hypothesized oxidative damage to DNA appear not to involve significant disruption of antioxidant defenses of cytotoxic effects resulting in lipid peroxidation. Generated free radicals may be related to the release of cyanide in oxidative metabolism.

B. Evidence in Animals/Key Events

Key Events: Strength, Consistency, Specificity, Dose-Response, Temporal Association, Biological Plausibility and Coherence

The hypothesis that acrylonitrile induces brain tumours through metabolically generated reactive oxygen species which cause damage to DNA is based in part on a number of incompletely reported *in vitro* studies, which indicate that free radicals (.OH, O_2.) and H_2O_2 generated perhaps in part by the release of cyanide, may be directly implicated in the oxidation of ACN and DNA damage (El-zahaby *et al.*, 1996, abstract; Mohamadin *et al.*, 1996, abstract; Ahmed *et al.*, 1996, abstract; Ahmed and Nouraldeen, 1996, abstract).

In more recent *in vitro* investigations, the results of which have also been presented incompletely at this time, Prow *et al.* (1997; abstract) reported that ACN inhibited gap junctional intercellular communication in a rat astrocyte cell line in a dose dependent manner, possibly through an oxidative stress mechanism (Kamendulis et al., 1999a). Similarly, Zhang *et al.* (1998; abstract) concluded that oxidative stress contributed to morphological transformation in Syrian hamster

[2] Including results of short-term toxicity studies in which the oxidative pathway has been induced prior to administration with acrylonitrile or antioxidants have been administered concomitantly with acrylonitrile

embryo cells, assayed with and without an antioxidant (Zhang et al., 2000a, in press). Jiang *et al.* (1998; abstract) reported oxidative damage (indicated by presence of 8-hydroxy-2'-deoxyguanosine) at all concentrations tested in a rat astrocyte cell line and Zhang et al. (2000b) reported morphological transformation of Syrian Hamster Embryo (SHE) cells by 8-hydroxy-2'-deoxyguanosine.

In vivo data relevant to consideration of consistency of tumours with observations of key events are restricted to those from short term studies in which effects of ACN on levels of GSH, reactive oxygen species, oxidative DNA damage and measures of oxidative stress have been investigated. Studies of the association between the degree of metabolism to the putatively active metabolite, subsequent measures of reactive oxygen species or oxidative DNA damage and tumours are not available. In cancer bioassays, tumours of the brain occurred at periods as early as 7 to 12 weeks following exposure; however, information on progression of the lesions is sparse since in very few of the early bioassays were there interim kills, and none included investigations of reversibility.

Following exposure of Sprague-Dawley rats to 0 or 100 ppm ACN in drinking water for 2 weeks, levels of GSH and reactive oxygen species in brain and liver and levels of 8-hydroxy-2'-deoxyguanosine (indicative of oxidative DNA damage) and activation of NF-$_{\kappa}$B (a transcription factor strongly associated with oxidative stress) in several tissues were determined. Concentrations of GSH in brain were decreased (Jiang et al. 1997; abstract).

Whysner *et al.* (1998a) reported no effects on concentrations of GSH in the brain of male Sprague-Dawley rats exposed to 3, 30 or 300 ppm ACN in drinking water for 3 weeks, though there was a significant increase in levels of 8-oxodeoxyguanosine in brain nuclear DNA at the two highest doses (NF-$_{\kappa}$B was also activated). In the liver, concentrations of nuclear DNA 8-oxodeoxyguanosine were also significantly increased at the two highest doses (Whysner et al., 1998a). In a bioassay with comparable dose levels, the incidence of brain and/or spinal cord tumours was significantly increased in male Sprague-Dawley rats exposed to 35 ppm (3.4 mg/kg-bw per day) ACN and higher for two years (Quast et al., 1980a).

In male F344 rats exposed for 21 days to 0, 1, 3, 10, 30 or 100 ppm ACN in drinking water, there were no significant differences between groups in levels of 8-oxodeoxyguanosine in the brain (Whysner et al., 1997). (Note the contrast with reported results in Sprague-Dawley rats where there was an increase in 8-oxodeoxyguanosine in brain at 30 and 300 ppm for 3 weeks).

In male Sprague-Dawley rats exposed for up to 94 days to 0 or 100 ppm in drinking water, concentrations of 8-oxodeoxyguanosine in brain nuclear DNA were significantly increased after three, 10 and 94 days of exposure (Whysner et al., 1998a). The endpoint for which changes were consistently observed in male Sprague-Dawley rats was the induction of oxidative DNA damage, including the accumulation of 8-oxodeoxyguanosine in the brain. The authors drew correlations between these results and the incidence of brain/spinal cord tumours that had been reported in carcinogenicity bioassays in which male Sprague-Dawley rats were exposed to ACN via drinking water. In the two-year drinking water bioassay with male Sprague-Dawley rats (Quast et al., 1980a), the incidence of brain and/or spinal cord tumours was significantly increased at 100 ppm (8.5 mg/kg-bw per day).

Increased levels of 8-oxodeoxyguanosine occur only in the anterior portion of the brain, which contains rapidly dividing glial cells (Whysner et al., 1998b).

Increases in brain tumours have been dose-related and observed at concentrations as low as 30 ppm in drinking water (equivalent to approximately 1 mg/kg bw/day). However, in shorter term studies, in one strain but not in another where brain tumours were also observed, there were increases in 8-oxodeoxyguanosine in the brain (Whysner et al., 1998a; 1998b). Though there was a dose-response relationship in a 21 day study for increases in 8-oxodeoxyguanosine in the brain of Sprague-Dawley rats at concentrations similar to those at which astrocytomas were observed in this strain of rats in carcinogenesis bioassays (Quast et al., 1980a), there was no such dose-response for F344 rats, at doses at which brain tumours were observed in long term studies (Biodynamics, 1980b).

In shorter term mechanistic studies, therefore, exposure to acrylonitrile has been associated with the accumulation of 8-oxodeoxyguanine in the DNA isolated from brain tissue, presumably via the action of reactive oxygen species generated during its metabolism. However, the predicted greater sensitivity of Sprague-Dawley rats versus Fischer 344 rats on the basis of determination of 8-oxodeoxyguanosine levels in brain in shorter term studies is not borne out by the results of carcinogenicity bioassays reported previously.

Moreover, acrylonitrile (and particularly the active metabolite 2-cyanoethylene oxide) has been mutagenic and produces DNA adducts in relevant assays (See "Other Modes of Action"). Therefore, direct interaction with DNA rather than indirect oxidative damage may be the critical key event.

Alternate Modes of Action

Potentially, acrylonitrile may induce brain tumours through direct interaction with DNA.

Acrylonitrile is weakly mutagenic in bacterial assays. However, the database on mutagenicity in mammalian cells *in vitro* and *in vivo* is inadequate as a basis of assessment because of limitations of the studies. Results of the few identified investigations in which the relative potency of acrylonitrile was compared to that of the epoxide metabolite are consistent with the oxidative pathway of metabolism being critical in genotoxicity.

Binding of 2-cyanoethylene oxide to nucleic acids has also been reported in *in vitro* studies at high concentrations (Hogy and Guengerich, 1986; Solomon and Segal, 1989; Solomon et al., 1993; Yates et al., 1993, 1994[1]). The formation of acrylonitrile-DNA adducts is increased substantially in the presence of metabolic activation. Under non-activating conditions involving incubation of calf thymus DNA with either acrylonitrile or 2-cyanoethylene oxide *in vitro*, 2-cyanoethylene oxide alkylates DNA much more readily than acrylonitrile (Guengerich et al., 1981; Solomon et al., 1984, 1993). Incubation of DNA with 2-cyanoethylene oxide yields 7-(2-oxoethyl)-guanine (Guengerich et al., 1981; Hogy and Guengerich, 1986; Solomon and Segal, 1989; Solomon et al., 1993; Yates et al., 1993, 1994) as well as other adducts. Compared with studies with rat liver microsomes, little or no DNA alkylation by acrylonitrile was observed with rat brain microsomes (Guengerich et al., 1981). DNA alkylation in human liver microsomes was much less than that observed with rat microsomes (Guengerich et al., 1981); though there was no glutathione S-

[1] Yates et al. (1994) also reported single and double strand breaks in plasmid DNA incubated with 2-cyanoethylene oxide.

transferase activity cytosol preparations from human liver exposed to acrylonitrile, there was some activity for 2-cyanoethylene oxide (Guengerich et al, 1981).

In *in vivo* studies in F344 rats administered 50 mg acrylonitrile/kg-bw intraperitoneally, 7-(2-oxoethyl)-guanine adducts were detected in liver (Hogy and Guengerich, 1986). Incorporation of acrylonitrile into hepatic RNA was observed following intraperitoneal administration to rats (Peter et al., 1983). However, no DNA adducts were detected in the brain, which is the primary target for acrylonitrile-induced tumorigenesis, in this or a subsequent study in which F344 rats received 50 or 100 mg acrylonitrile/kg-bw by subcutaneous injection (Prokopczyk et al., 1988). In contrast, in three studies from one laboratory, exposure of SD rats to 46.5 mg [^{14}C]acrylonitrile/kg-bw (50 µCi/kg-bw) resulted in apparent binding of radioactivity to DNA from liver, stomach, brain (Farooqui and Ahmed, 1983), lung (Ahmed et al., 1992a) and testicles (Ahmed et al, 1992b). In each tissue, there was a rapid decrease in radioactivity of DNA samples collected up to 72 hours following treatment.

It is not clear why acrylonitrile–DNA binding was detected in the brain in these studies and not by Hogy and Guengerich (1986) or Prokopczyk et al. (1988). The DNA isolation protocols and method for correction for contaminating protein in the DNA sample used by Hogy and Guengerich (1986) may have allowed a more stringent determination of DNA-bound material. Alternatively, the methods used to achieve greater DNA purity might have caused the loss of adducts or inhibited the recovery of adducted DNA. However, more likely, 7-oxoethylguanine and cyanoethyl adducts are of little consequence in induction of ACN-induced brain tumours and investigation of the role of cyanohydroxyethylguanine and other adducts in induction of these tumours is warranted**.**

Therefore, while the role of mutagenesis and the primary mutagenic lesion induced by acrylonitrile in carcinogenesis are uncertain, acrylonitrile–DNA adducts (in particular, 7-(2-oxoethyl)-guanine) can be induced *in vitro* and in the liver *in vivo*, although at levels considerably less than those associated with, for example, ethylene oxide. However, when measures were taken to eliminate contamination of samples by adducted protein and unbound acrylonitrile, acrylonitrile–DNA adducts were not detected in the brain. This is in contrast to observations for ethylene oxide, which is also associated with gliomas of the brain. If the methods used to achieve greater DNA purity did not cause the loss of adducts or inhibit the recovery of adducted DNA, this suggests that acrylonitrile-induced DNA damage and mutagenicity may occur by a mechanism other than the formation of acrylonitrile–DNA adducts. Alternatively, they may be associated with an uninvestigated adduct (e.g., cyanohydroxyethyl adducts, another putatively critical adduct in brain has never been investigated).

Moreover, several aspects of tumour development are characteristic of those induced by compounds or metabolites which interact directly with DNA. Tumours are systemic and occur at multiple sites in both sexes following both inhalation and ingestion sometimes at non-toxic doses or concentrations and at periods as early as 7 to 12 months following onset of exposure. The ratio of benign to malignant tumours is small.

C. Conclusion - Assessment of Postulated Modes of Action in Animals and Statement of Confidence

Available data are insufficient to support a consensus view on a plausible mode of action for acrylonitrile-induced brain tumours other than through direct interaction with DNA. While there is some indication in ongoing studies many of which have not been fully reported, that oxidative damage to DNA may play a role, available data are inadequate as a basis for delineation of a plausible sequence of events leading to cancer. For example, the origin of the oxidative damage is unclear. *In vivo* data on potential key events to serve as a basis for consideration, of the weight of evidence for the hypothesized mode of action are also limited, being restricted to those in several strains of animals exposed for 21 days. Moreover, the pattern of results of these studies is inconsistent with that in the cancer bioassays, based on the hypothesized mode of action. The predicted greater sensitivity of Sprague-Dawley versus Fischer rats on the basis of results of shorter-term studies in which 8-oxodeoxyguanine levels in brain have been determined is not borne out by the carcinogenesis bioassays.

With respect to a potential mode of induction of tumours involving direct interaction with DNA, there is evidence for the genotoxic potential of acrylonitrile *in vitro*, inadequate data *in vivo* and insufficient data on acrylonitrile-DNA adducts in the brain, though such adducts can be induced in the liver *in vivo*.

Hence, while there is some indication in ongoing studies in animals that oxidative damage to DNA may play a role in induction of tumours, the origin of the oxidative damage is unclear. Moreover, the pattern of results of studies of oxidative damage is inconsistent with relative sensitivities of various strains to induction of tumours based on the hypothesized mode of action.

II. Are Key Events in the Animal MOA Plausible in Humans?

Section not relevant in this case, since weight of evidence for postulated mode of action for carcinogenesis in animals is inadequate

III. Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans

Section not relevant in this case, since weight of evidence for postulated mode of action for carcinogenesis in animals is inadequate.

IV. Statement of Confidence; Analysis; Implications

This case represents one in which the weight of evidence for the hypothesized mode of action (i.e., through metabolically generated reactive oxygen species which cause damage to DNA) of the principal tumour type (i.e., astrocytomas of the brain) in animals is inadequate. In the context of the framework, then, human relevance for the hypothesized mode of action is not considered further and available data are insufficient to support deviation from default in dose-response analyses.

Though only one type of tumour was considered for purposes of this case study, for each of the additional tumours observed in animals following exposure to

acrylonitrile, weight of evidence for a hypothesized mode of action would need to be considered additionally but separately.

It should be noted, however, that while there have been reported increases in astrocytomas of the brain and spinal cord in almost all adequate bioassays in rats exposed to acrylonitrile, increases in specific cancers have not been consistently observed in epidemiological studies of occupationally exposed populations. Although there was some evidence in primarily early limited studies of excesses of lung cancer (Thiess et al., 1980), "all tumours" (Zhou and Wang, 1991) and colorectal cancer (Mastrangelo et al., 1993), there have been no consistent excesses of cancer in four recent well-conducted and well-reported epidemiological studies of occupationally exposed populations (Benn and Osborne, 1998; Blair et al., 1998; Swaen et al., 1998; Wood et al., 1998). However, a non-significant excess of lung cancer was noted in the most highly exposed quintile in the statistically most powerful investigation (Blair et al., 1998). A large deficit in cancer in one cohort in comparison with national rates also suggests an underreporting of cause of death (Wood et al., 1998). The power to detect moderate excesses was also small for some sites (stomach, brain, breast, prostate, lymphatic/hematopoietic) because of small numbers of expected deaths. (For example, the upper 95% confidence limits on the SMRs for brain cancer in the only recent cohort study in which it was reported (Swaen et al., 1998) was 378, indicating that an almost 400% excess could not be excluded; the lower 95% confidence limit was 64).

Unfortunately, the results of these epidemiological studies cannot contribute significantly to hazard characterization or dose-response analyses for acrylonitrile in part due to lack of a plausible mode of induction of tumours observed in the animal studies. Meaningful contribution of the observations is precluded by the relative paucity of data on exposure of workers in the relevant investigations and the wide range of the confidence limits on the SMRs for rare tumours whose relevance cannot be precluded due to lack of understanding of the mode of induction of tumours in animals. This observation has generic implications beyond this particular case study - i.e., that results of negative epidemiological studies are rarely informative in the context of hazard characterization or dose-response analyses, particularly where there is no plausible mode of induction of tumours, for which appropriate biomarkers of effect have been examined.

Data from animal studies consistently support that oxidation of acrylonitrile to 2-cyanoethylene oxide is likely a critical activation pathway in induction of tumours. Dose–response might optimally be expressed, therefore, in terms of amounts or rates of formation of reactive metabolites produced per volume of tissue in the critical organ.

Based on studies in microsomes, the liver is the major site of formation *in vivo* of 2-cyanoethylene oxide in rats, mice and humans and studies with inhibitory antibodies in human hepatic microsomes indicate that the 2E1 isoform of cytochrome P-450 is primarily involved in epoxidation (Guengerich et al., 1991; Kedderis et al., 1993a).

Studies in subcellular hepatic fractions indicate that there is an active epoxide hydrolase pathway for 2-cyanoethylene oxide in humans, which is inactive, although inducible, in rodents (Kedderis and Batra, 1993). A physiologically based pharmacokinetic model has been developed and verified for the rat (Gargas et al., 1995; Kedderis et al., 1996), and work is under way to scale it to humans. In a recent, although incompletely reported study, Kedderis (1997) estimated *in vivo*

activity of epoxide hydrolase in humans based on the ratio of epoxide hydrolase to P-450 activity in subcellular hepatic fractions multiplied by the P-450 activity *in vivo*. Human blood to air coefficients for acrylonitrile and 2-cyanoethylene oxide have also been recently determined, although incompletely reported at present (Kedderis and Held, 1998). Research is in progress to determine partition coefficients for other human tissues.

CASE STUDY 6

MODE OF ACTION: SUSTAINED CYTOTOXICITY AND CELLULAR REGENERATION

Kidney and Liver Tumours Associated with Chloroform Exposure

M.E. Meek, Health Canada

Introduction

Sustained cytotoxicity and regenerative cell proliferation are key events in the hypothesized modes of action for chemical induction of a range of animal tumors. The example included here is chloroform, which causes liver and kidney tumours in mice and kidney tumours in rats. This case presents an analysis involving several animal tumor types for which chemical-specific data relevant to assessment of the weight of evidence of the hypothesized mode of action is considerable. Although the weight of evidence varies, chemical-specific data for key events in the mode of action for formation of these tumors is available from animal studies and qualitative and quantitative analyses supports, with few exceptions, the potential applicability of the animal mode of action in humans. Subject to uncertainties outlined below, the overall weight of evidence indicates that these animal tumors are relevant and useful for evaluating human risk.

I. Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

A. Postulated Mode of Action

The hypothesized mode of action for chloroform-related liver and kidney tumours in mice and rats is similar and finds support in histopathologic and metabolic data from several different sources. However, the weight of evidence varies considerably, and, as a result, these tumours are addressed separately here. There is considerable information available concerning the potential mode of induction of these tumours by chloroform. This includes a range of metabolic studies. In addition, while there have been no cancer bioassays in which a range of intermediate endpoints has been investigated, proliferative response in target organs has been examined in numerous subsequent investigations following exposure via regimens similar to those in the long-term studies. The histopathology in the target organ for one of the more critical studies has also been reexamined (Hard *et al*., in press). These data have been generated to investigate primarily the hypothesized mode of action for tumour induction in rodents that requisite precursor steps to cancer are 1) generation of

phosgene/HCL by cyp2E1, 2) sustained cytotoxicity and 3) subsequent persistent regenerative cell proliferation.

B. Evidence in Animals/Key Events

Metabolism to phosgene, resulting from the oxidative pathway that predominates at low exposures, is believed to be the principal determinant of sustained toxicity and resulting persistent proliferation that is hypothesized to lead to a higher probability of spontaneous cell mutation and subsequent cancer.

Available data indicate that the toxicity of chloroform is attributable to metabolites. In the liver, for example, both the incidence and severity of toxicity correlate with the level of covalent binding of chloroform metabolites to tissue macromolecules, and phosgene is believed to be quantitatively responsible for the irreversible binding of chloroform metabolites to liver components (Pohl *et al*., 1980). The extent of chloroform-induced hepatic necrosis also correlates with the extent of covalent binding to protein in male and female rats and in male mice (Ilett *et al*., 1973; B.R. Brown *et al*., 1974). This covalent binding is more prevalent within the areas of necrosis (Ilett *et al*., 1973; Tyson *et al*., 1983), and the association of metabolism with toxicity is further supported by localization of binding to necrotic lesions (Ilett *et al*., 1973). The results of *in vitro* studies are consistent, in that irreversible binding to macromolecules in rat and human liver microsomes requires prior metabolism (Cresteil *et al*., 1979).

Increased covalent binding of chloroform metabolites in the liver also occurs when glutathione is depleted, while some degree of protection is conferred if glutathione or a precursor is administered (Stevens and Anders, 1981). Since covalent binding of a chloroform metabolite with glutathione precedes and becomes maximal prior to the chloroform-induced hepatic cytotoxicity, depletion of glutathione may contribute to the observed cytotoxicity as it does to covalent binding (Stevens and Anders, 1981).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis (Ilett *et al*., 1973; Smith and Hook, 1983, 1984). Strain- and sex-related differences in sensitivity of mice to nephrotoxicity are also correlated with the ability of the kidney to metabolize chloroform (Taylor *et al*., 1974; Clemens *et al*., 1979; Pohl *et al*., 1984; Smith *et al*., 1984; Mohla *et al*., 1988; Henderson *et al*., 1989; Hong *et al*., 1989). In an investigation in F344 rats, however, it was concluded that intrarenal bioactivation of chloroform by cytochrome P450 did not appear to play a major role in nephrotoxicity (Smith *et al*., 1985).

The primary, if not only, enzyme catalysing metabolism at low concentrations of chloroform is cytochrome P4502E1 (CYP2E1) (Brady *et al*., 1989; Guengerich *et al*., 1991).

Regional distribution of lesions in the liver of rats and mice also correlates well with the hepatic distribution of CYP2E1 and glutathione. The highest concentrations of CYP2E1 in both uninduced and induced rat and human liver are present in the centrilobular region (Ingelman-Sundberg et al., 1988; Tsutsumi et al., 1989; Johansson et al., 1990; Dicker et al., 1991). In comparison, concentrations of the phosgene-scavenging agent glutathione in the centrilobular region are only about half those in the periportal region (Smith et al., 1979).

Measures of cytotoxicity include histopathological effects and release of hepatic enzymes and labelling indices as surrogates for regenerative cell proliferation.

Key Events: Dose-Response Relationship/Temporal Association

In all cases where examined, sustained cytotoxicity and cellular proliferation were observed in the liver and kidney of the same strain of mice and rats exposed in a similar manner in short-term studies to concentrations or doses that induced tumours in these organs in cancer bioassays. However, the converse is not always true. Tumours have sometimes not been observed in cases where there have been sustained increases in damage and resulting proliferation in the same strain exposed to similar concentrations in the same manner in shorter-term studies, namely kidney lesions in B6C3F1 mice and F344 rats. These results are consistent with the hypothesis that, where chloroform causes tumours, toxicity and reparative hyperplasia are obligatory precursor steps. Tumours would not necessarily be expected whenever there is an increase in cell replication. The multiple susceptibility factors that produce tumours following cytotoxicity will depend on tissue-specific factors and will likely vary between species and strains. For example, in spite of the overt toxicity and sustained increased cell proliferation in the epithelial tissue of the nose in both rats and mice, no tumours have been noted in this tissue in any chronic studies, including the inhalation bioassay in which nasal tissues were carefully evaluated (Yamamoto, 1996).

Strength, Consistency, Specificity of Association of Tumour Response with Key Events:

Liver Tumours – Mice. Liver tumours are observed in B6C3F1 mice following administration of bolus doses by gavage in corn oil (NCI, 1976), but not following administration of the same daily doses in drinking water (Jorgenson et al., 1985). That dose rate is a critical determinant of tissue damage (e.g., being greater following bolus dosing by gavage compared with continuous administration) is consistent with the proposed mode of induction of tumours, with higher bolus doses leading to tissue damage. Doses at which tumours have been observed following administration in corn oil in the cancer bioassay are associated in shorter-term studies with sustained proliferative response in the liver of the same strain exposed similarly (Larson et al., 1994c; Pereira, 1994; Melnick et al., 1998). Sustained increases in proliferative response have not been observed following ingestion in drinking water of concentrations that did not induce increases in hepatic tumour incidence in the long-term bioassay (Larson et al., 1994a).

The incidence and severity of hepatic necrosis in the mouse liver have been related to the degree of covalent binding of chloroform metabolites to tissue proteins. The linking of metabolism to toxicity is underscored by localization of covalent binding to the necrotic lesions and the predictable variations in toxic response produced by pretreatment with inducers or inhibitors of cytochrome P450-mediated metabolism, specifically CYP2E1. There is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse liver. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative

metabolites) and protein binding in the liver of the strain of mice in which tumours have been observed (Ade *et al*., 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse liver tumours is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the liver of two strains of CYP2E1 null mice (Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan *et al*., 1999). There is a consistent association between CYP2E1 distribution, chloroform metabolism, pattern of covalent tissue binding and toxic injury to hepatocytes in mice.

Evidence of concordance between metabolism to reactive intermediates, sustained cytotoxicity, persistent regenerative proliferation and tumour development in the mouse liver is, therefore, very strong. Indeed, there is a wealth of information that indicates a relationship between sustained enhanced proliferative response and induction of liver neoplasia in the strain in which tumours have been observed (B6C3F1 mice).

Renal Tumours – Mice. Chloroform also induces renal tumours in BDF1 mice following inhalation (Yamamoto, 1996) and in ICI mice exposed by gavage in toothpaste (Roe *et al*., 1979), although at lower rates than liver tumours. The response is strain and sex specific, occurring only in males.

Evidence of concordance between metabolism to reactive intermediates, cytotoxicity, regenerative proliferation and tumour development in the mouse kidney, although strong, is not as robust as for the mouse liver, due primarily to the more limited data available on sustained enhanced proliferative response in the strains in which tumours have been observed. Indeed, this is limited to a single study in BDF1 mice, in which there was an increase in labelling index in the kidneys of males but not females at concentrations that induced renal tumours in this strain in the long-term inhalation bioassay (Templin *et al*., 1996c; Yamamoto, 1996). The available data concerning the relationship between sustained cellular proliferation and induction of renal tumours in another strain (B6C3F1) of mice indicate that sustained proliferative response is not always associated with tumours. In this strain, in shorter-term studies, there were sustained proliferative responses at doses at which kidney tumours were not observed in the relevant cancer bioassays following exposure by both gavage in corn oil and drinking water (NCI, 1976; Jorgenson *et al*., 1985; Larson *et al*., 1994a,c).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis, with strain and sex differences in sensitivity to nephrotoxicity being correlated with the ability of the kidney to metabolize chloroform. Similar to the liver, there is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse kidney. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative metabolites) and protein binding in the kidney of DBA/2J mice (Ade *et al*., 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse renal tumours is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the kidney of two strains of CYP2E1 null mice (Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan *et al*., 1999).

Renal Tumours – Rat. The weight of evidence for the hypothesized mode of induction of tumours in the rat kidney is considerably less than that for the mouse liver and kidney due primarily to limited data on intermediate endpoints in the only strain (Osborne-Mendel) in which increases in kidney tumours have been observed. These increases have been reported following exposure via both gavage in corn oil and drinking water (NCI, 1976; Jorgenson *et al*., 1985). There are also few identified data on the relationship between the metabolism of chloroform and induction of renal lesions in rats. In the F344 rat, there were sustained increases in proliferative response in shorter-term studies following administration of doses similar to those that induced tumours in Osborne-Mendel rats following administration by gavage in corn oil but not following ingestion in drinking water (Larson *et al*., 1995a,b). However, there are no bioassays in this strain following ingestion for direct comparison with these results. Sustained increases in labelling index were observed in the proximal tubules of F344 rats exposed to daily doses of 30 ppm (147 mg/m^3) and greater and at 90 ppm (441 mg/m^3) and greater at 5 days per week (Templin *et al*., 1996b). However, increases in kidney tumour incidence were not observed in this strain exposed to up to 90 ppm (441 mg/m^3) for 5 days per week in the only inhalation cancer bioassay (Yamamoto, 1996).

Based on studies conducted primarily in F344 rats in which tumours have not been observed, a mode of action for carcinogenicity in the kidney observed in the carcinogenesis bioassay in Osborne-Mendel rats based on sustained cytotoxicity and persistent tubular cell regeneration is, therefore, plausible. For Osborne-Mendel rats, the results of reanalyses of the original renal tissues (Hard and Wolf, 1999; Hard *et al*., in press), from both the drinking water bioassay (Jorgenson *et al*., 1985) and the gavage study (NCI, 1976), have been critical. They provide strong support for the contention that the mode of induction of these tumours is consistent with the hypothesis that sustained proximal tubular cell damage is a requisite precursor lesion for chloroform-induced tumours.

Biological Plausibility and Coherence of the Database

The organs in which chloroform-induced cytotoxicity and proliferative lesions are observed (liver, kidney and nasal passages) correlate well with the distribution of CYP2E1 both across and within species (Löfberg and Tjälve, 1986). This consistent pattern of response to chloroform across species and organs supports a conclusion that chloroform-induced neoplasia is dependent on sustained cytotoxicity coupled with persistent regenerative cell proliferation. This is further supported by the considerable weight of evidence indicating that chloroform is not genotoxic, with unconvincing evidence for direct DNA reactivity. Due principally to limitations of the available data, though, weak genotoxicity in the rat cannot be precluded, which detracts somewhat from the weight of evidence in this species, although it is unknown whether this might be a result of secondary effects on DNA.

The hypothesized mode of carcinogenesis for chloroform is in keeping with the growing body of evidence supporting the biological plausibility that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis. This has been addressed in numerous articles, including Ames and Gold (1990, 1996), Cohen and Ellwein (1990, 1991, 1996), Preston-Martin *et al*. (1990), Ames *et al*. (1993), Tomatis (1993), Cohen (1995), Cunningham and Matthews (1995), Butterworth (1996), Farber (1996) and Stemmermann *et al*. (1996). Enhanced cell

proliferation can lead to an increased frequency of spontaneous genetic damage either through errors that result from the infidelity of DNA replication or through the increased conversion of endogenous DNA changes into heritable genetic changes (Cohen and Ellwein, 1990, 1991, 1996; Ames *et al*., 1993; Cohen, 1995). Additionally, during periods of cell replication, heritable non-mutagenic modifications of the genome may occur that may lead to changes in gene expression, contributing to carcinogenesis (U.S. EPA, 1996b). This view that cell proliferation is a risk factor for carcinogenesis is not universally accepted, because strict correspondence between increased cell turnover and carcinogenic response is not always demonstrable (Melnick, 1992; Farber, 1996). However, as indicated above, in view of the complex interplay of factors involved in the carcinogenesis process, it is not surprising that acute measures of cell proliferation do not always indicate a one-to-one correlation. Among the factors to be considered are the kinetics of DNA adduct formation and repair, the balance between cell proliferation, differentiation and death, proliferation in the target cell compartments compared with that of non-target cells and the consequences of overt tissue toxicity.

Alternate Modes of Action

While the evidence is fairly convincing that chloroform acts principally through cytotoxic effects of phosgene and other products of oxidation, other possibilities involve mutagenicity. One possibility is that the effects of chloroform are a composite of metabolites from both oxidative and reductive pathways contributing to toxicity and carcinogenicity. However, several observations strongly support the predominant role of oxidative pathways in chloroform toxicity and make any significant role of reductive metabolism highly unlikely. Firstly, the macromolecular binding following administration of chloroform represents only a very small portion of the delivered dose. Secondly, the mechanisms of action related to the nature of the necrotic lesion, the time course of injury after single doses and the differences in cumulative damage on multiple exposures are very different for chloroform and carbon tetrachloride, the latter a compound for which the free radical (reductive) pathway is causative for toxicity. In addition, carbon tetrachloride, which is largely metabolized to a free radical, is not itself mutagenic. Based on these considerations, it was concluded that free radicals do not play a significant role in the toxicity or carcinogenicity of chloroform.

Another possibility is that minor pathways, associated with glutathione conjugation, produce mutagenic metabolites, as is believed to be the case for dichloromethane. However, there is little evidence for a significant direct conjugation pathway for chloroform. In studies with *Salmonella* tester strains with glutathione transferase T1-1 inserted into the bacterial genome and expressed during testing, a small increase in mutagenic activity (less than a factor of 2) was noted for chloroform at very high doses, even though positive controls with methylene chloride and bromochloromethane produced much larger responses (Pegram *et al*., 1997). Neither of these potential modes of action is believed to play a significant role in the observed toxicity and carcinogenicity of chloroform, although further investigation of weak genotoxicity in the rat is desirable.

C. Conclusion - Assessment of Postulated Modes of Action in Animals and Statement of Confidence:

In summary, then, chloroform has induced liver tumours in mice and renal tumours in mice and rats. The weight of evidence -- genotoxicity, sex and strain specificity and concordance of sustained cytotoxicity, persistent regenerative proliferation and tumours -- is consistent with the hypothesis that marked cytotoxicity concomitant with a period of sustained cell proliferation likely represents a secondary mechanism for tumor induction following exposure to chloroform. This is consistent with a non-linear dose–response relationship for induction of tumours. This cytotoxicity is primarily related to oxidation rates of chloroform to reactive intermediates, principally phosgene and hydrochloric acid. The weight of evidence for this mode of action is strongest for hepatic and renal tumours in mice and more limited for renal tumours in rats.

The evidence that supports an obligatory role of sustained cytotoxicity in the carcinogenicity of chloroform is considerable. Indeed, there are few compounds for which the supporting database in this regard is as complete, consistent and cohesive as it is for chloroform. Although there are some uncertainties, the weight of evidence in this regard is strongest for hepatic and renal tumours in mice. The evidence is more limited for renal tumours in rats, primarily due to the relative paucity of data in strains where tumours have been observed, on metabolism and intermediate endpoints and the relationship between them. Uncertainty could be reduced by additional information on metabolism, cytotoxicity and proliferative response in the strain in which tumours were observed (i.e., Osborne-Mendel rats) following long-term exposure to chloroform. Additional data on metabolism and chronic (e.g., 2-year) cytotoxic/proliferative response in the kidneys of F344 rats could also contribute to greater confidence in the hypothesized mode of action.

II. Are Key Events in the Animal MOA Plausible in Humans?

A. Concordance Analysis - Key Events

The concordance tables (Tables 1 and 2) succinctly illustrate the nature and relative weight of evidence for the hypothesized mode of action of chloroform in humans as well as experimental species

Table 1. Concordance Analysis - Key Events - Animals and Humans - Liver Tumours

Key Event	Animals	Humans
Generation of phosgene/HCL by cyp2E1	incidence/severity of toxicity correlate with covalent binding of metabolites in rats and mice, more prevalent in necrotic lesions	Irreversible binding to macromolecules in human liver microsomes requires prior metabolism; PBPK model based on human physiological parameters and metabolic parameters *in vitro* in eight human liver samples

Key Event	Animals	Humans
cytotoxicity	In all cases where examined, sustained cytotoxicity (as measured by histopathological effects and release of hepatic enzymes) in the liver of mice at doses that induce tumours	Liver also a target organ in humans based on reports of effects associated with occupational exposure
regeneration/ proliferation	In all cases where examined, persistent regenerative proliferation (as measured by labelling indices) in the liver of mice at doses that induce tumours	No data
Tumours	Mice	Inadequate epidemiological data

In general, chloroform elicits the same symptoms of acute toxicity in humans as in experimental animals; target organs are also similar. For example, there have been infrequent reports of renal tubular necrosis and renal dysfunction resulting from the use of chloroform as an anesthetic (Kluwe, 1981). Liver toxicity due to occupational exposure to chloroform has also been reported at concentrations in the range of 80–160 mg/m^3 (with an exposure period of less than 4 months) in one study and in the range of 10–1000 mg/m^3 (with exposure periods of 1–4 years) in another study. The mean lethal oral dose for an adult is estimated to be about 45 g, but there are large inter-individual differences in susceptibility (WHO, 1994).

Table 2. Concordance Analysis - Key Events - Animals and Humans - Kidney Tumours

Key Event	Mice	Rats	Humans
Generation of phosgene/HCL by cyp2E1	in mice, strain and sex-related differences correlate with metabolism; necrosis correlates with the degree of covalent binding;	few such data for rats and in F344 rats, nephrotoxicity not correlated with bioactivition	Quantitation in. PBPK model based on human physiological parameters and activity in the microsomal fraction of kidneys to that in the microsomal fraction of the liver *in vitro* supported by data on metabolism of two known substrates of CYP2E1 by

Key Event	Mice	Rats	Humans
			microsomal fractions of the kidney and liver from 18 humans
cytotoxicity	in mice, in all cases where examined, sustained cytotoxicity (as measured by histopathological effects and release of hepatic enzymes), at doses that induced tumours;	in rats in critical bioassay, cytotoxicity based on histopathological reexamination	Kidney also a target organ in humans based on reports of renal effects resulting from anaesthetic use of chloroform
regeneraton/proliferation	In all cases where examined, persistent regenerative proliferation (as measured by labelling indices) in the kidney of mice at doses that induce tumours, though data less than for liver;	studies in rats restricted to those in a strain where tumours have not been observed	No data
Tumours		mice and rats	Inadequate epidemiological data

Available epidemiological data do not allow conclusions with respect to the potential carcinogenicity of chloroform in humans. Some reports indicating an association between exposure to disinfection byproducts (DPBs) in drinking water and increased risks of bladder cancer fulfill, in part, traditional criteria of causality. However, some inconsistencies in reported differences between men and women and between smokers and non-smokers are difficult to explain. Moreover, it is not possible to attribute these excesses specifically to chloroform (ILSI, 1997); indeed, due to the relative paucity of exposure information in relevant studies, the sources of increased relative risks are unclear. Specific risks may be due to other DBPs, mixtures of by-products, other water contaminants or other factors for which chlorinated drinking water or THMs may serve as a surrogate (WHO, in press).

The information summarized in the concordance tables leads to the conclusion that the weight of evidence for the hypothesized mode of induction of tumours (i.e., metabolism by the target cell population, induction of sustained cytotoxicity by metabolites and subsequent persistent regenerative cell proliferation) is greatest for liver and kidney tumours in mice, followed by kidney tumours in rats. Though data in humans are limited, based on expected similar response in humans and in the absence of data to the contrary, the mode of action for tumours in animals for chloroform is considered to be qualitatively applicable to humans. Available data

confirm that target organs in populations exposed occupationally to high concentrations are similar to those in experimental animals (i.e., the kidney and liver).

III. Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans,

Other data relevant to the human relevance of the hypothesized mode of action address quantitative variations in rates of metabolism to the putatively toxic metabolite in target organs. Quantitative variations in response between animals and humans for the hypothesized mode of induction of tumours are likely to be a function primarily of variations in rates of metabolism to phosgene in the target tissue. The rates of formation of reactive metabolites of chloroform (namely phosgene) in animals and humans have been estimated pharmacokinetically based on models that include specific parameters related to metabolic rates, enzyme affinities and enzyme tissue distribution. The most refined animal model features two-compartment absorption (stomach and intestinal tract) and subdivision of the liver and kidney compartments into regions of high and low metabolic activity (ILSI, 1997). This "hybrid" animal model has been revised and extended to humans (ICF Kaiser, 1999).

For the human model, the physiological and anatomical parameters were derived from Brown *et al*. (1997) with the exception of the ventilation rate and cardiac output, which were related to an assumed breathing rate of23 m^3/day (Health Canada, 1994). Liver tissue subvolumes were assumed to be the same as in the rat, based on Tsutsumi *et al*. (1989) and Buhler *et al*. (1992), while kidney was subdivided into a 70:30 cortex:non-cortex ratio as described by ICRP (1992). Human metabolic parameters were taken from Corley *et al.* (1990); these had been determined *in vitro* in eight human liver samples. Kidney rate constants were based on the relationship of activity observed in the microsomal fraction of kidneys to the activity observed in the microsomal fraction of the liver based on *in vitro* results reported by Corley *et al*. (1990) but supported by data on metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans (Amet *et al*., 1997).

Results from the human model were compared with data on total metabolized parent and exhaled chloroform reported by Fry *et al*. (1972) in an investigation in which chloroform was administered to male and female volunteers in olive oil or gelatin capsules. Exhaled chloroform was measured for up to 8 hours following exposure, and the total percentage of the dose exhaled unchanged was calculated by extrapolation to infinite time. Human model simulations conducted using a single-compartment description of oral uptake were closer to the observations of Fry *et al*. (1972) than those estimated using a multi-compartment description. Therefore, while a multi-compartment description was necessary in the rat model, a single-compartment description of oral uptake was used in estimating human equivalent concentrations.

Quantitative variations in delivered dose to the target organ predicted by the physiologically based pharmacokinetic model for dose-response analysis (i.e., kidney) for mice, rats and humans are consistent with the magnitude of difference expected based on species variations in metabolic rates.

IV. Statement of Confidence; Decision Analysis; Implications

There is a high degree of confidence in the weight of evidence for an obligatory role for cytotoxicity in the carcinogenicity of chloroform, including a non-linear dose-response relationship for tumor induction in animals. Because histopathologic effects, rather than biochemical effects such as increases in urinary enzymes, are the most sensitive indicator of damage, it is difficult to envisage other information on markers of effect that might reasonably be collected in additional study in humans. For example, scientists are unlikely to recommend biopsies for this purpose.

Although toxicokinetic data only are available for quantifying relative sensitivity to chloroform in humans, expected similarities in the mode of action in animals and humans give little reason to expect qualitative differences in the response of human tissues to this chemical. Quantitative variations in chloroform metabolism in animal and human target cell populations have been characterized in a physiological toxicokinetic model. Variations in metablic parameters, particular in the kidney for humans, had the greatest impact in the sensitivity analysis. Data from *in vitro* studies exposing target tissue from both rats and humans to the putatively toxic metabolite of chloroform could provide additional information on relative sensitivity.

If performed on tissues from a number of individuals, additional *in vitro* data on the metabolism of chloroform in the human kidney and liver would be useful not only to reduce uncertainty in these values, but, potentially to address the issue of variability across the human population, In particular, it would be desirable to clarify whether the same metabolic pathways contribute to the potential for cytotoxicity in rodents and humans, specifically with respect to CYP2 E1 and other P450 isozymes.

The case presents considerable evidence for an obligatory role for cytotoxicity in the carcinogenicity of chloroform, consistent with a non-linear dose–response relationship for induction of tumours in animals and by inference, in humans. Unfortunately, few compounds will have a supporting database that is as complete, consistent and cohesive for mode of action and human relevance analysis as chloroform. While qualitatively applicable to humans, quantitative variations in response between animals and humans for the hypothesized mode of action are likely due to variations in rates of metabolism to phosgene in the target tissue. These quantitative variations between species in metabolism of chloroform by the target cell population have been characterized in a physiological toxicokinetic model. For this model, among those parameters considered in the sensitivity analysis to have most impact on output, uncertainty was greatest for the metabolic parameters particularly in the kidney for humans.

For chloroform, the data for analyzying human relevance are principally compound specific, including kidney and liver toxicity following exposure at high levels during use of chloroform as an anaesthetic or following occupational exposure. Quantitative scaling to humans in the PBPK model was based on more generic information on physiological parameters in humans and human metabolic parameters *in vitro*, supported by metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans (Amet *et al*., 1997).

Although this case study focused on mode of action analysis, it also provides information useful for dose-response analysis. Specifically, if data on precursor events are inadequate, the incidence of obligatory non-cancer precursor events (cytotoxicity and sustained regenerative proliferation) from interim kills in the critical cancer bioassay or tumours, would be most useful for the dose-response analysis in a

risk assessment for chloroform. In addition, in view of the weight of evidence for the role of the oxidative metabolites of chloroform in the induction of requisite damage and resulting tumours, dose–response might optimally be expressed in terms of amounts or rates of formation of reactive metabolites produced per volume of tissue in the critical organ.

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APPENDIX

This Appendix provides abbreviated definitions for several concepts and terms used throughout this report. Figure A-1 is adapted from the 1983 NAS report.

Risk Assessment. The risk assessment/risk management paradigm (NRC, 1983, NRC, l994) describes risk assessment in terms of four distinct analyses with the "risk characterization" end product destined for used in decision-making. A diagram and relevant definitions appear below.

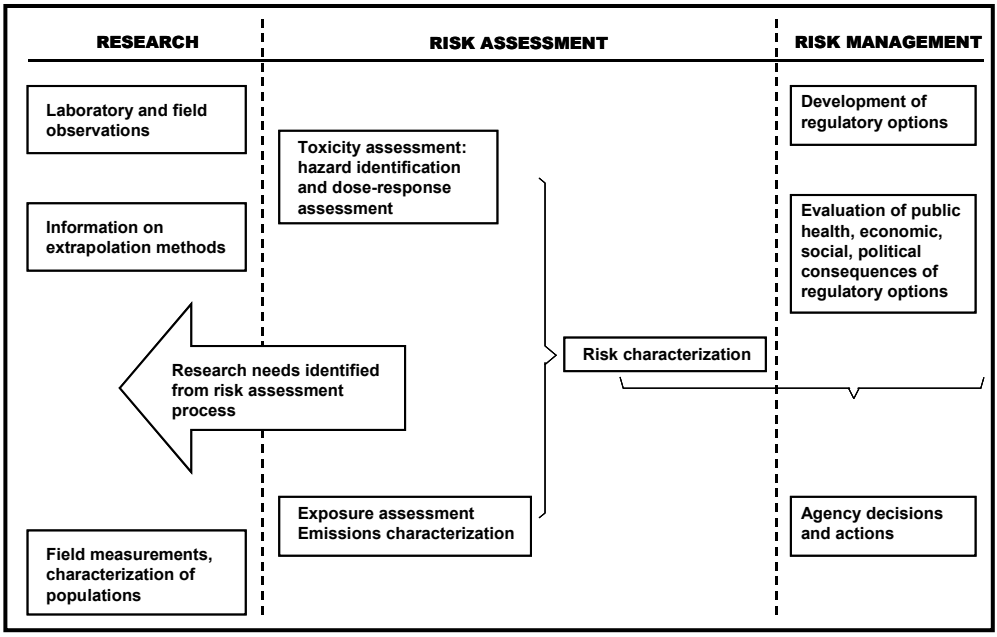

Figure A1. NRC Risk Assessment/Management Paradigm.

SOURCE: NRC, 1994.

Hazard Identification. The process of determining whether exposure to an agent can cause an increase in the incidence of a health condition such as cancer or birth defects. It involves describing the nature and weight of the evidence of causation. Many practitioners and guidance documents refer also to Hazard ***Characterization, t***he presentation of the weight of evidence on hazard and, if MOA data are available, the MOA for an endpoint (or spectrum of end-points) for, defining appropriate end-points for and approaches to, dose/concentration–response assessment.

Dose/Concentration–Response Assessment. The characterization of the relationship between the dose of an agent administered or received and the likelihood of an adverse effect.

Exposure Assessment. The qualitative and/or quantitative assessment of the nature, form and concentration of a chemical to which an identified population is exposed from all sources (air, water, soil, skin and diet)

Risk Characterization. The synthesis of data from exposure assessment, hazard identification and dose–response assessment into a summary that identifies clearly the strengths and weaknesses of the database, the criteria applied to the evaluation and validation of all aspects of methodology, uncertainties and data gaps, and conclusions reached, both qualitative and quantitative, including numerical risk values.

Toxicokinetics. How the body processes chemicals, including modeling and mathematical description of the interaction occurring at the interface of xenobiotics and tissues, as to such variables as similarities and differences in absorption, deposition, metabolism, elimination, and related parameters.

Toxicodynamics. How the body responds to chemicals, including differential sensitivity, modeling and mathematical description of the time course of disposition of xenobiotics in the whole organism.

Weight of Evidence. A systematic evaluation of factors bearing on the quality of all studies and other information, positive and negative, in the database under study.

Chapter 6

Categorical Default Uncertainty Factors - Interspecies Variation and Adequacy of Database

Meek, M. E. (2001)

Human & Ecological Risk Assessment 7: 157-163

Abstract

The potential development of categorical defaults for several components of uncertainty relevant to development of tolerable or reference concentrations/doses is considered - namely interspecies variation and adequacy of database. For the former, the adequacy of allometric scaling by body surface area as a species-specific default for oral tolerable or reference doses is considered. For the latter, the extent to which data from analyses of subchronic:chronic effect levels, Lowest Observed Adverse Effect Levels (LOAELs)/No Observed Adverse Effect Levels (NOAELs) and critical effect levels for complete versus incomplete datasets informs selection of defaults is examined. The relative role of categorical defaults for these aspects is considered in the context of the continuum of increasingly data-informed approaches to characterization of uncertainty and variability which range from default ("presumed protective") to "biologically-based predictive".

Key Words: uncertainty, variability, tolerable or reference dose/concentration, interspecies variation, body surface area scaling, adequacy of database, categorical default

Introduction

In considering the relative utility of categorical defaults in the development of tolerable or reference concentrations or doses, it is important that their potential contribution be understood in the context of a broader continuum of increasingly data-informed approaches to characterization of uncertainty and variability which range from default ("presumed protective") to more "biologically-based predictive". These approaches can be considered in the context of a range of options which increasingly incorporate more refined data to better inform selection of uncertainty or adjustment factors. In the most common cases, this involves incorporation of simple default values (often 10 x 10). Where data permit, categorical defaults, which permit more refinement through delineation of categories based on, for example, characteristics of the compounds themselves or of the species in which the critical effect has been determined as a basis of the reference or tolerable concentration or dose, can be developed. The latter include surface area to body weight scaling for different species or the approaches to development of reference concentrations for inhalation for various types of gases/particles adopted by the U.S. EPA (Jarabek, 1994). Additional data permit replacement of kinetic or dynamic components of interspecies or interindividual variation with compound specific adjustments in the context of the traditional default framework as developed by Renwick (1993) and revised by a Task Group to the International Programme on Chemical Safety (IPCS, 1994). More quantitative toxicokinetic data may permit replacement of the interspecies component for this aspect with a physiologically-based pharmacokinetic model-derived value. Though rarely the case, where there is fuller quantitative characterization of toxicokinetic and toxicodynamic aspects, a full biologically-based dose-response model can be adopted.

The approach along this continuum adopted for any single substance is necessarily determined principally by the availability of relevant data. It is, however, also a function of the purpose of an assessment and the weight of evidence considered appropriate to replace defaults. The extent of data available is, in turn,

often a function of the economic importance of the substance. In striking the appropriate balance along this continuum, it is helpful to bear in mind the definition of default in the Oxford Dictionary - i.e., a "culpable neglect of some duty or obligation".

However, the proportion of cases to date where there is sufficient information to serve as a basis for more predictive compound specific adjustment factors or biologically-based dose-response models, is small. For example, while there was provision to develop compound specific adjustment factors according to the scheme of Renwick (1993) and modifed by IPCS (1994), there were few of the 44 compounds on the first Priority Substances List under the Canadian Environmental Protection Act (CEPA) for which data were considered sufficient for replacement of default values. For interspecies variation in dynamics, information was sufficient to address peroxisome proliferation induced by DEHP. With respect to interspecies variations in kinetics, data were sufficient for dichloromethane and marginal for DEHP, styrene and tetrachloroethylene. However, in almost no cases, were the data sufficient to replace default values for intraspecies (interindividual) variations in either dynamics or kinetics with data-derived factors. The one possible exception was cadmium, where data on concentrations in the target organ (i.e., the renal cortex) in humans obviates the need to account additionally for interindividual variations in kinetics.

For the 25 compounds included on the second Priority Substances List under CEPA, the default component for interspecies kinetics was replaced by compound specific data-adjusted values for both chloroform and 2-butoxyethanol. A biologically motivated case-specific model was available for formaldehyde, thereby addressing all four subcomponents of the factors for interspecies and interindividual variation (Government of Canada, in press).

While there are few cases in which we have adequate data to replace defaults with compound-specific adjustments for interspecies and interindividual toxicokinetics and toxicodynamics, it is important to frame dose-response analysis in this manner to focus attention on gaps in the available information which, if filled, would permit development of more predictive Tolerable Intakes or Concentrations or estimates of carcinogenic potency. In view of the limited number of compounds for which such information is sufficient to develop compound specific adjustment factors, it also seems important and timely to investigate the feasibility of general application of more generic categorical defaults which, while incorporating less biological data than compound specific adjustment factors, are likely more predictive than straight default values.

In the text which follows, then, the potential utility of categorical defaults for several components of uncertainty relevant to development of tolerable or reference concentrations/doses is considered - namely interspecies variation and adequacy of database. For the former, the adequacy of allometric scaling by body surface area as a species-specific default is considered. For the latter, the extent to which data from analyses of subchronic:chronic effect levels, LOAELs/NOAELs and critical effect levels for complete versus incomplete datasets inform selection of defaults is examined.

Interspecies Variation

An aspect worthy of consideration in the context of categorical defaults in the

development of tolerable or reference intakes is cross species scaling on the basis of dose administered per unit of body mass raised to the 2/3 or 3/4 power. Traditionally, scaling on this basis, which derives from the principles of allometry that observable differences among species vary in a regular quantitative manner as a function of size, has been applied only in risk assessment for cancer. The basis for allometric scaling originates from empirical observations that certain anatomical compartments (*e.g.,* organ weights) and physiological processes (*e.g.,* blood flow, renal clearance) are scalable to some fractional power of body weight or to surface area. Surface area, in turn, can be scaled as a power of body weight (raised to the 2/3 power).

Even in the context of risk assessment for cancer, however, scaling by surface area has been applied inconsistently with some jurisdictions introducing it only in cases where the active moiety is the parent compound and others utilizing it in all cases. The value of the exponent applied (2/3 or 3/4) also varies among jurisdictions (U.S. EPA, 1992; Meek et al., 1994). In addition to these inconsistencies in its application in risk assessment for cancer, distinction between cancer and non-cancer in this respect needs to be reconsidered, since there is little apparent justification for the current variations in approach. This would result in different default factors being applied for interspecies variation in the development of tolerable or reference intakes for ingestion depending upon the species in which the critical effect level or benchmark dose was determined. For example, if a 0.3 kg rat receives a dose of 10 mg/kg-day of a carcinogen then the equivalent dose for a 70 kg human is 2.56 mg/kg-day using a scaling factor of 0.75. The dose ratio (rat dose divided by the human equivalent dose) in this case is equal to 3.9. Under this approach, the potency of a chemical (assessed in terms of human equivalent dose) is 3.9 times greater than when it is expressed as an administered rodent dose. Similarly, a dose ratio of 6.9 is derived for doses extrapolated to humans from a 0.03 kg mouse. If a scaling factor of 0.67 (2/3) is adopted, then the rodent:human dose ratios become 5.8 and 13.3 for rats and mice, respectively. In all cases, scaling the dose from a smaller animal to a larger one using a fractional power of body weight results in the assumed potency of the chemical increasing.

In work conducted recently for the Priority Substances Program, the output of PBPK modelling was compared to scaling by body surface area for a range of compounds. Preliminary results of this exercise indicate that the body surface area correction is most appropriate for simple, non-reactive, direct acting compounds eliminated by first order processes (McLaren-Hart and the Sapphire Group, 2000). Comparisons with PBPK output indicated that allometric scaling was conservative, but more applicable to acute, high dose vs. long term low level exposure. Consistent with conclusions of others (U.S. EPA, 1992), available data were considered insufficient as a basis to distinguish which exponent is most appropriate, i.e., 2/3 or 3/4.

In the interest of incorporating more data to better inform selection of defaults, therefore, it is recommended that consideration be given to incorporation of allometric scaling as a species-specific default for the component of uncertainty which addresses interspecies variation in the development of Tolerable or Reference Doses**.**

Database Deficiencies

Another area possibly amenable to replacement of single default uncertainty factors

with category specific values which could possibly draw on observations from empirical analyses of relevant data are those related to deficiencies of the database considered in the development of tolerable or reference doses or concentrations. Commonly, aspects addressed which relate to limitations of the database include lack of adequate data on developmental, chronic or reproductive toxicity, use of a LO(A)EL versus a NO(A)EL and inadequacies of the critical study. In the Priority Substances Program, inadequacies of the database are grouped and addressed by a factor of 1 to 100, to avoid undue conservatism. For example, inclusion of an element to address both "subchronic to chronic" extrapolation and "lack of a chronic study" is somewhat duplicative. Similarly, inadequacies of the database are addressed only where missing endpoints are likely to be more sensitive than that on which the Tolerable Intake/Concentrations are based. For example, there is no reason to address inadequacies of the database such as lack of developmental or reproductive studies in application of the uncertainty factor, if there is a convincing database which indicates that establishment of a Tolerable Concentration on the basis of irritant effects at the site of contact in the respiratory tract is likely to be protective (i.e., available data indicate that these effects always occur at lower concentrations).

There has been considerable effort devoted to examination of scientific databases as a potential basis for improvement of existing default values for components of uncertainty which address inadequacies of the database, particularly factors applied when tolerable or reference intakes or concentrations are based on subchronic rather than chronic studies, or on LOAELs rather than NOAELs (e.g., Nessel et al., 1995; BIBRA International, 1995, 1996; Baird et al., 1996; Dourson and Stara, 1983; McNamara, 1976; Weil and McCollister, 1963; Woutersen et al., 1984). There has also been some investigation of the impact of missing studies (Evans and Baird, 1998).

For example, in work conducted for the Priority Substances Program, toxicological data on 25 endpoints each for 30 compounds (for 18 of which, there were data in two species) were considered. Criteria for inclusion were that subchronic and chronic investigations which included analyses of liver or kidney as target organs conducted in the same species, strain and sex by the same research team about the same time were available. For each species (rat and mouse) and sex, NOAELs and LOAELs were determined for specific endpoints (decreased survival, impaired growth) and for lesions of individual organs, as were the overall NOAELs and LOAELs. This analysis was unique from similar recent studies in that results were analyzed by endpoint and organ (BIBRA International, 1995, 1996).

Consistent with the results of similar analyses, mean and median values of ratios of subchronic to chronic NOAELs and NOAELs to LOAELs were generally two- to three-fold less than the default value of 10 often adopted for each of these components of uncertainty. Median values were generally less than 3; indeed, for effects on the liver and kidney, median values were both 1.5. For the liver and kidney, all values of the ratio of NOAELs in subchronic:chronic studies were less than 10 (respective ranges of 0.13-8.6 and 0.06-8.4). Interestingly, ratios were consistently highest for hyperplastic changes (thyroid, parathyroid, spleen and lung); cases where hyperplastic changes progressed to tumours were excluded. Based on analyses of data on ratios of LOAELs:NOAELs in subchronic and chronic studies, the default value of 10 for this component of uncertainty is quite protective (All ratios were less than 5.5).

In work also conducted for the Priority Substances Program, the impact of missing data on estimates of the NOAEL was investigated for 38 pesticides for which a complete database was available (Evans and Baird, 1998). The impact of missing data was assessed by artificial censoring of the complete databases to exclude one or more bioassays, the critical NOAEL estimated and compared with the value derived from the complete database. The data were analyzed in different subsets depending on definition of complete database (Health Canada Priority Substances Program/U.S. EPA) and number of studies missing by multiple regression to provide point estimates and non-parametric analysis to provide distributional estimates. Results were provided based on two approaches for interspecies scaling - one based on body weight scaling and another based on surface area scaling.

Results indicated that both the number and type of bioassays available had an impact on the estimate of the critical NOAEL from an incomplete data set. Based on the two definitions of a complete data set and scaling assumptions, the chronic bioassay in rats appears to be the most informative and the developmental bioassay in rats the least informative. This relationship was consistent across the database and scaling assumptions. For the non-parametric analysis, for the HC definition of complete database, for the most informative bioassay (rat chronic), the adjustment factor was always less than 3; for the least informative bioassay (rat developmental), more than 70% of the adjustment was above 3 and some were as high as 640.

While it has been suggested that ratios observed upon empirical analyses might justify the use of smaller factors to address deficiencies of the database in the development of Tolerable or Reference Concentration or Doses (e.g., Nessel et al., 1995), it needs to be recognized that derived values are primarily a function of dose-spacing and limited by the representativeness of the small samples sizes of often similar types of compounds; moreover, the criteria for comparison varied considerably from investigation to investigation, with subchronic:chronic and NOAEL:LOAEL ratios being effect-specific in only one. Based on additional consultation with authors, some analyses were crudely constructed from secondary sources. While it may be that in future, this aspect will be more adequately assessed through examination of ratios of benchmark concentrations or doses, based on interface with several of the principal investigators, access to the raw data which served as the basis for these analyses could not be provided to facilitate more systematic investigation of a broader, more meaningful dataset.

Recently, in a quantitative investigation of the extent to which such ratios reveal true properties of a specified distribution, it has been concluded that: (1) Such dependencies are complex and produce pronounced systematic errors, and (2) that sampling errors associated with typical samples sizes (e.g., 50 chemicals) are non-negligible (Brand et al., 1999).

Conclusions

In the interest of incorporating more data to better inform selection of defaults, it is recommended that consideration be given to incorporation of allometric scaling as a species-specific default for the component of uncertainty which addresses interspecies variation in development of Tolerable or Reference Doses. Criteria for application need be considered, particularly in relation to whether the putatively toxic entity is parent compound or metabolite and the dose range of the effect level or benchmark dose on which the Tolerable intake or Reference Dose is based.

In relation to default values of components of uncertainty for deficiencies of the database (i.e., subchronic to chronic, LOAEL to NOAEL, lack of lifetime or developmental reproductive studies), due to their limitations, empirical analyses of relevant data are considered minimally informative, except to provide some confidence that the use of 10 fold default factors in this regard are protective. Limitations of characterization of dose-response in current studies may also preclude additional utility of benchmark analyses in better informing default values for these components of uncertainty in the development of tolerable or reference concentrations or doses. Compound specific data are clearly preferred as a basis for development of more predictive "adjustment" versus "uncertainty" factors for the toxicokinetic and toxicodynamic aspects of interspecies and interindividual (intraspecies) variation. In this regard, there has been considerable progress in an international initiative to improve understanding and develop guidance to facilitate convergence of assessments of the sufficiency of weight and nature of evidence to replace default methodologies to address uncertainties with compound specific adjustments. However, while developments in this area are promising, it needs to be recognized that there will continue to be many instances where generation of such data is unjustified and continued investigation of the potential utility of more generic categorical defaults is advised.

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Chapter 7

Toxicological Highlight – Biologically motivated computational modelling: Contribution to risk assessment

Meek, M. E. (2004)

Toxicological Sciences 82(1): 1-2

The article highlighted in this issue is "Human Respiratory Tract Cancer Risks of Inhaled Formaldehyde: Dose Response Predictions Derived From Biologically-Motivated Computational Modeling of a Combined Rodent and Human Dataset" by Rory Conolly, Julia Kimbell, Derek Janszen, Paul Schlosser, Darin Kalisak, Julian Preston, and Frederick Miller.

In the featured article, Conolly *et al.* describe the development of the human component of a biologically motivated computational model to predict exposure response at levels of formaldehyde less than those associated with squamous cell carcinomas (SCC) observed in Fischer 344 rats exposed by inhalation. The article addresses extension of the computational model to the entire respiratory tract of humans, complementing a previous description which presented modeling for the nasal airways of rats (Conolly et al. (2003). Extension to the entire respiratory tract is relevant for prediction of risk associated with oronasal breathing of humans, as occurs at higher exertion levels characteristic of those likely in the occupational environment.

The computational model incorporates regenerative cell proliferation as a required step in the induction of tumours by formaldehyde and a contribution from mutagenicity that has greatest impact at low exposures through modeling of complex functional relationships for cancer due to actions of formaldehyde on mutation, cell replication and exponential clonal expansion. Species variations in dosimetry are taken into account by computational fluid dynamics modeling of formaldehyde flux in various regions of the nose and a single path model for the lower respiratory tract of humans.

Specifically, the animal model includes an anatomically realistic three dimensional computational fluid dynamics model (CFD) of rat nasal airflow and site-specific flux of formaldehyde into the tissue in which the nasal SCC developed. Flux is the relevant dose metric for the two relevant non-cancer effects in the tissues: formation of DNA-protein crosslinks (DPX) and cytolethality/regenerative cellular proliferation (CRCP). A two-stage clonal growth model links the modes of action with mutation accumulation and tumour formation.

In the human component of the model described in this issue, predictions of regional dosimetry are based on human versions of the CFD model and a linked typical path model for the lower respiratory tract. Regional formation of DPX driven by the CFD-predicted flux of formaldehyde into tissue is predicted by a human DPX model based on scale up from rat and rhesus monkey DPX models. CRCP data were those for the rat. Baseline parameter values for the human clonal growth model were calibrated against human lung cancer incidence data (Peto et al., 1992; SEER, 2003).

The body of chemical specific and more generic biological information on which the model is based is extensive. Model structure is also consistent with the outcome of consideration of weight of evidence for mode of action of tumour induction in a formal framework analysis (Meek et al., 1993; Liteplo and Meek, 2003). The database on which this weight of evidence analysis and the computational exposure response model on formaldehyde draws is impressive, including a specifically designed inhalation bioassay in which extensive dose-response data on intermediate endpoints were collected (Monticello et al., 1991, 1996). The clonal expansion model also draws upon an impressive more generic body of work on relative roles of mutation and cell proliferation in cancer induction dating back over 20 years. The three dimensional computational fluid dynamics model contributes

considerably not only to formaldehyde-specific but generic understanding of site specificity as a function of airflow resulting from the complex anatomy of the nasal passages and lung of rats and humans.

The authors describe clearly uncertainties of the model structure and parameter values which include the lack of direct correlation between DPX and mutation (dose-response data for other potentially mutagenic lesions associated with formaldehyde not being available). Formal sensitivity analysis and Monte-Carlo based analysis of variability and uncertainty were also not conducted precluding identification of model parameters with greatest impact or presentation of confidence intervals around model generated risk predictions. However, in all cases, in the absence of relevant information, the authors have made conservative assumptions, thereby increasing confidence that action taken on the basis of resulting estimated risks would be health protective.

Important in the consideration of the robustness of any biologically motivated computational model and its acceptance, is peer input and review, which necessarily must address complexity and integration of a vast array of varying types of information. In this context, it is noteworthy that the computational model described in the highlighted article draws upon earlier input from a Steering Group of representatives of relevant agencies from two Governments, industry and a consulting group and incorporates revisions suggested by an external peer review workshop, representing a broad range of expertise including genetic toxicology, cancer biology, epidemiology, modeling and statistics (Report of Health Canada/U.S. EPA External Peer Review Workshop on Formaldehyde, Health Canada, 1998).

In addition to peer input and review, however, it is effective communication of the complex content of biologically motivated computational exposure response models that is likely the critically essential component of their acceptance. Presentation in the highlighted article is worthy of comment in this context. The authors have effectively and efficiently communicated the essential features of a highly complex model including the nature of the information on which the model draws, its limitations, assumptions made in the absence of relevant data and the comparison of resulting risk estimates with those for approaches incorporating less biological data, though formal sensitivity analysis was not conducted.

While all biologically motivated case specific models entail simplification of cancer biology which requires selection of a number of parameters and use of simplifying assumptions, they provide insight into the biological basis of responses observed experimentally and are the preferred basis for the extrapolation of data outside of the range of observation. Where underlying databases are robust and the models adequately developed taking into account peer input and review, they are clearly preferred over default methodology for dose-response consideration in risk assessment (It is notable in this context that default is assumed but not necessarily proven to be protective). Moreover, the significant, potentially adverse impact on advancing understanding of the modes of action of toxic chemicals and their resulting health effects of the lack of application in regulatory risk assessment of biologically motivated case-specific models should not be underestimated. Avoiding limitations of understanding resulting from inadequate communication is critical in this context and Conolly et al. make significant contribution in clearly, concisely and defensibly presenting their important work in the highlighted article.

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Chapter 8

Chemical-Specific Adjustment Factors (CSAF)

Meek, M. E. (2005)

Encyclopedia of Toxicology, Elsevier

Introduction

In the development of reference or tolerable concentrations or doses, where kinetic and/or dynamic data are adequate, commonly adopted default values for interspecies differences and human variability can be replaced by more certain chemical specific adjustment factors (CSAFs). Guidance for the development of CSAFs which represent part of a broader continuum of approaches to incorporate increasing amounts of data to reduce uncertainty, ranging from default ("presumed protective") to more "biologically-based predictive" (Meek, 2001), is available (IPCS, 2001).

Framework for Development of CSAFs

Renwick (1993) proposed a framework to address kinetics and dynamics separately in considering uncertainty related to interspecies differences and interindividual variability in the development of reference or tolerable concentrations/doses. Quantitation of this subdivision is supported by data on kinetic parameters and pharmacokinetic-pharmacodynamic (PKPD) modeling for a range of pharmacological and therapeutic responses to pharmaceutical agents (Renwick, 1993; Renwick and Lazarus, 1998).

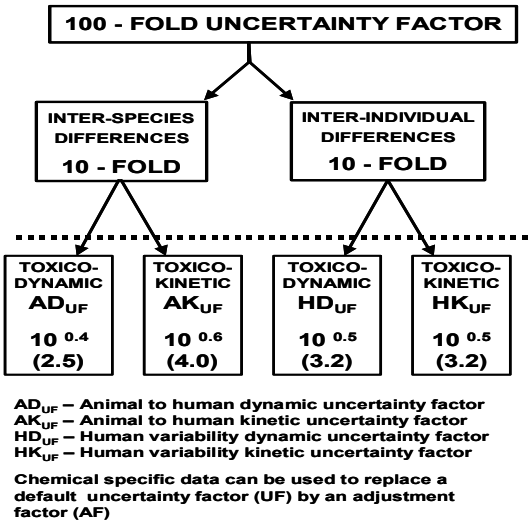

Figure 1 – the sub-divison of the 100-fold fold uncertainty factor to allow chemical- specific data to replace part of the default factor.

This framework allows the incorporation of quantitative chemical-specific data, relating to either toxicokinetics or toxicodynamics, to replace part of the usual 100-fold default uncertainty factor for interspecies differences or interindividual variability but collapses back to the usual 100-fold default in the absence of appropriate information (Figure 1). Owing to the nature of the data on which the subdivision is based, in the context of the framework, "toxicokinetics" relates to the movement of the chemical around the body (*i.e.* the absorption, distribution,

metabolism and excretion). "Toxicodynamics" relates specifically to the processes occurring in the target tissue(s), including metabolism.

Chemical -Specific Toxicokinetic Adjustment Factors - [AK_{AF}, HK_{AF}]

The chemical-specific adjustment factors for the toxicokinetic components of interspecies differences and interindividual variability are ratios of measurable metrics for internal exposure to the active compound such as area under the plasma or tissue-concentration time curve (AUC), the maximum measured concentration in blood (Cmax) or clearance (CL). For interspecies differences, this is generally determined on the basis of comparison of the results of *in vivo* kinetic studies with the active compound in animals and a representative sample of the healthy human population. For humans, relevant data on AUC, Cmax or CL are generally derived from *in vivo* experimentation in volunteers given very low doses of the relevant chemical. Alternatively, relevant information on such parameters may be derived from *in vitro* enzyme studies combined with suitable scaling to determine *in vivo* activity.

For interindividual variability, most often, factors responsible for clearance mechanisms are identified (e.g., renal clearance, CYP-specific metabolism, etc.) and a chemical-specific adjustment factor derived based on measured or physiologically based pharmacokinetically (PBPK) modelled human variability in the relevant physiological and biochemical parameters. The population distribution for the relevant metric (e.g., AUC, Cmax, renal clearance) for the active entity is analyzed and the CSAF (HK_{AF}) calculated as the difference between the central values for the main group and given percentiles (such as 95^{th}, 97.5^{th} and 99^{th}) for the whole population (Figure 2). These differences are analyzed separately for any potentially susceptible sub-group (Figure 2).

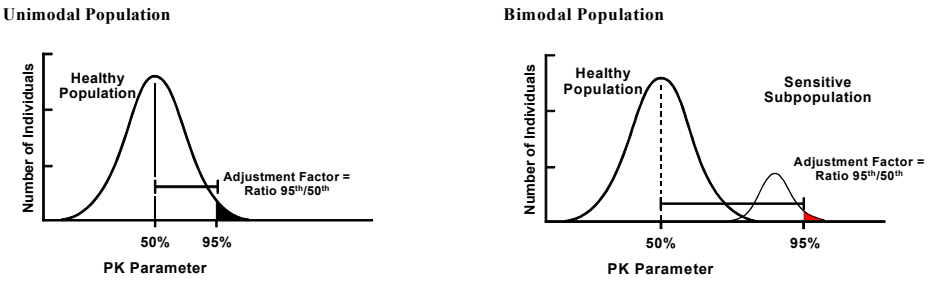

Figure 2 – Development of CSAFs for interindividual variability

Chemical-Specific Toxicodynamic Adjustment Factors [AD_{AF}, HD_{AF}]

Chemical-specific adjustment factors for toxicodynamic components are most simply, ratios of the doses which induce the critical toxic effect or a measurable related response *in vitro* in relevant tissues of animals and a representative sample of the healthy human population (interspecies differences) or in average versus sensitive

humans (interindividual variability). At its simplest, then, replacement of the dynamic component of the default factor for inter-species differences is the ratio of the effective concentrations in critical tissues of animals versus humans (e.g., $EC_{10\ animal}/EC_{10\ human}$) for interspecies differences and in healthy human and susceptible subpopulations for interindividual variability (e.g., the $EC_{10\ average}/EC_{10\ sensitive}$).

Guidance for Development of CSAF

IPCS (2001) provides guidance on several aspects of the development of CSAF, which are only briefly outlined here. For example, data for application in the four components of the framework must relate to the *active form of the chemical*. For the components of the framework addressing toxicokinetics [AK_{AF}], [HK_{AF}], *choice of the appropriate metric* is also an essential first step.

Choice of the appropriate endpoint is critical for the components addressing toxicodynamics [AD_{AF}], [HD_{AF}]. The selected measured endpoint must either be the critical effect itself or intimately linked thereto (with similar concentration-response and temporal relationships) based on an understanding of mode of action.

In addition, the metric for toxicokinetics or the measure of effects for toxicodynamics as a basis for CSAF needs careful consideration in relation to the delivery of the chemical to the target organ. Measures of various endpoints *in vivo* may represent purely toxicokinetics, or toxicokinetics and part or all of the toxicodynamic processes, as defined based on the subdivision of defaults. This necessitates consideration of the impact of specific data to replace the toxicokinetic and potentially a proportion or all of the toxicodynamic components of the default uncertainty factors.

For data that serve as the basis for all components, *relevance of the population, the route of exposure, dose/concentration* and *adequacy of numbers of subjects/samples* must also be considered and the potential impact on the validity of the calculated ratio addressed. For example, for *in vitro* studies which inform primarily dynamic components [AD_{AF}] [HD_{AF}], the quality of the samples should be considered, and evidence provided that they are representative of the target population, e.g. viability, specific content or activity of marker enzymes.

Conclusions

Consideration of relevant data in the context of a framework that addresses kinetic and dynamic aspects, explicitly, should result in greater understanding of contributing components and transparency in risk assessment. It is also hoped that consideration in this context will lead to clearer delineation and better common understanding of the nature of specific data required which would permit development of more informative measures of dose response.

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Chapter 9

Guidance for the Development of Chemical Specific Adjustment Factors – Integration with Mode of Action Frameworks

Meek, B. and Renwick, A. (2006)

Toxicokinetics and Risk Assessment, Eds. Lipscomb, J.C. and Ohanian, E.V., Informa Healthcare, New York, N.Y.

Introduction

Chemical-specific adjustment factors (CSAFs) provide for the incorporation of quantitative data on interspecies differences or human variability in either toxicokinetics or toxicodynamics (mode of action) to replace appropriately weighted components of default uncertainty factors commonly adopted in defining health-based guidance values. This requires subdivision of default uncertainty factors for interspecies differences and interindividual variation into toxicokinetic and toxicodynamic components.

CSAFs represent an intermediate step in a continuum of increasingly chemical specific data-informed approaches to extrapolation of dose-response in animals as a basis to provide guidance regarding acceptable limits of exposure for humans. Their application permits incorporation of partial information in the absence of the considerable data required to support full biologically-based dose-response models. They can be *compound-related* (e.g., based on more generic information on physiological processes such as glomerular filtration rates for substances where renal clearance is limiting) or *chemical specific* (e.g., based on experimental data on kinetics or dynamics such as metabolic rate constants specific for the individual chemical of interest).

In this chapter, guidance on CSAFs developed in a project of the International Programme on Chemical Safety (IPCS) initiative on *Harmonisation of Approaches to the Assessment of Risk from Exposure to Chemicals* is considered. The final guidance,[1] takes into account experience and comments received since the initial posting of the draft document on the IPCS website in 2001.

The guidance for adequacy of data to replace the default values for the toxicokinetic and toxicodynamic components of interspecies differences and human variability is presented in the context of several aspects including:

- determination of the active chemical species,
- choice of the appropriate kinetic measurement or parameter and
- experimental data, including
    - relevance of population,
    - relevance of route,
    - relevance of dose/concentration and
    - adequacy of number of subjects/samples.

Development of a CSAF is necessarily predicated on the basis of understanding of the mode of action by which a specific chemical induces its effect(s) (e.g., through action of the parent compound or metabolite). Mode of action is a description of the hypothesized processes that lead to induction of the relevant end-point of toxicity for which systematic consideration of the weight of evidence supports plausibility. It is distinguished from "mechanism of action" which implies a more detailed molecular description of causality, for which there is rarely sufficient information. This chapter explores the application of CSAFs in the context of recently developed extensions to mode of action frameworks to consider weight of evidence for human relevance, through presentation of a relevant case study. These frameworks outline a basis for transparent delineation of expert informed judgment

and as such, contribute to harmonization of approaches to hazard characterization for cancer and non-cancer effects.

The guidance developed in this project and described in part, herein, addresses criteria for the adequacy of quantitative data on toxicokinetics and toxicodynamics; as a result, it is also applicable to other approaches to dose response analyses (e.g., linear extrapolation from estimates of potency close to the experimental range or development of tumorigenic potencies). Development of CSAFs also results in delineation of appropriate avenues of research to enable more reliable assessments. The approaches described in this chapter are also amenable to presentation in a probabilistic context (rather than development of single measures for dose/concentration–response), where available data are sufficient to meaningfully characterize the distributions of interest.

Development of Chemical Specific/Compound Related Adjustment Factors: The Construct

Traditionally in assessment of non-cancer effects, default safety/uncertainty factors have been applied to develop health-based guidance values based on a measure of dose-response for critical effects. Most commonly, a value of 100 has been applied to no- or lowest-observed-adverse-effect levels (NOAELs or LOAELs) or benchmark doses for critical effects in chronic studies in animals to derive acceptable or tolerable daily intakes (TDI or ADI), or reference doses (RfD) for the general population.[2-5]

The NOAEL or benchmark dose/concentration is selected generally to be at or below the threshold in animals; uncertainty factors are then applied to estimate the subthreshold in ***sensitive human*** populations, with a 10 fold default factor addressing interspecies differences (i.e., the variation in response between animals and a representative healthy human population) and another 10 fold factor accounting for interindividual variability in humans (the variation in response between a representative healthy human population and sensitive subgroups). While additional factors are sometimes applied to account for deficiencies of the database, the 100 fold default value is common.

For practical purposes, the continuous process between external dose and toxic response (i.e., mode of action) can be subdivided into steps related to the fate of the chemical in the body and those related to the actions of the chemical on the body. These different aspects of the overall process represent major sources of interspecies differences and of human variability, the latter defining susceptible subgroups within the population. Measurements of response of humans to external doses *in vivo* represent both toxicokinetics and toxicodynamics and would be used directly as a basis for the NOAEL, LOAEL or benchmark dose (BMD) obviating the need for consideration of interspecies differences.

Development of CSAFs requires subdivision of each of the usual default uncertainty factors of 10 for interspecies differences and human variability into subfactors which address toxicokinetics and toxicodynamics. For application in this context, the continuum of processes leading to chemical toxicity was split at the level of delivery via the general circulation of the parent compound or of an active metabolite to the target tissue/organ: events up to this point were considered as toxicokinetics, and events within the target tissue/organ were considered as toxicodynamics. This split was chosen because the subdivision was derived largely from physiological differences between rodents and humans for interspecies

differences and from the clinical pharmacology literature for human variability, based on plasma concentration measurements (toxicokinetics) and data from *in vitro* studies or from modelling of data from *in vivo* studies in humans (toxicodynamics). In consequence, the data to replace a default subfactor for toxicokinetics will usually be based on the concentrations of the chemical or active metabolite in the general circulation.

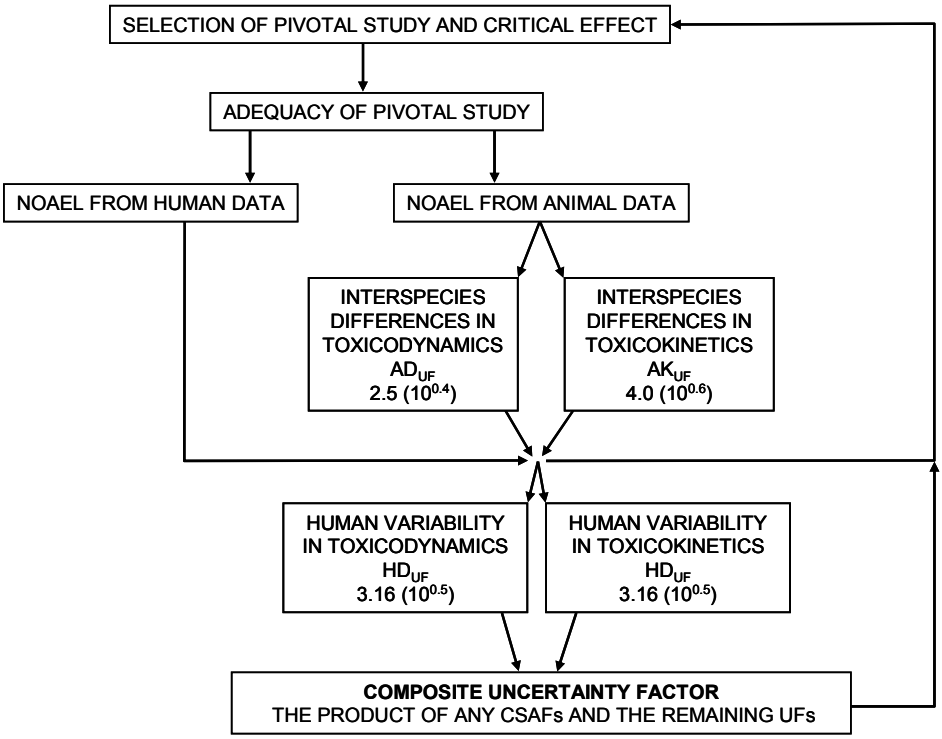
The quantitative split proposed by Renwick[6] and subsequently modified by an international review group[7] is consistent with data on kinetic parameters and pharmacokinetic-pharmacodynamic (PKPD) modeling for a range of pharmacological and therapeutic responses to pharmaceutical agents.[8] Based on such analyses, the 10-fold default factor for interspecies differences is subdivided into factors of 4 ($10^{0.6}$) for toxicokinetics and 2.5 ($10^{0.4}$) for toxicodynamics. This is consistent with the approximately 4-fold difference on a body surface area basis between rats (the most commonly used test species) and humans in basic physiological parameters such as cardiac output, and renal and liver blood flows, which are major determinants of clearance and elimination of chemicals. The factor for human variability is divided evenly into two sub-factors each of $10^{0.5}$ (3.16 or 3.2) (Figure 1).

Application of relevant data in the framework is predicated on the basis that measurements of the concentrations of the parent compound or its metabolites in the general circulation reflect major sources of interspecies differences and human variability in tissue/organ delivery. In consequence, the data to replace a default subfactor for toxicokinetics or toxicodynamics should be based on the concentrations of the chemical or active metabolite in the general circulation; if not, then the defaults for the remaining subfactors that were not replaced by a CSAF would need to be reconsidered.

This framework, then, allows the incorporation of quantitative chemical-specific data, relating to either toxicokinetics or toxicodynamics, to replace parts of the usual 100-fold uncertainty factor but collapses back to the usual 100-fold default in the absence of appropriate data. The framework is based, therefore, on a pragmatic split of defaults, for which the delineation between toxicokinetics and toxicodynamics is necessarily a function of the data from which it was derived. However, framing of the construct in this context does not prevent application, for example, of the output of physiologically based pharmacokinetic (PBPK) models which incorporate bioactivation and/or detoxification processes within the target tissue/organ. Rather, the fact that the model addresses additionally this aspect needs to be taken into consideration in applying both the chemical specific values which replace the toxicokinetic default and the remaining toxicodynamic default subfactor, for which information may not be available. For this reason, it must be determined whether quantitative modeling or measures of various parameters or endpoints represent purely toxicokinetics, or toxicokinetics and respective parts or all of the toxicodynamic processes as defined in the context of this framework. Their impact to replace the toxicokinetic and potentially a proportion or all of the toxicodynamic defaults needs then to be carefully considered.

Similar reconsideration of application in the context of the construct would be necessary if the model related to an effect at the site of contact. While the database on which the values for the subfactors was developed related to systemic effects produced after oral or intravenous dosage, the use of CSAFs and the approach described herein are applicable also to effects at the site of contact, but taking into

account that the toxicokinetic component would be direct delivery and not via the general circulation.



A = the animal to human extrapolation factor (based on quantification of interspecies differences)
H = the human variability factor (based on quantification of interindividual differences)
K = differences in toxicokinetics
D = differences in toxicodynamics
AF = the adjustment factor calculated from chemical-specific data
UF = the uncertainty factor, a default value that is used in the absence of chemical-specific data.

Figure 1. Introducing Quantitative toxicokinetic and toxicodynamic data into dose/concentration–response assessment (adapted from IPCS[7])

Chemical –Specific/Compound-Related Toxicokinetic Adjustment Factors - [AK_{AF}, HK_{AF}]

The CSAFs for the toxicokinetic components of interspecies differences and interindividual variability are ratios of measurable metrics for internal exposure to the active compound such as area under the plasma or blood concentration-time curve (AUC), the maximum observed concentration (Cmax) or clearance (CL). For

interspecies differences, this may be determined on the basis of comparison of the results of *in vivo* kinetic studies with the active compound in animals and a representative sample of the healthy human population. Estimates can also be derived from *in vitro* enzyme studies combined with suitable scaling to determine *in vivo* activity or by the scaling of *in vivo* data from animals to predict human equivalent values. Alternatively or necessarily (when metabolism or tissue uptake is non-linear), they are based on physiologically-based pharmacokinetic (PBPK) models in which data on partition coefficients for different tissues are combined with the organ blood flows for animals and for humans to predict delivery to and (often) the concentrations within the target tissue/organ. Partitioning of the chemical between the general circulation and target tissue/organ in PBPK models is usually based on measurements of the partition coefficient in animal tissues or other *in vitro* models.

In the context of the development of CSAFs, PBPK models may be subdivided into two types:

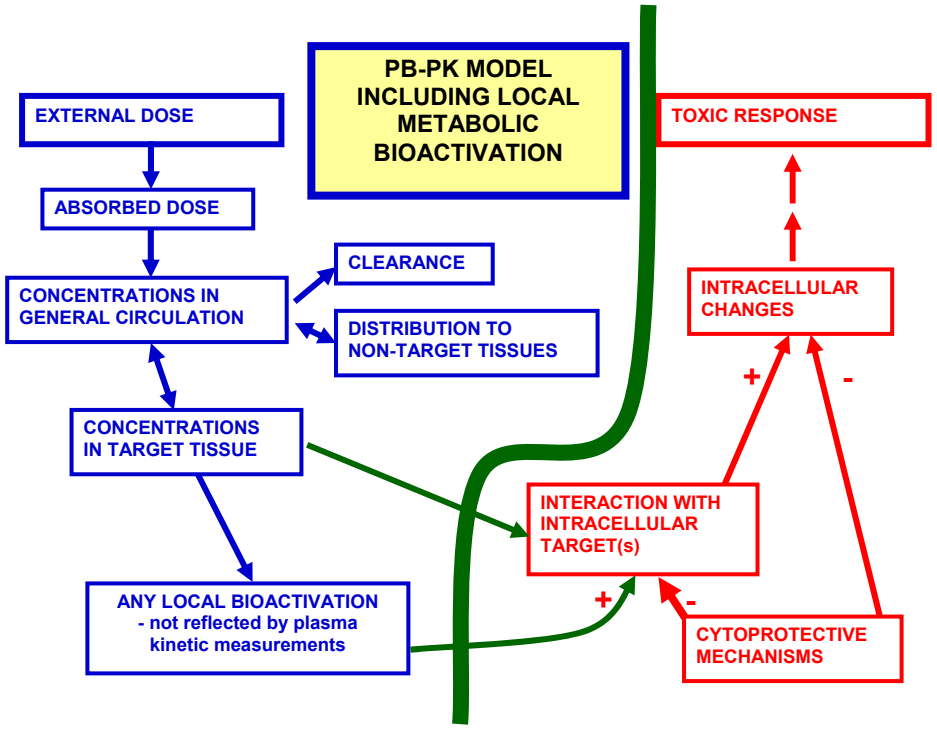
1) those that estimate the target tissue/organ dose of the parent compound or a circulating active metabolite; and
2) those that additionally incorporate bioactivation and detoxification processes that occur within the target tissue/organ.

Type 1 PBPK models are purely "toxicokinetic" in nature and consistent with the types of data on which the subdivision of the default uncertainty factors of 10 into toxicokinetic and toxicodynamic subfactors was based. Type 2 PBPK models include parts of the overall process (Figure 2) such as bioactivation or detoxification within the cells of the target tissue, that are not reflected in plasma-based toxicokinetic measurements and therefore reflect processes affecting the tissue "response" and in this context should be considered as part of toxicodynamics.

In addition to these PBPK models for systemic delivery, mathematical models can define delivery when the target tissue/organ is the site of contact, such as inhalation delivery to the lungs, as well as uptake and metabolic processing within the target tissue/organ. Such models differ from the database of systemic effects resulting from oral or intravenous exposure on which the values for the subfactors presented in Figure 1 are based. Although the approach described herein is applicable to effects at the site of contact, the toxicokinetic component would be related to direct delivery versus delivery via the general circulation and consideration would need to be given to the appropriateness of the default values for the particular model on a case-by-case basis.

For interindividual variability, while this adjustment factor could potentially be addressed on the basis of *chemical-specific in vivo* kinetic studies in a sufficiently broad range of subgroups of healthy and potentially susceptible populations to adequately define the population distribution, this may not be practicable or even possible. The population distribution for the relevant metric (e.g., AUC, Cmax, renal clearance) for the active entity is analyzed and the CSAF (HK_{AF}) calculated as the difference between the central values for the main group and given percentiles (such as 95^{th}, 97.5^{th} and 99^{th}) for the whole population (Figure 3). These differences are analyzed separately for any potentially susceptible sub-group. Often chemical-specific data are not available but the factors responsible for the clearance mechanisms are identified (renal clearance, CYP-specific metabolism, etc.); in such circumstances, a *compound-related* adjustment factor could be derived based on

measured or PBPK-modelled human variability in the relevant physiological and biochemical parameters.[9,10]



Note that "concentrations" refers to the relevant active form delivered by the general circulation and may be the parent compound or active metabolite produced in another tissue and delivered to the target tissue or organ.

Figure 2. Components of a toxic response

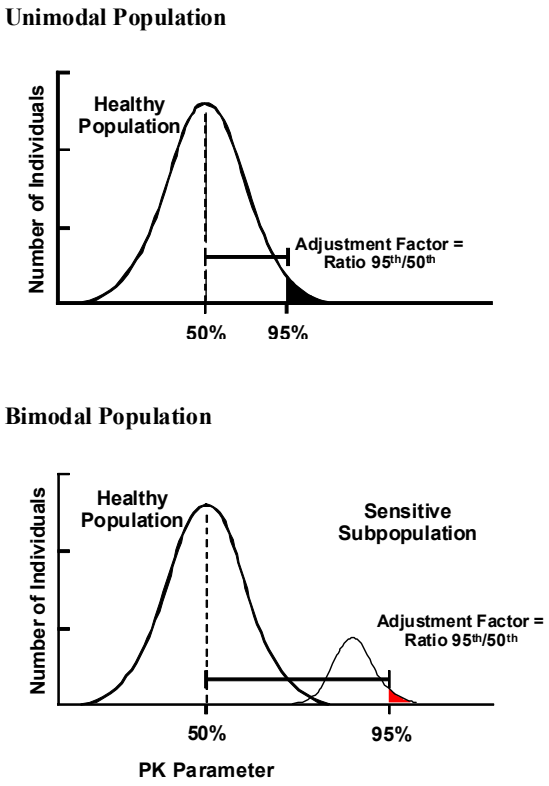


Figure 3. Development of CSAFs for interindividual variability (based on Meek et al.[13])

Chemical-Specific Toxicodynamic Adjustment Factors [AD_{AF}, HD_{AF}]

CSAFs for the toxicodynamic components are most simply, ratios of the concentrations which induce the critical toxic effect or a measurable related response (based on understanding of mode of action) *in vitro* in relevant tissues of animals and a representative sample of the healthy human population (interspecies differences) or in average versus sensitive humans (interindividual variability). At its simplest, then, replacement of the dynamic component of the default factor for inter-species differences is the ratio of the effective concentrations in critical tissues of animals versus humans. If the concentration associated with a 10% response was selected (EC_{10}), then the ratio for the CSAF would be ($EC_{10\ animal}/EC_{10\ human}$) for interspecies differences and ($EC_{10\ average}/EC_{10\ sensitive}$) for interindividual variability in healthy human and susceptible subpopulations. Measurements should be derived under experimental conditions where there has been control for variations in toxicokinetics.

Hence, CSAFs for interspecies differences and interindividual variability in toxicodynamics could be derived from *in vitro* studies, from *in vivo* studies in which the toxicokinetic component has been delineated (e.g., by kinetic-dynamic link models in which concentrations or amounts in the general circulation or at the site of action are related to the response by an empirical mathematical link-function formula) or from *ex vivo* experimentation (i.e., studies in which measurements are made *in vitro* following an *in vivo* exposure).

Not all *in vitro* or *in vivo* biological measurements represent processes that are critical to the development of the *in vivo* toxic response. There are frequently numerous sequential steps in producing a toxic response, and biomarkers of early changes may not reflect the critical toxicodynamic process. In order to serve as a surrogate marker for toxic effect, the measurements should be representative, both qualitatively and quantitatively, of the critical toxic end-point, based on an understanding of the mode of action by which specific chemicals induce their effects.

Chemical Specific/ Compound-Related Composite Factor for Interspecies Differences and Human Variability (CF)

The composite factor (CF) is the product of 4 different factors, each of which could be a chemical-specific or compound-related adjustment factor (AF) or a default uncertainty factor (UF):

$CF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$

where A = interspecies; H = human variability; K = kinetics; D = dynamics.

CFs should be developed for several effects which might be considered critical to ensure that resulting tolerable, acceptable or reference intakes/concentrations are sufficiently protective.

It is important to recognize that depending on the nature of the data, the CF can be either greater than or less than the usual 100-fold default. If the CF for an effect considered potentially critical based on assessment of the entire database is similar to or exceeds the normal default (i.e., 100), then the resulting tolerable intake or concentration should be protective for most other toxic effects. If, however, the CF for a potentially critical effect is less than the normal default, a different toxic effect with a higher NOAEL/NOAEC combined with a default uncertainty factor could become critical as a basis for a Tolerable Intake or Concentration (Figure 4).

In the vast majority of cases, the quantitative toxicokinetic or toxicodynamic data necessary to define a CSAF will not be available necessitating the usual NOAEL/uncertainty factor approach. The default uncertainty factors (AK_{UF}, AD_{UF}, HK_{UF} and HD_{UF}) are based on the usual default values (10 for each of interspecies differences and human variability), so this guidance remains compatible with the current default procedures.

Application of such a framework even in the absence of relevant data to replace default values is encouraged, in order to focus attention on gaps in the available information which, if filled, would permit development of more predictive measures of dose/concentration response. In this manner, it should contribute to a better common understanding of the appropriate nature of relevant data, thereby

facilitating their development and incorporation in dose/concentration response assessment for regulatory purposes.

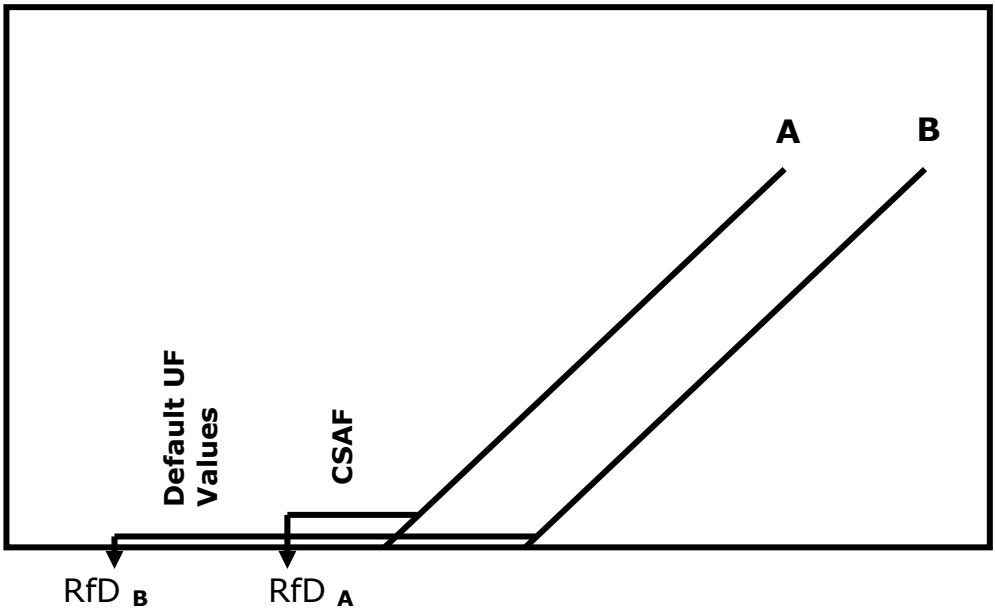


Figure 4. Potential quantitative relationship for development of RfD values based on default UF values or CSAF values

Guidance for Development of CSAF

The development of CSAF requires an understanding of mode of action for the critical effect(s) under consideration, in particular, whether or not effects are induced by the parent compound or a metabolite. "Mode of action" (MOA), the hypothesized processes that lead to induction of the relevant end-point of toxicity for which systematic consideration of the weight of evidence supports plausibility, is described in the context of "key events," which are measurable parameters critical to the effect under consideration. Key events may include, for example, metabolism to the active entity, cytotoxicity and associated regenerative proliferation, deposition of crystals in the target tissue, hormonal perturbations, etc.

Data for application in the four components of the framework for CSAFs *must relate to the active form of the chemical* – i.e., taking into consideration early metabolic key events. Information that is relevant in this regard includes data on the mode of toxicity of structural analogues, the effects of metabolites administered directly, the influence of induction or inhibition of metabolism of the chemical on the critical effect and variations in patterns of toxicity with metabolic profiles across species, strains and sexes.

For the components of the framework addressing toxicokinetics [AK_{AF}], [HK_{AF}], *choice of the appropriate kinetic metric* is an essential first step. Observation of the effect only following administration of an intravenous bolus dose or administration by gavage compared with continuous administration in diet or drinking water may indicate the importance of dose rate (i.e., Cmax being the appropriate metric). In the absence of such data, a reasonable assumption is that effects resulting from sub-chronic or chronic exposure are related to the AUC, especially for chemicals with long half-lives, while acute toxicity could be related to either the AUC or the Cmax. Alternatively, the AUC is a reasonable default because there are likely to be greater species differences or human variability in AUC or 1/CL than in Cmax.[11]

Choice of the appropriate endpoint is critical for the components addressing toxicodynamics [AD_{AF}], [HD_{AF}]. The selected measured endpoint must either be the critical effect itself or intimately linked thereto (with similar concentration-response and temporal relationships) based on an understanding of mode of action.

As indicated above, the framework for CSAF is based on a pragmatic split of default uncertainty factors, for which the defined delineation between toxicokinetics and toxicodynamics has been determined by the nature of the quantitative data on which the subdivision was based. However, for all datasets, the relevant parameter for toxicokinetics or the measure of effects for toxicodynamics being considered as a basis for quantitative comparison to replace default for either interspecies differences or human variability, needs to be carefully considered in relation to the delivery of the active chemical (parent compound or metabolite) to the target organ. Apparently toxicokinetic measurements may represent purely toxicokinetics (e.g., area under the curve) or toxicokinetics and part or all of the toxicodynamic processes (e.g., a PBPK model which incorporates tissue metabolism). This may necessitate consideration, in some cases, of replacement of the toxicokinetic and potentially a proportion or all of the toxicodynamic default for inter-species differences or human variability. For example, development of a factor developed on the basis of a PBPK model which incorporates quantitative data on metabolic activation within the target tissue, would replace the toxicokinetic and the toxicodynamic default if metabolic activation was the critical step in determining overall sensitivity of the species or individual.

The *relevance of the population studied* must also be considered in the development of a CSAF. For the subfactors in kinetics [AK_{AF}] [HK_{AF}], for which the data are normally derived *in vivo*, this entails that the human population investigated is sufficiently representative of the subpopulation at risk for the adverse effect detected in the animal studies (e.g., males if the critical effects are those on the testes, pregnant females if critical effects are developmental, relevant age group). If not, the impact of any discrepancy on the validity of the calculated ratio needs to be considered. For *in vitro* studies which inform primarily dynamic components [AD_{AF}] [HD_{AF}], the quality of the samples should be considered, and evidence provided that they are representative of the target population, e.g., viability, specific content or activity of marker enzymes.

The *relevance of the route of exposure* needs to be considered in relation primarily to i*n vivo* kinetic studies in animals and humans. If the route of exposure for the toxicokinetic study in either animals or humans does not match that of the toxicity study on which the effect level or benchmark dose/concentration is based (which should also be the route by which humans are normally exposed), then the impact of route-to-route extrapolation will need to be critically assessed in relation to the development of a CSAF.

The *relevance of dose/concentration* needs also to be considered for all components. Ideally, CSAFs for toxicokinetics [AK_{AF}, HD_{AF}] are based on comparison of kinetic parameters determined in animals exposed to doses similar to the critical NOAEL, effect level or benchmark dose/concentration to those determined in human kinetic studies, where exposure is similar to the human equivalent concentration. Any discrepancies should be assessed for their potential impact on the dose metric and the validity of the resulting CSAF. For *in vitro* investigations which inform primarily CSAFs for toxicodynamic aspects [AD_{AF}] [HD_{AF}], relevant studies should include a suitable number of concentrations to adequately characterise the concentration-response relationship. Where the concentration-response curves in animals and humans or in different human subgroups are parallel, selection of the point for comparison can be anywhere between 10 and 90% response on the dose response curve. Where the curves are not parallel, the point for comparison should be the lowest point on the concentration-response curve that provides reliable information without extrapolation below the experimental data (e.g., the EC_{10}).

The *adequacy of numbers of subjects/samples* needs also to be considered in relation to all components of the framework. For interspecies comparisons in both kinetics and dynamics [AK_{AF}] [AD_{AF}], the numbers of animals and humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency for each species. As pragmatic advice, it is suggested that for both *in vivo* and *in vitro* measurements, the number of subjects within the population, or within the major sub-groups (if there are two or more groups on which the estimate of central tendency is based), should be such that the standard error (standard deviation of the sample divided by the square root of the sample size) is less than 20% of the mean. Based on practical experience, this would normally translate to a minimum of 5 subjects/samples, unless the coefficient of variation is very low. For considerations of factors related to within human variability (HK_{AF}, HD_{AF}), the numbers of humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency and of the population distribution.

Integrating Development of CSAFs with Mode of Action Frameworks – An Example

Examples of the development of CSAFs have been presented previously.[12-15] In the period since the posting of the initial guidance on CSAFs, however, frameworks for systematic and transparent consideration of the weight of evidence for humans of the relevance of hypothesized modes of action in animals have been developed. These frameworks set the scene for more fulsome consideration of relevant implications for dose-response, including development of CSAFs.[16-18]

An example is presented here which integrates consideration of relevant data in a weight of evidence framework for human relevance of cancer and pre-cursor key events (in hazard characterization) and subsequently, development of a CSAF for **AK_{AF}** that addresses the total combined oral, dermal and inhalation exposures to chloroform. The example includes information from two previous analyses[19,20] where external doses were translated to an internal dose (dose metric) through PBPK modeling as a basis for species extrapolation.

For chloroform, the weight of evidence for genotoxicity, sex and strain specificity and concordance of sustained cytotoxicity, persistent regenerative proliferation and tumours (liver and kidney tumours in mice and kidney tumours in

rats) is consistent with the hypothesis that marked cytotoxicity concomitant with a period of sustained cell proliferation likely represents a critical key (and potentially rate limiting) event for tumor induction following exposure. This cytotoxicity is primarily related to oxidation rates of chloroform to reactive intermediates, principally phosgene and hydrochloric acid.[19]

A concordance analysis for human relevance for the hypothesized mode of induction of tumours in animals exposed to chloroform is summarized in Table 1 for the liver and kidney. The information summarized in the concordance tables leads to the conclusion that the weight of evidence for the hypothesized mode of induction of tumours (i.e., metabolism by the target cell population, induction of sustained cytotoxicity by metabolites and subsequent persistent regenerative cell proliferation) is greatest for liver and kidney tumours in mice, followed by kidney tumours in rats.

Uncertainty could be reduced by additional information on metabolism, cytotoxicity and proliferative response in the strain in which tumours were observed (i.e., Osborne-Mendel rats) following long-term exposure. Additional data on metabolism and chronic (e.g., 2-year) cytotoxic/proliferative response in the kidneys of F344 rats would also contribute to greater confidence in the hypothesized mode of action. Though data in humans are limited, based on expected similar response in humans and in the absence of data to the contrary, the mode of action for chloroform-induced tumours in animals is considered to be qualitatively applicable to humans. Limited available data in humans confirm that target organs in populations exposed to high concentrations are similar to those in experimental animals (i.e., the kidney and liver).

Toxicokinetic comparisons in both rats and humans indicate that critical to the MOA is the requirement for a metabolized dose sufficient to produce cytotoxicity and subsequent cellular regeneration. This analysis, then, sets the stage for identification of critical pre-cursor non-cancer key events for which subsequent quantitation of interspecies differences and interindividual variability in dose response analysis is relevant. This includes application of a physiologically-based pharmacokinetic model as a basis to consider interspecies variation in rates of formation of reactive metabolites in the target tissue which constitutes a reasonable basis for replacement of the default subfactor for interspecies differences in toxicokinetics with a CSAF (AK_{AF}).

In view of the convincing weight of evidence for the hypothesized mode of action, the optimum approach to quantitation of exposure–response might involve analysis of the incidence of essential noncancer precursor events (cytotoxicity and regenerative proliferation) from interim kills in the critical cancer bioassay (i.e., that in which tumours were observed at lowest dose, following administration by the route most relevant to humans – i.e., continuously in drinking water) on the basis of rates or amounts of oxidative metabolites produced per volume of tissue in the critical organ. Unfortunately, data on precursor lesions were not collected in the critical bioassay. Re-examination of a proportion of the slides from several of the dose groups, however, confirmed histopathological changes consistent with the hypothesis that sustained tubular cytotoxicity and regenerative hyperplasia in the critical study led to renal tubular tumor induction, though the data amenable for quantitation of exposure–response in this investigation were limited.

Table1. Concordance analysis - key events - animals and humans - liver and kidney tumours – chloroform

Key Event	Animals- Liver	Humans – Liver	Weight of Evidence
Metabolism by cyp2E1	Incidence/severity of toxicity correlate with covalent binding of metabolites in rats and mice, more prevalent in necrotic lesions	Irreversible binding to macromolecules in human liver microsomes requires prior metabolism; PBPK model based on human physiological parameters and metabolic parameters *in vitro* in eight human liver samples	considerable in animals, limited in humans
Sustained cytotoxicity	In all cases where examined, sustained cytotoxicity (as measured by histopathological effects and release of hepatic enzymes) in the liver of mice at doses that induce tumours	Liver also a target organ in humans based on reports of effects associated with occupational exposure	considerable in animals, limited in humans
Persistent, regenerative proliferation	In all cases where examined, persistent regenerative proliferation (as measured by labelling indices) in the liver of mice at doses that induce tumours	No data	considerable in animals, none in humans
Liver Tumours	Mice	Inadequate epidemiological data	considerable in animals, inadequate in humans
Metabolism by cyp2E1	In mice, strain and sex-related differences correlate with metabolism; necrosis correlates with the degree of covalent binding; few such data for rats and in F344 rats, nephrotoxicity not correlated with bioactivition	Quantitation in. PBPK model based on human physiological parameters and activity in the microsomal fraction of kidneys to that in the microsomal fraction of the liver *in vitro* supported by data on metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans	considerable in mice, unconvincing in rats and limited in humans
Sustained cytotoxicity	In mice, in all cases where examined, sustained cytotoxicity (as measured by histopathological effects and release of hepatic enzymes), at doses that induced tumours; in rats in critical bioassay, cytotoxicity based on histopathological reexamination	Kidney also a target organ in humans based on reports of renal effects resulting from anesthetic use of chloroform	considerable in mice though less data than for liver, less in rats and limited in humans
Kidney tumours	Mice and rats	Inadequate epidemiological data	Considerable in animals; inadequate in humans

There have been numerous subsequent shorter-term investigations of the proliferative response in the liver and kidney of various strains of mice and rats exposed to doses and concentrations of chloroform similar to those administered in the cancer bioassays in which tumors have been observed. However, for renal tumors, most of these investigations have been conducted in a strain of rat (F344) which varies from that, in which increases in renal tumors in the critical bioassay were observed (i.e., the Osborne-Mendel rat). Moreover, limited available data indicate that patterns of response (e.g., sex specific) between the two strains vary. Available data are also inadequate as a basis of characterization of the relative sensitivity of the two strains to cytotoxicity.

Since quantitative data on the incidence of precursor lesions for cancer in the strain of interest are inadequate to meaningfully characterize exposure–response, a tumorigenic concentration was developed based on the incidence of tubular cell adenomas and adenocarcinomas in the critical bioassay in Osborne-Mendel rats[21].

In order to describe dose-response in the context of rates of formation of active metabolites in the target tissue (renal cortex), a PBPK model was developed and extended to humans. In the animal component of the PBPK model, the liver and kidney were described as individual sites of metabolism including regions of both high and low activity. The maximum rate of metabolism in the kidney was scaled to the maximum rate in the liver based on relative tissue volumes and a proportionality constant. In the human component of the PBPK model, the kinetics were described by a single-compartment, based on simulation of an available study in humans in which metabolized and exhaled chloroform were determined for up to 8 hours following administration to male and female volunteers in olive oil or gelatin capsules. Liver tissue subvolumes were assumed to be the same as in the rat, while the kidney was subdivided into a 70:30 cortex:noncortex ratio, as per reference man described by the International Commission on Radiological Protection. Blood flow to the kidney was also split between the cortex (90%) and non-cortex (10%). Human metabolic parameters were those determined *in vitro* in eight human liver samples. Kidney metabolic rate constants were based on the relationship of activity observed in the microsomal fraction of kidneys to the activity observed in the microsomal fraction of the liver based on *in vitro* results but supported by data on metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans.

The combined incidence of renal adenomas and adenocarcinomas and the more limited histological data on cytotoxicity in the critical study were considered in the context of the mean rate of metabolism (i.e., cumulative metabolite formed per gram of tissue in the kidney cortex). Benchmark doses for tumours and histological lesions were converted to internal dose metrics based on the animal component of the PBPK model (amount metabolized per time per unit volume renal cortex tissue – 4 mg/hr/L). The human component was then run to estimate the total external exposure associated with this fixed value for the internal dose metric. Because it was assumed that all chloroform exposure (oral, dermal inhalation) was due to chloroform in drinking water, and because the critical effect was independent of dose route, the combined equivalent human exposure was expressed in units of mg/kg/day. The external exposure of humans associated with the relevant measure of exposure–response, i.e., the estimated mean rate of metabolism in the kidney cortex associated with a 5% increase in tumor risk (TC_{05}) based on the PBPK model, was

92.8 mg/kg b.w./day. This was compared with the comparable external dose for rats of 41.2 mg/kg b.w./day.

The external dose-adjusted ratio of the human dose metric to the animal dose metric was [4 mg/hr-L/92.77 mg/kg/day] divided by [4 mg/hr-L/41.17 mg/kg/day] and this factor (0.44) constitutes the basis for development of an $\mathbf{AK_{AF}}$, in lieu of the default value of 4. Since the PBPK model incorporates the amount metabolized per time per unit volume tissue (a component of toxicodynamics in the context of the default subfactors for CSAF), retention of the full default factor of 2.5 for species differences in toxicodynamics is considered conservative. The resulting interspecies CSAF would be 0.44 x 2.5 = 1.1. With no data to inform the intraspecies extrapolation, the default value of 10 would be retained for human variability, and the composite factor would be 1.1 x 10 = 11.

For the PBPK model, among those parameters considered in the sensitivity analysis to have most impact on output, uncertainty was greatest for the metabolic parameters particularly in the kidney for humans. As a result, additional *in vitro* data on the metabolism of chloroform in the human kidney and liver would be useful not only to reduce uncertainty in derived values, but, potentially to address the issue of variability across the human population.

Conclusions

Consideration of relevant data in the context of a framework that addresses kinetic and dynamic aspects, explicitly, should result in greater understanding of contributing components and transparency in risk assessment. It is also hoped that consideration in this context will lead to clearer delineation and better common understanding of the nature of specific data required which would permit development of more informative measures of dose response.

Integration with recently developed frameworks for consideration of the weight of evidence for hypothesized modes of action for both cancer and non-cancer effects additionally contributes to transparency in risk assessment through explicit delineation and consideration of appropriate key events for subsequent dose-response analysis. The example presented here which includes consideration of the mode of action for induction of tumours and subsequent dose-response analysis for non-cancer precursor key events is illustrative of the manner in which these analytical frameworks for hazard characterization and dose-response analyses are contributing to the harmonization of approaches for cancer and non-cancer effects.

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Chapter 10

Chloroform: Exposure Estimation, Hazard Characterization, and Exposure-Response Analysis

Meek, M.E., Beauchamp, R., Long, G., Moir, D., Turner, L. and Walker, W. (2002)

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ABSTRACT

Chloroform has been assessed as a Priority Substance under the **Canadian Environmental Protection Act.**

The general population in Canada is exposed to chloroform principally through inhalation of indoor air, particularly during showering, and ingestion of tap water. Data on concentrations of chloroform in various media were sufficient to serve as the basis for development of deterministic and probabilistic estimates of exposure for the general population in Canada.

On the basis of data acquired principally in studies in experimental animals, chloroform causes hepatic and renal tumors in mice and renal tumors in rats. The weight of evidence indicates that chloroform is likely carcinogenic only at concentrations that induce the obligatory precursor lesions of cytotoxicity and proliferative regenerative response. Since this cytotoxicity is primarily related to rates of formation of reactive, oxidative metabolites, dose–response has been characterized in the context of rates of formation of reactive metabolites in the target tissue. Results presented here are from a "hybrid" physiologically based pharmacokinetic (PBPK) animal model that was revised to permit its extension to humans.

The relevant measure of exposure–response, i.e., the mean rate of metabolism in humans associated with a 5% increase in tumor risk (TC_{05}), was estimated on the basis of this PBPK model and compared with tissue dose measures resulting from 24-h multimedia exposure scenarios for Canadians based on midpoint and 95th percentiles for concentrations in outdoor air, indoor air, air in the shower compartment, air in the bathroom after showering, tap water, and food.

Nonneoplastic effects observed most consistently at lowest concentrations or doses following repeated exposures of rats and mice to chloroform are cytotoxicity and regenerative proliferation. As for cancer, target organs are the liver and kidney. In addition, chloroform has induced nasal lesions in rats and mice exposed by both inhalation and ingestion at lowest concentrations or doses. The mean rate of metabolism associated with a 5% increase in fatty cysts estimated on the basis of the PBPK model was compared with tissue dose measures resulting from the scenarios described above, and lowest concentrations reported to induce cellular proliferation in the nasal cavities of rats and mice were compared directly with midpoint and 95th-percentile estimates of concentrations of chloroform in indoor air in Canada.

The degree of confidence in the underlying database and uncertainties in estimates of exposure and in characterization of hazard and dose–response are delineated.

INTRODUCTION

Chloroform has been assessed as a Priority Substance under the *Canadian Environmental Protection Act* (CEPA). As summarized briefly here, this assessment was composed of population exposure estimation, hazard characterization, exposure–response analyses, and risk characterization, along with a discussion of the uncertainties and degree of confidence associated with the evaluation. In view of the volume of relevant information, the scope of this text is necessarily restricted to the most important sources of human exposure and those effects considered critical. Additional information on sources of exposure not addressed herein (e.g., consumer

products and swimming pools) and effects not considered critical is included in the more extensive Supporting Documentation and Assessment Report, which served as the basis for preparation of this paper, available from the Health Canada Existing Substances website. These documents were externally reviewed by experts identified in the Acknowledgements.

Chloroform is used as a solvent, in the production of other chemicals, and in some imported pesticide formulations. Significant releases of chloroform arise indirectly through reactions of chlorine with organic chemicals and as a by-product during the addition of chlorine to drinking water and wastewaters for disinfection. Sources of chloroform in food are not clearly understood, although migration from packaging solvents, glues, and inks has been documented, and the use of chlorinated water by bottling plants may explain the presence of chloroform in some beverages.

POPULATION EXPOSURE

Concentrations in Environmental Media

Identified relevant data on concentrations of chloroform in environmental media in Canada included those on concentrations in 8807 samples of ambient air collected during the 1990s in the National Air Pollution Surveillance (NAPS) program (Dann, 1998) and those from a national survey of the indoor air of 754 Canadian homes (Concord Environmental Corporation, 1992; Health Canada, 1999). Although national surveys are available (Williams et al., 1995), estimates of intake in drinking water were based on monitoring data for samples collected within water treatment plants and their distribution systems from the provinces and territories, which included much larger numbers of samples over an extended time frame (detected in 96% of 6607 samples of drinking water obtained from Canadian provinces and territories in the 1990s). These data were also more representative of the water supplies of a larger proportion of the population and lead to more conservative estimates of intake, although they were collected and analyzed by less consistent, less reliable, and less comparable methodology than for the national surveys (Health Canada, 1999).

Exposure to volatile components of tap water, including chloroform, through inhalation and/or dermal absorption may be greater than exposure through ingestion of tap water from the same source (Shimokura et al., 1998). Common domestic water uses, including showering and bathing, involve far greater volumes of water daily than that ingested, as well as extensive dermal contact. In addition, concentrations of chloroform and other volatile organic compounds increase when water is heated, such as in domestic hot water tanks (Weisel & Chen, 1994; Benoit et al., 1998), and the contaminants cannot volatilize, as the domestic hot water tank and associated plumbing form a closed system, with no headspace**.**

In view of the limited focus of currently available experimental data sets on measured levels of chloroform in the air of showers or exhaled breath during showering, estimates of the average concentration of chloroform in the air of a shower compartment during a 10-min shower were developed for the present assessment based on measured concentrations of chloroform in drinking water supplies across Canada (Health Canada, 1999). Lower estimates were based on an assumed concentration of chloroform of 50 µg/L, which is approximately the mean

concentration of chloroform in water in Canada (i.e., 47.3 µg/L, according to provincial/territorial data). Assuming water flow rates of 5 or 10 L/min, a water temperature of 40°C, a transfer efficiency of 50%, a 10-min shower duration, and minimum air exchange, the range of average concentrations of chloroform in the air of the shower compartment was estimated as 417–833 µg/m^3. With similar assumptions, but a concentration of chloroform in water of 166 µg/L (i.e., the 95th percentile of the distribution of concentrations, according to provincial/territorial data; Health Canada, 1999), the range of average concentrations of chloroform in the air of the shower compartment was estimated as 1382–2765 µg/m^3 (Health Canada, 1999). This approach, though limited, takes into account the considerable variability in levels of chloroform in water supplies across Canada.

No data were available on the concentrations of chloroform in the air of a bathtub enclosure or in the breathing zone of a bather as a function of the concentration of chloroform in the water in the tub. Therefore, no estimates of intake by inhalation during bathing could be derived. Consequently, the total intakes of chloroform during typical baths and showers are currently assumed to be approximately equal based on the considerations that most adults bathe less frequently than they shower (United States Environmental Protection Agency [U.S. EPA], 1997), a bath faucet is less efficient than a shower head at stripping chloroform from water, and the water contacting the skin is not being constantly replaced by "newer" water (as in a shower). On the other hand, the average duration of baths is typically longer than the average duration of showers (U.S. EPA, 1997), resulting in longer periods of both inhalation and dermal exposure to chloroform.

Identified data on concentrations of chloroform in foodstuffs in Canada were restricted to detection in 13 samples of various beverages (i.e., juices, soft drinks, milk) and dry foods (decaffeinated coffee and tea) purchased in Ottawa, Ontario, and 41 of 47 additional samples of foods and beverages also purchased from supermarkets in Ottawa. The highest concentrations reported (i.e., 50, 83, and 129 µg/kg) were in butter (Page & Lacroix, 1995). Data from Canada and the United States (Heikes, 1987; Daft, 1988a, 1988b; Miller & Uhler, 1988; Heikes et al., 1995; McNeal et al., 1995) were sufficient to serve as a basis for estimating the minimum, midpoint, and maximum concentrations of chloroform for 131 of the 181 foods for which per capita daily intake rates (i.e., g/d) are available for estimation of the daily intake of chemical substances from the ingestion of foods and beverages (Environmental Health Directorate [EHD], 1998; Health Canada, 1999). Detectable concentrations were present in 79 of the 131 food items, while concentrations were less than the limits of detection in the remaining 52 items. The midpoint estimates of concentrations were greater than 100 µg/kg in 12 food items (i.e., butter, margarine, vegetable fats and oils, baby food cereal, pizza, marine fish, fresh fish, crackers, pancakes, veal, beef roast, and cheese).

Deterministic Estimates of Exposure to Chloroform for the General Population

Point estimates of the average daily intake (per kg body weight), based on the data described briefly above and on reference values for body weight, inhalation volume, and amounts of food and drinking water consumed daily, were calculated for six age groups. Average intake was estimated to range from 0.6 to 10.3 µg/kg body weight per day. The upper value in this range is for infants 0–6 mo of age and is based on

the assumption that infants are exclusively formula fed during this period, with powdered infant formula reconstituted with tap water containing the maximum annual mean concentration of chloroform (i.e., 89.4 µg/L) as determined from provincial/territorial data. If it is assumed instead that infants are fed table-ready foods containing the same concentrations of chloroform as assumed for the remaining five age groups, the estimated average daily intakes for infants are much lower, ranging from 0.2 to 1.1 µg/kg body weight per day; for the six age groups, the average daily intakes then range from 0.2 to 6.9 µg/kg body weight per day.

Upper bounding estimates of the daily intake, based on the maximum reported concentrations of chloroform in indoor and outdoor air and in drinking water in Canada and on the maximum reported concentrations in foods in Canada and the United States, were also developed for six age groups. These were also based on the reference values for body weight, inhalation volume, and amounts of food and drinking water consumed daily (EHD, 1998). Upper bounding estimates of daily intake ranged from 40 to 95 µg/kg body weight per day. It is assumed that infants are fed table-ready foods only and that their average intake of total tap water is 0.3 L/d (EHD, 1998). If it is assumed instead that infants are exclusively formula fed and that powdered infant formula is prepared with tap water containing the maximum reported concentration in Canada (i.e., 1224 µg/L), the upper bounding estimate of total daily intake for infants is more than twice as high (i.e., 147.6 µg/kg body weight per day, with 130.6 µg/kg body weight per day resulting from ingestion of total tap water).

The contribution of outdoor air to the estimates of average total daily intakes is considerably less than the contributions from indoor air, food, and water, which are approximately similar in magnitude. The contributions of outdoor air and food to the upper bounding estimates of total daily intake are considerably less than the contributions from indoor air and tap water. On the basis of these deterministic estimates, the main pathways of exposure to chloroform for the general population in Canada are inhalation of indoor air and ingestion of tap water. It is also apparent from these deterministic estimates that the average daily intake from a single daily 10-min shower can exceed the intake from all other exposure pathways.

Probabilistic Estimates of Exposure to Chloroform for the General Population

Probabilistic estimates of exposure for six age groups of the general population were developed based on distributions of the concentrations of chloroform in outdoor air, indoor air, and drinking water in Canada from the same sources that served as the basis for the deterministic estimates (Health Canada, 1999). Age group-specific lognormal distributions of daily intake rates for these media were also assumed (EHD, 1998). Data were considered insufficient to develop probabilistic estimates of exposure from ingestion of foods or from showering (Health Canada, 1999).

Probabilistic estimates were generated in an Excel (Microsoft Corporation, 1997) spreadsheet using Crystal Ball (Decisioneering, Inc., 1996). Age group-specific body weights and rates of intake of air and tap water were assumed to be lognormally distributed and are characterized by their geometric means and standard deviations (EHD, 1998). A normal distribution of hours per day spent outdoors is assumed, characterized by an arithmetic mean and standard deviation of 3.0 ± 2.0 h

(EHD, 1998) and truncated at 0 and 9 h. The same distribution is assumed for each of the age groups (Health Canada, 1999).

Two scenarios were developed for estimating daily intakes from exposure to chloroform in outdoor and indoor air and tap water. In a scenario for general population exposure, the following distributions of concentrations were assumed. For outdoor air, this was based on the distribution of chloroform in the air of 8807 samples collected during the 1990s in the NAPS program (Dann, 1998). For indoor air, it was based on the estimated geometric mean and standard deviation of an assumed lognormal distribution of chloroform in the indoor air of 754 Canadian homes (Concord Environmental Corporation, 1992; Health Canada, 1999). For tap water, the distribution of chloroform in the treated drinking water of 6607 samples, based on provincial/territorial data, was assumed.

In a reasonable worst case (RWC) exposure scenario, the following distributions of concentrations were assumed. For outdoor air, the distribution of chloroform in air was that in 800 samples collected during the 1990s from four sites adjacent to major roadways in the NAPS program (Dann, 1998). For indoor air, this was again based on the estimated geometric mean and standard deviation of an assumed lognormal distribution of the concentrations of chloroform in the indoor air of 754 Canadian homes (Concord Environmental Corporation, 1992; Health Canada, 1999), since these data were inadequate as a basis to define a subset of concentrations for use in the RWC scenario. For tap water, the distribution of chloroform in the treated drinking water of 2597 samples, based on data from Manitoba and Alberta only, where reported concentrations were highest, was assumed.

Simulations of 10,000 trials were run 5 times each using two sampling methods (i.e., Monte Carlo random and Latin Hypercube) to gauge the reproducibility of the parameters estimated. For the average population scenario, the 95th percentiles of the distribution of intakes from inhalation and ingestion of drinking water for five age groups of the general population (i.e., 0.5 yr to 60+ yr of age) range from 4.9 to 12.9 µg/kg body weight per day (Health Canada, 1999). Similar estimates were obtained from each of the two sampling methods. The relative standard deviations (for n = 5 simulations of 10,000 trials each) of the upper-percentile estimates of intake did not exceed 5%, indicating a high degree of reproducibility.

For the RWC scenario, the 95th percentiles of the distribution of intakes from inhalation and ingestion of drinking water for five age groups of the general population (i.e., 0.5 yr to 60+ yr of age) range from 7.0 to 19.1 µg/kg body weight per day (Health Canada, 1999). Similar estimates were obtained from each of the two sampling methods. The relative standard deviations (for n = 5 simulations of 10,000 trials each) of the upper-percentile estimates of intake did not exceed 7%, indicating a high degree of reproducibility.

For both the population exposure and RWC scenarios, due to limitations of the data concerning the daily intake rate of total tap water by infants (EHD, 1998), probabilistic estimates could not be developed for the sixth age group (i.e., birth to 0.5 yr).

HAZARD CHARACTERIZATION

Effects observed most consistently at lowest concentrations or doses following short-term and subchronic repeated exposures to chloroform in rats and mice are cytotoxicity and regenerative proliferation in the liver (centrilobular region) and kidney (cortical region). Chloroform has also induced nasal lesions in rats and mice exposed by both inhalation and ingestion at similarly lowest concentrations or doses. In carcinogenicity bioassays in rats and mice following exposure both orally and by inhalation, chloroform has been carcinogenic in the mouse liver and in the male rat and male mouse kidney. The carcinogenic response has varied with different routes and vehicles of exposure and among sexes, species, and strains.

Effects on the hematological, neurological, and immunological systems have been observed less consistently and only at concentrations higher than those reported to induce effects on the liver, kidney, and nose (see Assessment Report). Teratogenic effects have not been reported. Developmental/reproductive effects have been restricted to those observed most often at dose levels that caused other manifestations of systemic toxicity in the same studies, primarily hepatic effects. Such effects have also only been observed at doses greater than the lowest values reported in other studies to induce effects on the liver, kidney, or nose.

Limited identified data in humans indicate that the principal target organs (i.e., liver and kidney) are similar to those in experimental animals. There have been infrequent reports of renal tubular necrosis and renal dysfunction resulting from the use of chloroform as an anesthetic (Kluwe, 1981). The lowest levels at which liver toxicity due to occupational exposure to chloroform has been reported are in the range of 80–160 mg/m^3 (with an exposure period of less than 4 mo) in one study and 10–1000 mg/m^3 (with exposure periods of 1–4 yr) in another study (World Health Organization [WHO], 1994). Available epidemiological data are inadequate to assess the carcinogenicity of chloroform in human populations.

There is considerable information on the potential mode of induction of liver and kidney tumors by chloroform in rats and mice, including a range of metabolic studies. In addition, while there have been no cancer bioassays in which a range of intermediate endpoints has been investigated, proliferative response in target organs has been examined in numerous subsequent investigations following exposure via regimens similar to those in the long-term studies. The histopathology in the target organ for one of the more critical studies has also been reexamined (Hard et al., 2000). These data have been generated to investigate primarily the hypothesized mode of action for tumor induction in rodents that requisite precursor steps to cancer are metabolism of chloroform by the target cell population, induction of sustained cytotoxicity by metabolites, and subsequent persistent regenerative cell proliferation.

Since liver and kidney cancer are putatively the critical effects of long-term exposure to chloroform, this section focuses primarily on information relevant to assessment of the weight of evidence of cancer, genotoxicity, and additional information relevant to assessment of the weight of evidence for this hypothesized mode of action, including concordance of observations of tumors with histopathological evidence of cytotoxicity and proliferative response and the relationship of metabolism with tissue damage. Although the hypothesized modes of induction of liver and kidney tumors are similar, the weight of evidence varies, and, as a result, they are addressed separately here.

Carcinogenicity

Summaries of tumor incidence in the identified carcinogenesis bioassays are presented in Table 1 for the liver and Table 2 for the kidney.

TABLE 1. Summary of Liver Tumor Response to Chloroform (Modified from ILSI, 1997)

Exposure	Dose levels in protocol (mg/kg body weight)[a]	Dose[b] (mg/kg body weight)[a]	Duration (weeks)	Strain	Sex	Response (%)[c]	Reference
Mouse							
Corn oil	0 138 277	138	78	B6C3F1	Male	27 (POS)[d]	NCI (1976)
Corn oil	0 238 477	238	78	B6C3F1	Female	74 (POS)[d]	NCI (1976)
Water	0 34 65 130 263	263	104	B6C3F1	Female	(Neg.)[e]	Jorgenson et al. (1985)
Inhalation	0 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Male	7	Yamamoto (1996)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Female	8	Yamamoto (1996)
Toothpaste	0 17 60	17	104	ICI	Male	26	Roe et al. (1979)
Toothpaste	0 17 60	60	104	ICI	Female	(Neg.)[e]	Roe et al. (1979)
Rat							
Corn oil	0 90 180	180	111	O-M[f]	Male	6	NCI (1976)
Corn oil	0 100 200	200	111	O-M[f]	Female	–4	NCI (1976)
Water	0 19 38 81 160	160	104	O-M[f]	Male	(Neg.)[e]	Jorgenson et al. (1985)
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Male	(Neg.)[e]	Yamamoto (1996)

Exposure	Dose levels in protocol (mg/kg body weight)[a]	Dose[b] (mg/kg body weight)[a]	Duration (weeks)	Strain	Sex	Response (%)[c]	Reference
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Female	(Neg.)[e]	Yamamoto (1996)
Water	One dose level only	>100	185	Wistar	Male	(Neg.)[e]	Tumasonis et al. (1987)
Water	One dose level only	>150	185	Wistar	Female	25 (POS)[d,g]	Tumasonis et al. (1987)
Toothpaste	0 15 75 165	165	80	S-D[h]	Male	0	Palmer et al. (1979)
Toothpaste	0 15 75 165	165	80	S-D[h]	Female	0	Palmer et al. (1979)

[a] Unless otherwise specified.
[b] Lowest dose giving a positive response or highest dose giving a negative response.
[c] Percent increase of tumor rate over controls; ( ) for decrease over controls.
[d] POS = statistically significant increase in liver neoplasms.
[e] Actual tumor data not given.
[f] Osborne-Mendel rats.
[g] Treated animals survived longer than controls (185 vs. 145 weeks).
[h] Sprague-Dawley rats.

TABLE 2. Summary of Kidney Tumor Response to Chloroform (Modified from ILSI, 1997)

Exposure	Dose levels in protocol (mg/kg body weight)[a]	Dose[b] (mg/kg body weight)[a]	Duration (weeks)	Strain	Sex	Response (%)[c]	Reference
Mouse							
Corn oil	0 138 277	138	78	B6C3F1	Male	–2	NCI (1976)
Corn oil	0 238 477	238	78	B6C3F1	Female	0	NCI (1976)
Water	0 34 65 130 263	263	104	B6C3F1	Female	(Neg.)[d]	Jorgenson et al. (1985)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	30 ppm	104	BDF1	Male	14 (POS)[e]	Yamamoto (1996)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Female	(Neg.)[d]	Yamamoto (1996)
Toothpaste	0 17 60	60	104	ICI	Male	21 (POS)[e]	Roe et al. (1979)
Toothpaste	0 17 60	60	104	ICI	Female	(Neg.)[d]	Roe et al. (1979)
Toothpaste	One dose level only	60	104	C57BL	Male	(Neg.)[d]	Roe et al. (1979)
Toothpaste	One dose level only	60	104	CBA	Male	(Neg.)[d]	Roe et al. (1979)
Toothpaste	One dose level only	60	104	CF/1	Male	(Neg.)[d]	Roe et al. (1979)
Corn oil	0 90 180	180	111	O-M[e]	Male	24 (POS)[f]	NCI (1976)
Corn oil	0 100 200	200	111	O-M[e]	Female	4	NCI (1976)
Water	0 19 38 81 160	160	104	O-M[e]	Male	13 (POS)[f]	Jorgenson et al. (1985)
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Male	(Neg.)[d]	Yamamoto (1996)

Exposure	Dose levels in protocol (mg/kg body weight)[a]	Dose[b] (mg/kg body weight)[a]	Duration (weeks)	Strain	Sex	Response (%)[c]	Reference
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Female	(Neg.)[d]	Yamamoto (1996)
Water	One dose level only	>100	185[d,g]	Wistar	Male	7	Tumasonis et al. (1987)
Water	One dose level only	>150	185[d,g]	Wistar	Female	0	Tumasonis et al. (1987)
Toothpaste	0 15 75 165	165	80	S-D[h]	Male	0	Palmer et al. (1979)
Toothpaste	0 15 75 165	165	80	S-D[h]	Female	0	Palmer et al. (1979)

[a] Unless otherwise specified.
[b] Lowest dose giving a positive response or highest dose giving a negative response.
[c] Percent increase of tumor rate over controls; ( ) for decrease over controls.
[d] Actual tumor data not given.
[e] Osborne-Mendel rats.
[f] POS = statistically significant increase in renal neoplasms.
[g] Treated animals survived longer than controls (185 vs. 145 weeks).
[h] Sprague-Dawley rats.

Liver Chloroform is carcinogenic in the male and female mouse liver (National Cancer Institute [NCI], 1976), but only following gavage in a corn oil vehicle. It was not carcinogenic in the liver of mice exposed in drinking water (Jorgenson et al., 1985), although the daily doses were similar to those administered by corn oil gavage (NCI, 1976). Similarly, it was not carcinogenic by inhalation, despite an exposure escalation strategy that achieved final concentrations that were several fold greater than those considered acutely lethal (Yamamoto, 1996). Thus, neither the daily dose nor the cumulative dose of chloroform was predictive of tumor outcome following exposure in drinking water.

In a single study, there was an increased incidence of liver tumors in female Wistar rats following administration of chloroform in drinking water. The control group in this investigation was small, and the longer survival of the exposed females (185 wk) compared with the control females (145 wk) complicates interpretation of the results (Tumasonis et al., 1985, 1987). In other studies in which chloroform was administered in drinking water, by gavage in corn oil, or by inhalation to various strains of rats, the incidence of liver neoplasia was not increased (NCI, 1976; Palmer et al., 1979; Jorgenson et al., 1985; Yamamoto, 1996).

Kidney Chloroform has induced renal tumors in both rats and mice, but only in males. Renal tubular cell tumors were observed in mice exposed to chloroform by inhalation (Yamamoto, 1996) or in toothpaste preparations (Roe et al., 1979) and in rats exposed by corn oil gavage (NCI, 1976) or in drinking water (Jorgenson et al.,

1985). The responses varied with route of exposure, administration vehicle, and strain.

There was a significant increase in renal tumors in male BDF1 mice following inhalation (Yamamoto, 1996) and in male ICI mice exposed in either toothpaste or arachis oil (Roe et al., 1979). However, there were no renal tumors in male B6C3F1 mice following exposure to chloroform by corn oil gavage (NCI, 1976) or in drinking water (Jorgenson et al., 1985). Responses were positive in Osborne-Mendel rats (drinking water) (Jorgenson et al., 1985) but not in F344 rats (inhalation) (Yamamoto, 1996) or Sprague-Dawley rats (toothpaste) (Palmer et al., 1979).

In a recent study reported currently only as an abstract, Gollapudi et al. (1999) exposed transgenic $p53^{+/-}$ mice (who respond most effectively to mutagenic carcinogens) to chloroform by gavage in corn oil at doses up to 140 mg/kg body weight per day (males) or 240 mg/kg body weight per day (females) for up to 26 wk. Wild-type mice were also included in the protocol. Although renal tubular regeneration and proliferation of renal tubular epithelial cells were observed in males, there were no treatment-related increases in the incidence of any tumors.

The bioassay in which tumors were observed at lowest concentration or dose following exposure in a manner similar to that of humans (i.e., continuously in drinking water or by inhalation), namely kidney tumors in male rats, was that of Jorgenson et al. (1985). In this bioassay, male Osborne-Mendel rats were exposed to 0 (n = 330), 200 (n = 330), 400 (n = 150), 900 (n = 50), or 1800 mg/L (n = 50) in drinking water (corresponding to time-weighted average daily doses of 0, 19, 38, 81, and 160 mg/kg body weight) for 2 yr. Matched controls received an amount of water without chloroform equal to that consumed by the 1800 mg/L group. Clinical chemistry indicated renal impairment in control animals but not in the groups receiving 900 or 1800 mg/L. Renal impairment in the 200 and 400 mg/L dose groups was mild. These results are consistent with the occurrence of severe chronic nephropathy in the control animals associated with caloric overload on an *ad libitum* diet and a protective effect of dietary restriction in the high-dose exposed groups associated with reduced consumption of drinking water. Consistent with these results, mortality was decreased in the matched control group and inversely related to concentration of chloroform in the exposed groups. Data on organ weights were not provided. The only clear dose-related effect was an increase in renal tubular cell adenomas and adenocarcinomas, with combined incidence being significantly increased at the top dose. The incidence of tubular cell adenomas and adenocarcinomas (combined) was 4/301, 1/50, 4/313, 4/148, 3/48, and 7/50 ($p < 0.01$) ($p < 0.001$ for trend) for control, matched control, and the 19, 38, 81, and 160 mg/kg body weight groups, respectively.

Kidney tissue from the Jorgenson et al. (1985) investigation has recently been microscopically reevaluated for evidence of cytotoxicity and regeneration (Hard & Wolf, 1999; Hard et al., 2000). Detailed results of this reevaluation are presented in Table 3. This reexamination included a portion of animals in the untreated control group and all animals in the four dose groups sacrificed at 104 wk (the number of tissues in each group for which it was possible to evaluate chloroform-related cytotoxicity ranged from 16 to 48). Kidneys from rats at interim time points were also examined; however, slides from water-matched controls and the low-dose group at 2 yr were not available. Slides from the 104-wk sacrifices were read independently by each of three authors; those for interim time points for selected dose groups were evaluated in blinded fashion by one of the authors. Toxic injury in proximal tubular epithelial cells was observed in all high-dose (1800 mg/L, the dose at which there

was a statistically significant increase in tumor incidence) males at all time points and approximately half of animals receiving the second highest dose (900 mg/L) for 18 or 24 mo. None of the other treatment groups or controls had these characteristic changes. The chloroform-associated alterations were characterized by slightly increased basophilia, cytoplasmic vacuolation, karyomegaly, anisokaryosis, nuclear crowding, and mild tubular hyperplasia. The cytotoxic tubular lesions, occasional foci of atypical tubular hyperplasia, and incipient renal tubular tumors were all located in the mid to deep cortex.

TABLE 3. Pertinent Histopathological Findings in Kidneys of Male Osborne-Mendel Rats in Drinking Water Bioassay by Jorgenson et al. (1985) (from Hard et al., 2000)

Group	No. of months on test	Total in group	No. of rats examined[a]	Effective number evaluated for cytotoxicity[b]	Percentage of effective number with lesions[c] of chloroform cytotoxicity	Mean grade of chronic progressive nephropathy[d]	Percent renal adenomas and carcinomas reported by Jorgenson et al. (1985)
Untreated	24	330	43	24	0	3.6	1.3
control	18	20	19	19	0	2.7	
	12	20	20	20	0	1.8	
	6	20	20	20	0	0.9	
Water-	24	50	0				2.0
matched	18	18	18	18	0	1.4	
control	12	19	19	19	0	1.1	
	6	19	19	19	0	0.9	
1800	24	50	49	46	100	0.9	14.0
ppm	18	20	18	17	100	0.9	
	12	19	18	18	100	0.6	
	6	20	20	20	95	0.6	
900 ppm	24	50	48	48	50[e]	1.7	6.3
	18	20	19	10	58[e]	1.6	
	12	20	20	20	33[e]	1.0	
	6	20	20	20	25[e]	0.8	
400 ppm	24	150	40	40	0	2.9	2.7
	18	20	20	19	0	2.3	
200 ppm	24	330	0				1.3
	18	20	16	16	0	2.3	

[a] Discrepancy in numbers attributed to missing slides.
[b] Excludes rats in which autolysis, end-stage chronic progressive nephropathy, or other diffuse disease process prevented evaluation of chloroform-related cytotoxicity.
[c] Histological changes indicative of tubule injury, e.g., nuclear crowding, cytoplasmic vacuolation, and faint basophilia in the mid to deep cortex.
[d] Spontaneous, age-related chronic progressive nephropathy; scores by D.C. Wolf, U.S. EPA.
[e] Chloroform-associated lesions were of a much lower grade than at 1800 ppm.

Although a systematic evaluation was not possible due to degradation of the slides and frequent autolytic change, the authors confirmed that such changes were also present in males of the same strain in the NCI (1976) bioassay in which exposure was by corn oil gavage. An incidental finding was the striking difference in the dimensions of renal tumors induced by chloroform administered by corn oil gavage (approximately 2-fold greater) compared with those arising from exposure via drinking water in the investigation by Jorgenson et al. (1985) (Hard et al., 2000).

Cytotoxicity and Regenerative Proliferative Response

Following exposure by inhalation, effects at the site of contact are limiting, with proliferation in the nasal passages being reported at concentrations as low as 2 ppm (9.8 mg/m^3) in both rats and mice for 6 or 7 h/d for periods ranging from 4 to 7 d (Larson et al., 1996; Templin et al., 1996b). At 5 ppm (25 mg/m^3) and 10 ppm (49 mg/m^3), ossification of the nasal septum was observed in BDF1 mice and F344 rats, respectively, exposed for 5 d/wk for 2 yr (Yamamoto, 1996). Other observations included necrosis and respiratory metaplasia of the olfactory epithelium and goblet cell hyperplasia in the respiratory epithelium in both rats and mice, although it was not specified at which concentrations these effects were observed. In spite of the overt toxicity and increased cell proliferation in these epithelial tissues in the nose, no tumors have been noted in this tissue in any of the chronic toxicity studies, including the Yamamoto (1996) inhalation study. At 10 ppm (49 mg/m^3), cell proliferation and histopathological lesions were reported in the nasal passages of rats exposed for 6 h/d for 1–3 d and mice exposed for 6 h/d for 4–7 d (Mery et al., 1994; Templin et al., 1996b). In one study (Larson et al., 1994b), moderate hepatic changes were observed in mice exposed to 10 ppm (49 mg/m^3) for 6 h/d for 7 d. At concentrations of 25–30 ppm (123–147 mg/m^3), effects on the kidney and liver in rats and mice, including increases in organ weights, histopathological lesions, and increases in proliferation, were observed following exposure for periods ranging from 4 d to 6 mo.

Following administration in drinking water, renal effects were reported at the lowest doses in rats and mice, with hepatic effects observed at higher doses. Regenerative proliferation was observed following a 3-wk exposure to 17 and 40 mg/kg body weight per day in rats and mice, respectively (200 mg/L in drinking water) (Larson et al., 1994a, 1995b). Histological alterations in the liver of F344 rats were reported at 58 mg/kg body weight per day after a 4-d exposure (Larson et al., 1995b).

In protocols with bolus administration, the weight of the liver was affected in rats at the lowest dose following gavage in corn oil for 4 d (10 mg/kg body weight per day), while there were histological changes in the liver at higher doses (34 mg/kg body weight per day) (Larson et al., 1995a, 1995b). At 15 and 30 mg/kg body weight per day, fatty cysts in the liver were observed in dogs exposed to chloroform in toothpaste base in gelatin capsules 6 d/wk for 7.5 yr (Heywood et al., 1979). Data on incidence are presented in Table 4. There were no treatment-related increases in tumors. At the high dose, there were significant increases in serum glutamate–pyruvate transaminase (SGPT) levels at 6 wk of treatment. At the low dose, significant increases in SGPT levels were observed at 34 wk and after. Similar effects were not observed in the vehicle control or untreated control groups**.** At 34 mg/kg body weight per day, effects upon kidney and liver were reported in mice (Larson et

al., 1994b); proliferation and lesions in the olfactory epithelium (Larson et al., 1995a) and lesions in the ethmoid region of the nasal passages (Dorman et al., 1997) were observed at this dose in rats.

TABLE 4. Fatty Cyst Incidence in Chronic Dog Study (Heywood et al., 1979)

Group	No. of dogs examined histologically	No. of dogs with nodules in liver	No. of dogs with fatty cysts	
			Occasional or minimal	Moderate or marked
Males				
30 mg/kg body weight per day	7	0	1	6
15 mg/kg body weight per day	7	1	0	6
Vehicle control	15	0	7	1
Untreated	7	1	2	0
Alternative nonchloroform toothpaste	8	0	2	0
Females				
30 mg/kg body weight per day	8	4	0	7
15 mg/kg body weight per day	8	1	2	3
Vehicle control	12	3	3	0
Untreated	5	1	1	0
Alternative nonchloroform toothpaste	7	1	0	0

There have been many investigations of compensatory hyperplasia in the liver and kidney as monitored by labeling index, as a surrogate measure for cytotoxicity induced by chloroform. Studies on cell proliferation have been conducted in BDF1 and B6C3F1 mice and F344 and Osborne-Mendel rats, by gavage, drinking water, or inhalation, for exposure periods ranging from 1 d up to 22 wk, with cell proliferation being quantified by bromodeoxyuridine immunohistochemistry. Increases in hepatic labeling index may be sustained at the initial levels, but more often are transient and likely represent regenerative growth in response to cell death produced by repeated exposures to chloroform. A brief summary of the results of these studies is presented here.

Liver, Oral Exposure Chloroform administered to female B6C3F1 mice by corn oil gavage for 4 d increased the hepatic labeling index at 238 and 477 mg/kg body weight per day but not at 90 mg/kg body weight per day. The hepatic labeling index was still elevated at 3 wk relative to controls but was decreased relative to the hepatic index observed at the 4-d time point (Larson et al., 1994a). These results are consistent with the observation of liver tumors in females of this strain following administration of similar doses (238 and 477 mg/kg body weight per day) for 78 wk (NCI, 1976). Melnick et al. (1998) exposed female B6C3F1 mice by gavage in corn oil for 3 wk and reported a significant increase in hepatic labeling index at 110, 238, and 477 mg/kg body weight per day (top two doses similar to those administered to females in the NCI [1976] bioassay); their protocol did not include a dose level of 90

mg/kg body weight per day. In male B6C3F1 mice administered chloroform by corn oil gavage (Larson et al., 1994b), the hepatic labeling index was increased relative to controls in the 34–277 mg/kg body weight per day dose range after 4 d of administration. After 3 wk of administration, the hepatic labeling index was not elevated compared with controls at 34 and 90 mg/kg body weight per day but was still significantly elevated at 138 and 277 mg/kg body weight per day. At 138 mg/kg body weight per day, the increase in proliferation was less than at 4 d; at 277 mg/kg body weight per day, it was similar to that observed at 4 d (Larson et al., 1994b). These results (i.e., sustained increases in proliferative response at similar doses) are consistent with increases in hepatic tumor incidence observed in the NCI (1976) bioassay in males at 138 and 277 mg/kg body weight per day.

Pereira (1994) examined the hepatic labeling index in female B6C3F1 mice over longer periods (263 mg/kg body weight by corn oil gavage for 159 d). Initially (at 5 d), marked regenerative hyperplasia was observed. The hepatic labeling index remained elevated but declined, relative to the increase observed at 5 d, steadily to the last observation point (159 d of administration). However, the increase in labeling index over respective controls was relatively constant over the period of administration, ranging from 5 times the control value at 5 d to 7 times at 159 d.

Exposures of female B6C3F1 mice to chloroform in drinking water at concentrations similar to those used in the Jorgenson et al. (1985) bioassay (60–1800 mg/L) did not increase the hepatic labeling index after 4 d or 3 wk of administration (Larson et al., 1994a). This is consistent with the lack of increase in hepatic tumor incidence observed in this strain in this cancer bioassay and contrasts with the results described above in which similar daily doses administered by gavage increased cellular proliferation and tumors.

In male F344 rats administered chloroform by corn oil gavage, an increase in hepatic labeling index was observed following 4 d of exposure to 90 and 180 mg chloroform/kg body weight and 3 wk of administration of 180 but not 90 mg/kg body weight (Larson et al., 1995b). However, the degree of elevation relative to controls was less after 3 wk (4.5 times control) than after 4 d (17 times control). These doses were similar to those administered to male Osborne-Mendel rats in the NCI (1976) bioassay in which tumors were observed at 180 mg/kg body weight per day. In female F344 rats administered chloroform by corn oil gavage, the hepatic labeling index was increased following 4 d or 3 wk of administration at doses between 100 and 400 mg/kg body weight per day (Larson et al., 1995a). This increased hepatic labeling index was sustained through 3 wk of exposure at the 200 and 400 mg/kg body weight doses. While it declined relative to the increase observed at 4 d, there was still an approximate 3-fold increase in relation to controls between 4 d and 3 wk.

An increase in hepatic labeling index was not observed in male F344 rats administered chloroform at concentrations up to 1800 mg/L in drinking water for either 4 d or 3 wk (Larson et al., 1995b), consistent with the lack of increase in liver tumors observed in Osborne-Mendel rats exposed to similar concentrations in the cancer bioassay of Jorgenson et al. (1985).

Liver, Inhalation Exposure In inhalation studies, Larson et al. (1996) exposed B6C3F1 mice to chloroform for up to 13 wk in dosing regimens with varying days per week exposures. An increase in hepatocyte labeling index was observed at 30 ppm (147 mg/m^3) in females exposed on a daily basis for 3 and 6 wk, but not at 13 wk. Increases at 90 ppm (441 mg/m^3) were observed in both sexes at most durations of

exposure, where exposure was for 7 d/wk. However, exposure was for 5 d/wk only in a 13-week protocol. Butterworth et al. (1998) exposed female B6C3F1 (*lacI* transgenic) mice on a daily basis for up to 180 d and reported no increase in hepatocyte labeling index at 10 ppm (49 mg/m^3), a "borderline" response at 30 ppm (147 mg/m^3), and "substantial regenerative cell proliferation" at 90 ppm (441 mg/m^3) at all time points. Templin et al. (1998) exposed BDF1 mice for 5 d/wk for up to 13 wk and reported regenerative proliferation in both sexes only at 90 ppm (441 mg/m^3), the highest concentration. At 13 wk, the increase was observed only in females. At this concentration, in the only identified inhalation carcinogenesis bioassay that was conducted in the same strain of mice, borderline increases in hepatic tumor incidence were reported (Yamamoto, 1996). These mice were exposed for 6 h/d, 5 d/wk.

Templin et al. (1996b) exposed male and female F344 rats to various regimens for up to 13 wk. Exposure was daily, with the exception of the 13-week protocol, in which exposures were for either 5 or 7 d/wk. Increased labeling index in both sexes was observed only at the highest chloroform concentration of 300 ppm (1470 mg/m^3), for exposures of both 5 and 7 d/wk. However, no increases in hepatic tumor incidence were reported in this strain exposed to up to 90 ppm (441 mg/m^3) in the only identified inhalation carcinogenesis bioassay (Yamamoto, 1996), in which exposure was for 5 d/wk.

Kidney, Oral Exposure For kidneys, increased cell proliferation has been observed mainly in the proximal convoluted tubules of the cortex, extending into the straight proximal tubules of the outer stripe of the outer medulla at higher doses.

In male B6C3F1 mice, chloroform doses of 0, 34, 90, 138, and 277 mg/kg body weight per day by gavage induced a dose-dependent increase in labeling index of proximal tubules at all doses after 4 d (Larson et al., 1994b). At 3 wk, there was a diminution in the response at all dose levels, and only at doses of 138 and 277 mg/kg body weight per day, were the labeling indices significantly elevated over controls (Larson et al., 1994b). There were no increases in renal tumor incidence in the cancer bioassay in which male mice of this strain were exposed to 138 or 277 mg/kg body weight per day in corn oil for 78 wk (NCI, 1976). After administration of chloroform in drinking water to female B6C3F1 mice for 3 wk, the labeling index in the medulla was significantly increased at 200 mg/L (Larson et al., 1994a). This was the same as the lowest dose in the Jorgenson et al. (1985) study, in which renal tumors were not observed in this strain following administration in drinking water of concentrations up to 1800 mg/L.

Gollapudi et al. (1999) reported proliferation of renal tubular epithelial cells in male transgenic p53$^{+/-}$ mice exposed to 140 mg/kg body weight per day by gavage in corn oil for 13 wk.

Although a carcinogenesis bioassay in F344 rats exposed via ingestion is not available as a basis for comparison, dose-dependent increases in renal cell proliferation have also been demonstrated in this strain following administration by corn oil gavage (Larson et al., 1995a, 1995b). In the male F344 rat, the labeling index in the renal cortex was increased only at the highest dose (180 mg/kg body weight per day) after 4 d of administration in corn oil. In the parallel studies in female F344 rats, dose-dependent increases in the labeling index in the renal cortex

were observed at doses of 100 mg/kg body weight per day and above administered by corn oil gavage at both 4 d and 3 wk (Larson et al., 1995a).

Templin et al. (1996a) compared male F344 and Osborne-Mendel strains after a single gavage exposure at an observation point of 2 d. Although the authors concluded that these strains were about equally susceptible to chloroform-induced renal injury, a statistically significant increase in labeling index was observed at a much lower dose (10 mg/kg body weight) in the kidney of the Osborne-Mendel rat than in the F344 rat (90 mg/kg body weight) (Templin et al., 1996a). This is the only published investigation of chloroform-associated renal cell proliferation as measured by labeling index in the Osborne-Mendel rat, a function primarily of the lack of commercial availability of pathogen-free colonies of this strain.

Chloroform administered to male F344 rats at concentrations in drinking water similar to those in the cancer bioassay of Jorgenson et al. (1985) produced no increase in renal cell labeling index at either 4 d or 3 wk (Larson et al., 1995b). This attests to the greater sensitivity of male Osborne-Mendel rats, in which renal tumors in males were observed at the highest dose in the study by Jorgenson et al. (1985).

Kidney, Inhalation Exposure In a 13-week inhalation study in which chloroform was administered to B6C3F1 mice at doses ranging from 0.3 up to 90 ppm (1.5 to 441 mg/m^3) (Larson et al., 1996), the renal labeling index in male mice was significantly increased at concentrations of 30 and 90 ppm (147 and 441 mg/m^3) when exposure was for 7 d/wk and at 10 ppm (49 mg/m^3) when exposure was for 5 d/wk. It was not increased in female mice at any concentration. Similar results were reported by Templin et al. (1998) in an assay in BDF1 mice, with daily exposure. An inhalation cancer bioassay has not been conducted with either of these strains.

A 7- to 10-fold increase in labeling index over controls was observed in the kidneys of male, but not female, BDF1 mice exposed to 30 and 90 ppm (147 and 441 mg/m^3), but not 5 ppm (25 mg/m^3), chloroform by inhalation for 4 consecutive days (Templin et al., 1996c). This observation supports the contention that the increased incidence of kidney tumors in males at the highest exposure concentration of 90 ppm (441 mg/m^3) in the 2-yr inhalation bioassay in BDF1 mice (Yamamoto, 1996) was likely associated with regenerative cell proliferation.

Inhalation of chloroform for 4 d, 3 wk, 6 wk, and 13 wk produced an increase in labeling index in the epithelial cells of the proximal tubules of the renal cortex of F344 rats at doses above 30 ppm (147 mg/m^3) with daily exposure and at 90 ppm (441 mg/m^3) and above when exposure was for 5 d/wk. Increases were similar in both male and female rats (Templin et al., 1996b). Renal tumors have not been observed, however, in either sex of this strain exposed to up to 90 ppm (441 mg/m^3) in the only identified inhalation cancer bioassay (Yamamoto, 1996).

Metabolism and Relationship with Tissue Damage

Both oxidative and reductive pathways of chloroform metabolism have been identified, although data *in vivo* are limited. The oxidative pathways generate reactive metabolites, including, perhaps exclusively, phosgene (Pohl et al., 1977; Pohl & Krishna, 1978) (determined *in vitro*, with phenobarbital induction). Phosgene is produced by the oxidative dechlorination of chloroform to trichloromethanol, which spontaneously dehydrochlorinates (Mansuy et al., 1977; Pohl et al., 1977). The reductive pathway generates the dichloromethylcarbene free radical (Wolf et al.,

1977; Tomasi et al., 1985; Testai & Vittozzi, 1986) (determined *in vitro* and *in vivo*, both with and without phenobarbital induction). The metabolism of chloroform proceeds through a P450-dependent activation step, regardless of whether oxidative or reductive reactions are occurring. The balance between oxidative and reductive pathways depends on species, tissue, dose, and oxygen tension.

The electrophilic metabolite phosgene binds covalently to nucleophilic components of tissue proteins (Pohl et al., 1980). It also interacts with other cellular nucleophiles (Uehleke & Werner, 1975) and binds to some extent to the polar heads of phospholipids (Vittozzi et al., 1991). Alternatively, phosgene reacts with water to release carbon dioxide and hydrochloric acid (Fry et al., 1972; D.M. Brown et al., 1974). Carbon dioxide is the major metabolite of chloroform generated by the oxidative pathway *in vivo*. The interaction of phosgene with glutathione results in the formation of *S*-chlorocarbonyl glutathione, which can either interact with an additional glutathione to form diglutathionyl dithiocarbonate (Pohl et al., 1981) or form glutathione disulfide and carbon monoxide (Ahmed et al., 1977; Anders et al., 1978). Incubation of mouse renal microsomes with glutathione increases production of these metabolites from chloroform and decreases irreversible binding to proteins and further metabolism to carbon dioxide (Smith & Hook, 1984). Reduced glutathione is capable of scavenging essentially all chloroform metabolites produced in incubations with mouse liver microsomes when chloroform concentrations are not too high (Vittozzi et al., 1991). The relative importance of the minor pathways of phosgene metabolism depends upon the availability of glutathione, other thiols, and other nucleophilic compounds, such as histidine and cysteine.

Dehydrochlorination of trichloromethanol produces 1 mol of hydrochloric acid, and hydrolysis of phosgene produces 2 more, so that 3 mol of hydrochloric acid are produced in the conversion of chloroform to carbon dioxide. Both products of oxidative activation, phosgene and hydrochloric acid, can cause tissue damage. Phosgene, as noted above, can bind covalently to cellular nucleophiles. Local acidification consequent to hydrochloric acid generation may also be cytotoxic.

Available data indicate that the toxicity of chloroform is attributable to metabolites. In the liver, for example, both the incidence and severity of toxicity correlate with the level of covalent binding of chloroform metabolites to tissue macromolecules, and phosgene is believed to be quantitatively responsible for the irreversible binding of chloroform metabolites to liver components (Pohl et al., 1980). The extent of chloroform-induced hepatic necrosis also correlates with the extent of covalent binding to protein in male and female rats and in male mice (Ilett et al., 1973; B.R. Brown et al., 1974). This covalent binding is more prevalent within the areas of necrosis (Ilett et al., 1973; Tyson et al., 1983), and the association of metabolism with toxicity is further supported by localization of binding to necrotic lesions (Ilett et al., 1973). The results of *in vitro* studies are consistent, in that irreversible binding to macromolecules in rat and human liver microsomes requires prior metabolism (Cresteil et al., 1979).

Increased covalent binding of chloroform metabolites in the liver also occurs when glutathione is depleted, while some degree of protection is conferred if glutathione or a precursor is administered (Stevens & Anders, 1981). Since covalent binding of a chloroform metabolite with glutathione precedes and becomes maximal prior to the chloroform-induced hepatic cytotoxicity, depletion of glutathione may contribute to the observed cytotoxicity as it does to covalent binding (Stevens &

Anders, 1981).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis (Ilett et al., 1973; Smith & Hook, 1983, 1984). Strain- and sex-related differences in sensitivity of mice to nephrotoxicity are also correlated with the ability of the kidney to metabolize chloroform (Taylor et al., 1974; Clemens et al., 1979; Pohl et al., 1984; Smith et al., 1984; Mohla et al., 1988; Henderson et al., 1989; Hong et al., 1989). In an investigation in F344 rats, however, it was concluded that intrarenal bioactivation of chloroform by cytochrome P450 did not appear to play a major role in nephrotoxicity (Smith et al., 1985).

The toxicity of chloroform has, therefore, traditionally been attributed principally to the electrophilic metabolite phosgene. However, Vittozzi and coworkers (Testai et al., 1990; Vittozzi et al., 1991) have argued that reductive activation of haloalkanes in physiologically hypoxic tissues, including the centrilobular region of the liver, where haloalkane hepatotoxicity is largely localized, should be given greater consideration. Physiological partial pressures of oxygen in the liver range from 0.13 to 8 kPa (1 to 60 mmHg), with a mean around 2.7 kPa (20 mmHg), with the lowest values located in the centrilobular region (de Groot & Noll, 1989). Although the dichloromethylcarbene radical could account for many of the reactive properties of chloroform, a large amount of circumstantial evidence argues against the significance of the anaerobic pathway of chloroform metabolism under normal conditions. The anaerobic pathway is observable only in phenobarbital-induced (or naphthoflavone-induced) animals or in tissues prepared from them; microsomes from uninduced animals display negligible reducing activity (Testai & Vittozzi, 1986). Chloroform is relatively ineffective compared with other haloalkanes as a source of free radicals or binding to P450 enzymes, even under the most favourable of test conditions (de Groot & Noll, 1989). Large species differences also exist in the ability of liver microsomes to catalyze reductive activation of chloroform, with microsomes derived from rats and humans being among the least active in this regard (Butler, 1961; Vittozzi et al., 1991) and microsome preparations from mice being only slightly active (Butler, 1961).

Direct experimental evidence has linked oxidative metabolism with tissue toxicity. The reactive intermediates generated by the oxidative and reductive pathways of chloroform metabolism bind to phospholipids differently. Oxidative products bind to the polar heads of the phospholipid molecule, while reductive metabolites bind to the fatty acid tails (De Biasi et al., 1992). This feature has been used experimentally *in vitro* to distinguish covalent binding resulting from chloroform oxidation from that resulting from chloroform reduction. Ade et al. (1994) investigated the amount of chloroform binding to proteins and to the polar heads and fatty acid portions of phospholipids in microsomes prepared from kidneys of uninduced DBA/2J mice. Protein and lipid binding were correlated with hormonal status (males, females, and testosterone-treated females) only under aerobic conditions, indicating that oxidative metabolism is involved in the gender-specific renal toxicity of chloroform. Combining data from previous (Testai et al., 1990; De Biasi et al., 1992) and current studies, Ade et al. (1994) also demonstrated a direct linear correlation between adducts to polar phospholipid heads and adducts to protein in microsomal preparations from livers of B6C3F1 mice and kidneys of DBA/2J mice, even under different experimental conditions. Of the total binding to microsomal phospholipids at 20% oxygen partial pressure, less than 25% was to the fatty acid

tails (and presumably derived from reductive processes). Supplementation of an incubation medium with 3 mM glutathione under room air completely abolished binding to liver microsomal lipids (Testai et al., 1990, 1992), but a small amount of residual binding to kidney microsomal lipids persisted under these conditions (Ade et al., 1994).

Convincing evidence of the role of oxidative metabolism in the toxicity of chloroform has also been acquired recently in male B6C3F1, Sv/129 wild-type, and Sv/129 CYP2E1 null mice exposed by inhalation for 4 d. Although B6C3F1 and Sv/129 wild-type mice exposed to chloroform alone had extensive hepatic and renal necrosis with significant regenerative cell proliferation and minimal toxicity in the nasal turbinates with focal periosteal proliferation, these effects were not observed in mice pretreated with a P450 inhibitor (1-aminobenzotriazole), and no adverse effects were observed in the Sv/129 CYP2E1 null mice (Constan et al., 1999).

These observations strongly support the conclusion that under normal conditions, reductive metabolism of chloroform in liver and kidney is minor and reductive dechlorination is not a quantitatively significant pathway in the human bioactivation of chloroform. However, because the reductively generated metabolites of chloroform are not effectively scavenged by glutathione, they may have contributed to the marked lipid peroxidation observed at high substrate concentrations and low oxygen tensions in experimental studies *in vitro* (Testai et al., 1990, 1992; Ade et al., 1994).

The primary, if not only, enzyme catalyzing metabolism at low concentrations of chloroform is cytochrome P4502E1 (CYP2E1) (Brady et al., 1989; Guengerich et al., 1991). CYP2E1, induced by ethanol, *n*-hexane, secondary ketones, isopropanol, and imidazole, is active in the metabolism of a wide variety of low-molecular-weight compounds in addition to the haloalkanes. The dominant involvement of CYP2E1 is confirmed by studies with chemical inducers of this isozyme, which lead to marked increases in metabolism of chloroform in microsomes from treated rats (Brady et al., 1989). In contrast, treatment with phenobarbital, which reduces the amount of CYP2E1 (Nakajima et al., 1995a, 1995b), inhibits chloroform metabolism (Brady et al., 1989). Brady et al. (1989) have also demonstrated competitive substrate inhibition by CYP2E1 antibodies in rat liver microsomes, indicating that CYP2E1 is responsible for at least 80% of the microsomal metabolism of chloroform at lower doses.

In earlier studies, reviewed in Pohl (1979), it had been demonstrated that inducers of the CYP2B family could also increase the conversion of chloroform to carbon dioxide. Since it is presumed that metabolism through the pathway that generates carbon dioxide results in covalent binding to tissue components, these studies indicated that the CYP2B pathway may also generate reactive intermediates. Nakajima et al. (1991, 1995a, 1995b) proposed that CYP2E1 is a lower-K_m enzyme that is entirely responsible for metabolism of chloroform at low chloroform concentrations, while CYP2B1/2 is a high-K_m isozyme whose activity is demonstrable only at high chloroform concentrations. Studies with purified reconstituted enzyme systems also indicate that CYP2E1 is active and CYP2B1 inactive in the metabolism of chloroform at low substrate concentrations (Brady et al., 1989). Although not optimized to demonstrate comparison between *in vitro* and *in vivo* determinations, Mohla et al. (1988) estimated the K_m values for the two isozymes isolated from the kidneys of BALB/c mice to be 0.6 ± 0.2 mM (CYP2E1) and 20.2 ± 6.8 mM (CYP2B1).

The results of recent studies in SV/129 CYP2E1 null mice (Constan et al., 1999) indicate, however, that the role of CYP2B is minimal, even at high doses.

The regional distribution of lesions in the liver of rats and mice also correlates well with the hepatic distribution of CYP2E1 and glutathione. The highest concentrations of CYP2E1 in both uninduced and induced rat and human liver are present in the centrilobular region (Ingelman-Sundberg et al., 1988; Tsutsumi et al., 1989; Johansson et al., 1990; Dicker et al., 1991). In comparison, concentrations of the phosgene-scavenging agent glutathione in the centrilobular region are only about half those in the periportal region (Smith et al., 1979).

Rats were administered ^{14}C-chloroform to identify those tissues with chloroform-metabolizing capability and investigate the generation of carbon dioxide and incorporation of ^{14}C into macromolecules *in vitro* (Löfberg & Tjälve, 1986). There were correlations between the ability of tissues to metabolize chloroform *in vivo* and *in vitro* and between sites accumulating metabolites *in vivo* and *in vitro*. Because radiolabel was accumulated in trichloroacetic acid-insoluble material, it was assumed to represent covalently bound metabolite. Tissues with chloroform-metabolizing ability included liver; kidney cortex; tracheal, bronchial, olfactory, and respiratory nasal mucosa; and esophageal, laryngeal, tongue, gingival, cheek, nasopharyngeal, pharyngeal, and soft palate mucosa. Of these, the liver was the most active, followed by the nose and kidney.

Genotoxicity

In previous assessments, it has been concluded that the weight of evidence of the genotoxicity of chloroform is negative (WHO, 1994; International Life Sciences Institute [ILSI], 1997). For the latter review, for example, it was concluded that chloroform has little, if any, capability to induce gene mutation, chromosomal damage, or DNA repair and does not appear capable of inducing unscheduled DNA synthesis *in vivo*, although there is some evidence of low-level binding to DNA (WHO, 1994).

In an independent review of identified genotoxicity studies for this assessment, chloroform was negative in the vast majority of *in vitro* assays in *Salmonella typhimurium* and *Escherichia coli*. However, it was positive/weakly positive in four strains of *Salmonella* (Varma et al., 1988) and weakly positive in one strain (Pegram et al., 1997). Although results have been mixed for sister chromatid exchange (SCE) tests, consistently negative results were reported for unscheduled DNA synthesis in a wide range of animal and human cells. *In vivo*, there are three negative and one equivocal mouse micronuclei studies. There have also been one positive micronuclei assay in rat kidney (Robbiano et al., 1998), one positive micronuclei assay in rat liver (Sasaki et al., 1998), one positive assay for chromosomal aberrations in rat bone marrow (Fujie et al., 1990), and one weakly positive investigation of chromosomal aberrations in hamster bone marrow (Hoechst, 1987). For other *in vivo* endpoints, results have been consistently negative, with the exception of the weak DNA binding reported by Colacci et al. (1991) and Pereira et al. (1982), mixed results for sperm abnormalities, and a positive SCE in mouse bone marrow (Morimoto & Koizumi, 1983).

While the weight of evidence of the genotoxicity of chloroform is negative overall, the possibility of a weak positive response in rats cannot be excluded on the basis of the observation that one of the only positive (although marginal) results in

the *Salmonella* gene mutation assay *in vitro* was observed in a TA1535 strain transfected with the rat glutathione-*S*-transferase T1-1 gene (Pegram et al., 1997) and the fact that there was at least some activity in all of the identified *in vivo* studies in rats (Fujie et al., 1990; Robbiano et al., 1998; Sasaki et al., 1998). While each of these results was acquired in nonstandard tests and can be considered questionable in its own right, their collective interpretation gives rise to uncertainty about conclusions concerning the weight of evidence of the genotoxicity of chloroform; further investigation of the nature of the induction of these effects (i.e., whether direct or secondary) is desirable.

Data on the genotoxicity of the metabolites of chloroform are limited. For example, investigations of the genotoxic potential of phosgene, the highly reactive oxidative metabolite of chloroform, have not been identified (ILSI, 1997). There are some data relevant to the assessment of the genotoxicity of reductive metabolites of chloroform. In an assay in which copies of the rat glutathione transferase gene were engineered into *Salmonella*, bromodichloromethane produced mutagenic conjugates, while the effects of chloroform were only marginal (Pegram et al., 1997). Additional supportive evidence that direct interaction of reductive metabolites of chloroform with DNA is unlikely is provided by carbon tetrachloride. While this compound is metabolized almost exclusively by reductive pathways to free radicals that cause severe liver toxicity, most data indicate that it is not mutagenic (Morita et al., 1997).

Assessment of Weight of Evidence for Mode of Action — Cancer

Metabolism to phosgene, resulting from the oxidative pathway that predominates at low exposures, is believed to be the principal determinant of sustained toxicity and resulting persistent proliferation that is hypothesized to lead to a higher probability of spontaneous cell mutation and subsequent cancer. Measures of cytotoxicity include histopathological effects and release of hepatic enzymes and labeling indices as surrogates for regenerative cell proliferation.

Chloroform causes liver and kidney tumors in mice and kidney tumors in rats. Although the hypothesized modes of induction of these tumors are similar, the weight of evidence varies considerably. Liver tumors are observed in B6C3F1 mice following administration of bolus doses by gavage in corn oil (NCI, 1976), but not following administration of the same daily doses in drinking water (Jorgenson et al., 1985). That dose rate is a critical determinant of tissue damage (e.g., being greater following bolus dosing by gavage compared with continuous administration) is consistent with the proposed mode of induction of tumors, with higher bolus doses leading to tissue damage. Doses at which tumors have been observed following administration in corn oil in the cancer bioassay are associated in shorter-term studies with sustained proliferative response in the liver of the same strain exposed similarly (Larson et al., 1994b; Pereira, 1994; Melnick et al., 1998). Sustained increases in proliferative response have not been observed following ingestion in drinking water of concentrations that did not induce increases in hepatic tumor incidence in the long-term bioassay (Larson et al., 1994a).

The incidence and severity of hepatic necrosis in the mouse liver have been related to the degree of covalent binding of chloroform metabolites to tissue proteins. The linking of metabolism to toxicity is underscored by localization of covalent binding to the necrotic lesions and the predictable variations in toxic response

produced by pretreatment with inducers or inhibitors of cytochrome P450-mediated metabolism, specifically CYP2E1. There is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse liver. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative metabolites) and protein binding in the liver of the strain of mice in which tumors have been observed (Ade et al., 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse liver tumors is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the liver of two strains of CYP2E1 null mice (Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan et al., 1999). There is a consistent association between CYP2E1 distribution, chloroform metabolism, pattern of covalent tissue binding, and toxic injury to hepatocytes in mice.

Evidence of concordance between metabolism to reactive intermediates, cytotoxicity, regenerative proliferation, and tumor development in the mouse liver is, therefore, very strong. Indeed, there is a wealth of information that indicates a relationship between sustained enhanced proliferative response and induction of liver neoplasia in the strain in which tumors have been observed (B6C3F1 mice).

Chloroform also induces renal tumors in BDF1 mice following inhalation (Yamamoto, 1996) and in ICI mice exposed by gavage in toothpaste (Roe et al., 1979), although at lower rates than liver tumors. The response is strain and sex specific, occurring only in males.

Evidence of concordance between metabolism to reactive intermediates, cytotoxicity, regenerative proliferation, and tumor development in the mouse kidney, although strong, is not as robust as for the mouse liver, due primarily to the more limited data available on sustained enhanced proliferative response in the strains in which tumors have been observed. Indeed, this is limited to a single study in BDF1 mice, in which there was an increase in labeling index in the kidneys of males but not females at concentrations that induced renal tumors in this strain in the long-term inhalation bioassay (Templin et al., 1996c; Yamamoto, 1996). The available data concerning the relationship between sustained cellular proliferation and induction of renal tumors in another strain (B6C3F1) of mice indicate that sustained proliferative response is not always associated with tumors. In this strain, in shorter-term studies, there were sustained proliferative responses at doses at which kidney tumors were not observed in the relevant cancer bioassays following exposure by both gavage in corn oil and drinking water (NCI, 1976; Jorgenson et al., 1985; Larson et al., 1994a, 1994b).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis, with strain and sex differences in sensitivity to nephrotoxicity being correlated with the ability of the kidney to metabolize chloroform. Similar to the liver, there is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse kidney. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative metabolites) and protein binding in the kidney of DBA/2J mice (Ade et al., 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse renal tumors is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the kidney of two strains of CYP2E1 null mice

(Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan et al., 1999).

The weight of evidence for the hypothesized mode of induction of tumors in the rat kidney is considerably less than that for the mouse liver and kidney due primarily to limited data on intermediate endpoints in the only strain (Osborne-Mendel) in which increases in kidney tumors have been observed. These increases have been reported following exposure via both gavage in corn oil and drinking water (NCI, 1976; Jorgenson et al., 1985). There are also few identified data on the relationship between the metabolism of chloroform and induction of renal lesions in rats. In the F344 rat, there were sustained increases in proliferative response in shorter-term studies following administration of doses similar to those that induced tumors in Osborne-Mendel rats following administration by gavage in corn oil but not following ingestion in drinking water (Larson et al., 1995a, 1995b). However, there are no bioassays in this strain following ingestion for direct comparison with these results. Sustained increases in labeling index were observed in the proximal tubules of F344 rats exposed to daily doses of 30 ppm (147 mg/m^3) and greater and at 90 ppm (441 mg/m^3) and greater at 5 d/wk (Templin et al., 1996b). However, increases in kidney tumor incidence were not observed in this strain exposed to up to 90 ppm (441 mg/m^3) for 5 d/wk in the only inhalation cancer bioassay (Yamamoto, 1996).

Based on studies conducted primarily in F344 rats in which tumors have not been observed, a mode of action for carcinogenicity in the kidney observed in the carcinogenesis bioassay in Osborne-Mendel rats based on cytotoxicity and tubular cell regeneration is, therefore, plausible. For Osborne-Mendel rats, the results of reanalyses of the original renal tissues (Hard & Wolf, 1999; Hard et al., 2000), from both the drinking water bioassay (Jorgenson et al., 1985) and the gavage study (NCI, 1976), have been critical. They provide strong support for the contention that the mode of induction of these tumors is consistent with the hypothesis that sustained proximal tubular cell damage is a requisite precursor lesion for chloroform-induced tumors.

In all cases where examined, therefore, sustained cytotoxicity and cellular proliferation were observed in the liver and kidney of the same strain of mice and rats exposed in a similar manner in short-term studies to concentrations or doses that induced tumors in these organs in cancer bioassays. However, the converse is not always true. Tumors have sometimes not been observed in cases where there have been sustained increases in damage and resulting proliferation in the same strain exposed to similar concentrations in the same manner in shorter-term studies, namely kidney lesions in B6C3F1 mice and F344 rats. These results are consistent with the hypothesis that, where chloroform causes tumors, toxicity and reparative hyperplasia are obligatory precursor steps. Tumors would not necessarily be expected whenever there is an increase in cell replication. The multiple susceptibility factors that produce tumors following cytotoxicity will depend on tissue-specific factors and will likely vary between species and strains. For example, in spite of the overt toxicity and sustained increased cell proliferation in the epithelial tissue of the nose in both rats and mice, no tumors have been noted in this tissue in any chronic studies, including the inhalation bioassay in which nasal tissues were carefully evaluated (Yamamoto, 1996).

The organs in which chloroform-induced cytotoxicity and proliferative lesions are observed (liver, kidney, and nasal passages) correlate well with the distribution

of CYP2E1 both across and within species (Löfberg & Tjälve, 1986). This consistent pattern of response to chloroform across species and organs supports a conclusion that chloroform-induced neoplasia is dependent on cytotoxicity coupled with regenerative cell proliferation. This is further supported by the considerable weight of evidence indicating that chloroform is not genotoxic, with unconvincing evidence for direct DNA reactivity. Due principally to limitations of the available data, though, weak genotoxicity in the rat cannot be precluded, which detracts somewhat from the weight of evidence in this species, although it is unknown whether this might be a result of secondary effects on DNA.

The hypothesized mode of carcinogenesis for chloroform is in keeping with the growing body of evidence supporting the biological plausibility that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis. This has been addressed in numerous articles, including Ames & Gold (1990, 1996), Cohen & Ellwein (1990, 1991, 1996), Preston-Martin et al. (1990), Ames et al. (1993), Tomatis (1993), Cohen (1995), Cunningham & Matthews (1995), Butterworth (1996), Farber (1996), and Stemmermann et al. (1996). Enhanced cell proliferation can lead to an increased frequency of spontaneous genetic damage either through errors that result from the infidelity of DNA replication or through the increased conversion of endogenous DNA changes into heritable genetic changes (Cohen & Ellwein, 1990, 1991, 1996; Ames et al., 1993; Cohen, 1995). Additionally, during periods of cell replication, heritable nonmutagenic modifications of the genome may occur that may lead to changes in gene expression, contributing to carcinogenesis (U.S. EPA, 1996b). This view that cell proliferation is a risk factor for carcinogenesis is not universally accepted, because strict correspondence between increased cell turnover and carcinogenic response is not always demonstrable (Melnick, 1992; Farber, 1996). However, as indicated above, in view of the complex interplay of factors involved in the carcinogenesis process, it is not surprising that acute measures of cell proliferation do not always indicate a one-to-one correlation. Among the factors to be considered are the kinetics of DNA adduct formation and repair, the balance between cell proliferation, differentiation, and death, proliferation in the target cell compartments compared with that of nontarget cells, and the consequences of overt tissue toxicity.

While the evidence is fairly convincing that chloroform is active principally through cytotoxic effects of phosgene and other products of oxidation, several other possibilities in which mutagenicity might play a role were also considered. One is that the effects of chloroform are a composite of those of metabolites from both oxidative and reductive pathways, contributing to toxicity and carcinogenicity. However, several observations strongly support the predominant role of oxidative pathways in chloroform toxicity and make any significant role of reductive metabolism highly unlikely. Firstly, the macromolecular binding following administration of chloroform represents only a very small portion of the delivered dose. Secondly, the mechanisms of action related to the nature of the necrotic lesion, the time course of injury after single doses, and the differences in cumulative damage on multiple exposures are very different for chloroform and carbon tetrachloride, the latter a compound for which the free radical (reductive) pathway is causative for toxicity. In addition, carbon tetrachloride, which is largely metabolized to a free radical, is not itself mutagenic. Based on these considerations, it was concluded that free radicals do not play a significant role in the toxicity or carcinogenicity of chloroform.

Another possibility is that minor pathways, associated with glutathione

conjugation, produce mutagenic metabolites, as is believed to be the case for dichloromethane. However, there is little evidence for a significant direct conjugation pathway for chloroform. In studies with *Salmonella* tester strains with glutathione transferase T1-1 inserted into the bacterial genome and expressed during testing, a small increase in mutagenic activity (less than a factor of 2) was noted for chloroform at very high doses, even though positive controls with methylene chloride and bromochloromethane produced much larger responses (Pegram et al., 1997). Neither of these other two potential modes of action is believed to play a significant role in the observed toxicity and carcinogenicity of chloroform, although further investigation of weak genotoxicity in the rat is desirable.

In summary, then, chloroform has induced liver tumors in mice and renal tumors in mice and rats. The weight of evidence of genotoxicity, sex and strain specificity, and concordance of cytotoxicity, regenerative proliferation, and tumors are consistent with the hypothesis that marked cytotoxicity concomitant with a period of sustained cell proliferation likely represents a secondary mechanism for the induction of tumors following exposure to chloroform. This is consistent with a nonlinear dose–response relationship for induction of tumors. This cytotoxicity is primarily related to rates of oxidation of chloroform to reactive intermediates, principally phosgene and hydrochloric acid. The weight of evidence for this mode of action is strongest for hepatic and renal tumors in mice and more limited for renal tumors in rats.

EXPOSURE–RESPONSE ANALYSIS

While target organs in populations exposed occupationally to high concentrations of chloroform are similar to those in experimental animals (i.e., the kidney and liver), the levels at which effects (i.e., dysfunction and necrosis) occur are not well documented and are inadequate as a basis to meaningfully characterize exposure–response.

Cancer

Available data are consistent with a mode of action for the carcinogenicity of chloroform that is a secondary consequence of cytotoxicity and associated reparative cell proliferation induced by oxidative metabolites. Hence, where chloroform causes tumors, oxidative metabolism, cytotoxicity, and reparative hyperplasia are considered obligatory precursor steps. Based on this mode of action, the optimum approach to quantitation of exposure–response would be as follows: data on noncancer precursor events (cytotoxicity and regenerative proliferation) from interim kills in the critical cancer bioassay could be analyzed on the basis of rates or amounts of oxidative metabolites produced per volume of tissue in the critical organ.

Liver tumors in male and female mice have been induced only by administration of bolus doses in corn oil (NCI, 1976) or at lethal concentrations following inhalation (Yamamoto, 1996). Kidney tumors have been reported in male mice following ingestion in a toothpaste vehicle (Roe et al., 1979) or inhalation (Yamamoto, 1996), but at concentrations in the latter that cause severe kidney necrosis and acute lethality. Renal tumors in rats have been observed, however, in an adequate and relevant study in which the route and pattern of exposure were

similar to those of humans (i.e., continuously in drinking water) (Jorgenson et al., 1985).

The critical carcinogenesis bioassay for quantitation of exposure–response for this assessment is, therefore, that of Jorgenson et al. (1985). Unfortunately, there were no data collected in this bioassay that might serve as the basis for quantitation of exposure–response for precursor lesions such as cytotoxicity or regenerative hyperplasia. A proportion of the slides from several dose groups were recently reexamined, however (Hard & Wolf, 1999; Hard et al., 2000). While this reexamination confirmed histopathological changes consistent with the hypothesis that sustained tubular cytotoxicity and regenerative hyperplasia led to renal tubular tumor induction, data amenable for quantitation of exposure–response in this investigation were limited but are provided to permit at least crude comparison.

There have been numerous subsequent shorter-term investigations of the proliferative response in the liver and kidney of various strains of mice and rats exposed to doses and concentrations of chloroform similar to those administered in the cancer bioassays in which tumors have been observed. However, for renal tumors, most of these investigations have been conducted in the F344 rather than the Osborne-Mendel rat, in which increases in renal tumors have been observed.

Limited available data indicate that the proliferative response in the F344 rat is not an appropriate surrogate for characterization of exposure–response for an intermediate endpoint for renal tumors in the Osborne-Mendel rat. For example, there is no indication of sex-specific variation in the proliferative response in the kidney of F344 rats (Larson et al., 1995a, 1995b), although the increase in renal tumors in Osborne-Mendel rats is sex specific (i.e., restricted to males). In addition, in metabolic studies in F344 rats, intrarenal activation by cytochrome P450 was not implicated as a determinant of nephrotoxicity (Smith et al., 1985). Available data are also inadequate as a basis of characterization of the relative sensitivity of the two strains to cytotoxicity. In the single study in which proliferative response was examined in Osborne-Mendel rats (Templin et al., 1996a), it was concluded that they were about as susceptible as F344 rats to chloroform-induced renal injury, based on comparison 2 d following a single gavage administration. However, a statistically significant increase in labeling index was observed at a much lower dose in the Osborne-Mendel rats (10 mg/kg body weight) than in the F344 rats (90 mg/kg body weight). This latter observation may have been a function of the low value in controls for the Osborne-Mendel rats, attesting to the fact that these data are inadequate in themselves to characterize variations in sensitivity of the two strains. Rather, the results of this study contribute inasmuch as they are not inconsistent with a mode of action of induction of tumors involving tubular cell regeneration in Osborne-Mendel rats.

Since quantitative data on the incidence of precursor lesions for cancer in the strain of interest are inadequate to meaningfully characterize exposure–response, a tumorigenic concentration has been developed for this purpose, based on the incidence of tubular cell adenomas and adenocarcinomas in the bioassay of Jorgenson et al. (1985).

Physiologically Based Pharmacokinetic (PBPK) Modelling In view of the weight of evidence for the role of oxidative metabolites in induction of requisite damage and resulting tumors, dose–response for cancer for chloroform is optimally expressed in terms of amounts or rates of formation of reactive metabolites in the target tissue. These rates have been estimated pharmacokinetically based on models that include

specific parameters related to metabolic rates, enzyme affinities, and enzyme tissue distribution.

Characterization of exposure–response for cancer associated with exposure to chloroform in the context of rates of formation of reactive metabolites in the target tissue is considered appropriate in view of the sufficiency of the evidence to support the following assumptions inherent in PBPK modelling:

- In both experimental animals and humans, metabolism of chloroform by CYP2E1 is responsible for production of the critical reactive metabolite, phosgene.
- The ability to generate phosgene and phosgene hydrolysis products determines which tissue regions in the liver and kidney are sensitive to the cytotoxicity of chloroform.
- This dose–effect relationship is consistent within a tissue, across gender, and across route of administration, and it may also be consistent across species.

In the first extensive model for chloroform, developed by Corley et al. (1990), liver and kidney were described individually and were sites of metabolism for chloroform. The maximum velocity of metabolism in the kidney was scaled to the maximum velocity in the liver based on relative tissue volumes and a proportionality constant. In order to fit gas uptake data, terms were added to allow for loss and resynthesis of metabolizing enzyme. Reitz et al. (1990) modified the Corley et al. (1990) model to include description of a pharmacodynamic endpoint, the induction of cytotoxicity in the liver. Two dose surrogates were considered: average daily macromolecular binding and cytotoxicity. The latter was chosen as the dose surrogate best reflecting carcinogenicity. Gearhart et al. (1993) modified tissue to blood partition coefficients and metabolism according to body temperature and were able to fit gas uptake data without the need to describe enzyme loss and resynthesis. Borghoff and coworkers (Dix et al., 1994; Dix & Borghoff, 1995) incorporated absorption from the stomach as well as the intestinal tract and also accounted for gastric emptying time. Lilly (1996) developed a model for bromodichloromethane that featured subdivision of the liver and kidney compartments into regions of high and low metabolic activity. The combination of this approach with the two-compartment absorption model of Borghoff and coworkers resulted in the most recent PBPK model for chloroform in animals (ILSI, 1997).

For the present assessment, the "hybrid" animal model of the ILSI Expert Panel (ILSI, 1997) was revised and extended to humans and modified to permit accommodation of multimedia exposures (ICF Kaiser, 1999). Several variants of the ILSI model in rats were developed. In all, the $V_{max}KC$ for the kidney adopted in the ILSI model was revised, based on the equation and appropriate values reported by Corley et al. (1990). The resulting value was 0.094 (proportionality constant A = (V/Skidney)/(V/Sliver); $V_{max}KC$ = (A*VKC* $V_{max}LC$)/VLC = 0.094).

In one variant, physiological and anatomical parameters for the ILSI model were updated based on more recent data reported by Brown et al. (1997). In addition, the ILSI model considered water consumption in rats as a 12 h on, 12 h off cycle, whereas one variant of the model developed for this assessment also considered and incorporated actual water consumption patterns by male rats over a 24-h period (Yuan, 1993; ICF Kaiser, 1999). Physiological and metabolic parameters

for the various variants are presented in Table 5.

For the present assessment, a model was also developed for the dog. The physiological and anatomical parameters were taken from Brown et al. (1997), while metabolic parameters were based on the average of rat and human parameters reported by Corley et al. (1990). The fractional subvolumes for the liver were assumed to be the same as those reported for the rat by ILSI (1997), which were estimated by quantitative evaluation of immunohistochemically stained slides of liver lobule reported by Tsutsumi et al. (1989) and Buhler et al. (1992).

For the human model, the physiological and anatomical parameters were also derived from Brown et al. (1997), with the exception of ventilation rate and cardiac output, which were related to an assumed breathing rate of 23 m^3/d (Health Canada, 1994).

The partition coefficients and rate constants in ILSI (1997) were maintained. Liver tissue subvolumes were assumed to be the same as in the rat, based on Tsutsumi et al. (1989) and Buhler et al. (1992), while kidney was subdivided into a 70:30 cortex:noncortex ratio, as described by ICRP (1992). Human metabolic parameters were taken from Corley et al. (1990); these had been determined *in vitro* in eight human liver samples. Kidney rate constants were based on the relationship of activity observed in the microsomal fraction of kidneys to the activity observed in the microsomal fraction of the liver based on *in vitro* results reported by Corley et al. (1990) but supported by data on metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans (Amet et al., 1997).

Results from the human model were compared with data on total metabolized parent and exhaled chloroform reported by Fry et al. (1972) in an investigation in which chloroform was administered to male and female volunteers in olive oil or gelatin capsules. Exhaled chloroform was measured for up to 8 h following exposure, and the total percentage of the dose exhaled unchanged was calculated by extrapolation to infinite time. Human model simulations conducted using a single-compartment description of oral uptake were closer to the observations of Fry et al. (1972) than those estimated using a multicompartment description. Therefore, while a multicompartment description was necessary in the rat model, a single-compartment description of oral uptake was used in estimating human equivalent concentrations.

The model was also modified to permit assessment of exposure to chloroform from all likely sources, including air, water, and food. The exposure scenario was modeled within a 24-h day and included inhalation, ingestion, and dermal absorption from one 10-min shower, a brief washing-up period before retiring, discrete periods of food and water consumption, and inhalation of chloroform at various concentrations (ICF Kaiser, 1999).

TABLE 5. Physiological and Metabolic Parameter Values Used to Exercise the Physiologically Based Model

	Rat[a]	Rat[b]	Dog	Human
Weights (kg)				
Body	0.40	0.40	15.0	70.0
% of body weight				
Fat	0.063	0.124	0.145	0.2142
Kidney	0.0071	0.0073	0.0055	0.0044
Liver	0.0253	0.0366	0.0329	0.0257
Rapidly perfused	0.0439	0.0621	0.0836	0.0709
Slowly perfused	0.77	0.594	0.548	0.4368
Fractional tissue subvolumes (kg)				
Liver periportal (fraction of liver volume)	0.58	0.58	0.58	0.58
Liver centrilobular (fraction of liver volume)	0.42	0.42	0.42	0.42
Kidney cortical (fraction of kidney volume)	0.76	0.76	0.73	0.70
Kidney noncortical (fraction of kidney volume)	0.24	0.24	0.27	0.30
Flows (L/h)				
Alveolar ventilation (L/h for 1-kg animal)	15.0	24.2	28.5	24.0
Cardiac output (L/h for 1-kg animal)	15.0	14.4	30.9	16.5
% of cardiac output				
Fat	0.05	0.07	0.07	0.052
Kidney	0.25	0.141	0.173	0.175
Liver	0.25	0.183	0.297	0.227
Slowly perfused	0.19	0.336	0.277	0.249
Partition coefficients				
Blood/air	20.8	20.8	20.8	7.43
Fat/air	203.0	203.0	203.0	280.0
Kidney/air	11.0	11.0	11.0	11.0
Liver/air	21.1	21.1	21.0	17.0
Rapidly perfused/air	21.1	21.1	21.0	17.0
Slowly perfused/air	13.9	13.9	13.9	12.0
Metabolic constants				
VmaxC for liver (mg/h for 1-kg animal)	6.44	6.44	11.025	15.7
Km for liver (mg/L)	0.543	0.543	0.4955	0.448
VmaxC for kidney (mg/h for 1-kg animal)	0.355 (0.094)[c]	0.067	0.078	0.089
Km for kidney (mg/L)	0.543	0.543	0.4955	0.448
Absorption rate constants for water (/h)				
kSL (from stomach)	2.5	2.5	NA	5.0
kIL (from upper gastrointestinal [GI] tract)	0.5	0.5	NA	0.0
kSI (rate constant from stomach to upper GI tract)	3.5	3.5	NA	0.0
Absorption rate constants for oil gavage (/h)				
kSL	1.5	1.5	1.5	NA
kIL	0.5	0.5	0.5	NA
kSI	1.8	1.8	1.8	NA

[a] Based on ILSI (1997).
[b] Updated with more recent information provided by Brown et al. (1997).
[c] Estimated using the equation provided by Corley et al. (1990).

Various dose metrics have been considered in exposure–response analyses for chloroform. ILSI (1997) investigated four dose metrics in their "hybrid" animal model in relation to the labeling indices (assumed to be representative of response for cytotoxicity, the intermediate endpoint in induction of cancer) in the liver and kidney of exposed F344 rats. As would be expected based on the hypothesized mode of action, the fit for two of these — namely, the total amount of phosgene produced and the maximum concentration of chloroform reached in each experimental dosing interval with proliferative response — was poor. Of the other two, the mean and maximum rates of phosgene production during each experimental dosing interval, the fit with the labeling indices was best for maximum rate (VRAMCOR) (ILSI, 1997). For the current assessment, maximum rate of metabolism per unit kidney cortex volume (VRAMCOR) and mean rate of metabolism per unit kidney cortex volume during each dose interval (VMRATEK) were considered.

Although similar, the fit of the data on tumor incidence for VRAMCOR (p = 0.97) was slightly better than that for VMRATEK (p = 0.84). However, human equivalent concentrations for the former could be developed only for the lower 95% confidence limit of the Tumorigenic Concentration$_{01}$ (TC_{01}), since the maximum rate of human metabolism in the kidney is less than that in the rat. The maximum rate of metabolism that can be achieved in the human kidney, based on metabolic parameters included in the model (approximately 8.1 mg/h/L), was between the animal dose metrics associated with the Benchmark Concentration$_{01}$ (BMC_{01}) and the lower 95% confidence limit of the BMC_{05}.

The results of the exposure–response assessment presented here are, therefore, those for the combined incidence of renal adenomas and adenocarcinomas in Jorgenson et al. (1985) versus VMRATEK, fit to the following model (Howe, 1995):

$$P(d) = q_0 + (1 - q_0) \cdot \left[1 - e^{-q_1 d - \ldots - q_k d^k} \right]$$

where d is dose, k is the number of dose groups in the study, $P(d)$ is the probability of the animal developing the effect at dose d, and $q_i > 0$, $i = 1,\ldots, k$ are parameters to be estimated. The model was fit to the incidence data using THRESH (Howe, 1995), and the Benchmark Dose$_{05}$s (BMD_{05}s) were calculated as the concentration D that satisfies:

$$\frac{P(D) - P(0)}{1 - P(0)} = 0.05$$

Results of the model fitting are presented in Figure 1. The relevant measure of exposure–response, i.e., the mean rate of metabolism (VMRATEK) in humans associated with a 5% increase in tumor risk (TC_{05}) estimated on the basis of the PBPK model, is 3.9 mg/h/L (95% lower confidence limit, 2.5, chi-square = 0.04, degrees of freedom = 1, P-value = 0.84). This dose rate would result from continuous lifetime exposure to 3247 mg/L in water or 30 ppm (147 mg/m^3)

chloroform in air. Respective lower 95% confidence limits for these values are 2363 mg/L and 15 ppm (74 mg/m^3).

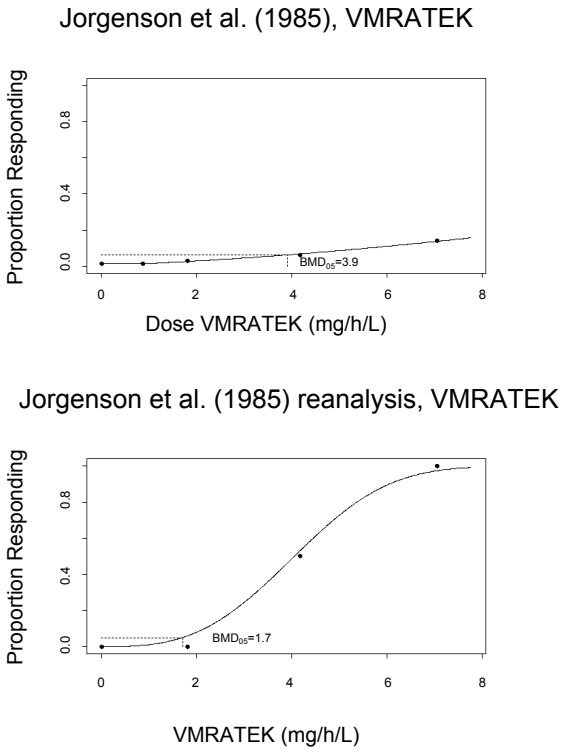

FIGURE 1. Tumorigenic Tissue Dose (Humans) for Combined Incidence of Renal Adenomas and Adenocarcinomas in Osborne-Mendel Rats (Jorgenson et al., 1985)

Although data on dose–response were less robust than those for the cancer bioassay, a benchmark dose was developed, for comparison purposes, for histological lesions in the kidney in the reanalysis of a subset of the slides from the Jorgenson et al. (1985) bioassay. Results of the model fitting are presented in Figure 1. The mean rate of metabolism (VMRATEK) in humans associated with a 5% increase in histological lesions characteristic of cytotoxicity is 1.7 mg/h/L (95% lower confidence limit, 1.4, chi-square = 3.9, degrees of freedom = 2, P-value = 0.14). This dose rate would result from continuous lifetime exposure to 1477 mg/L in water or 6.8 ppm (33.3 mg/m^3) in air. These values are approximately 2-fold less than those presented above, based on the more robust data on tumor incidence.

Nonneoplastic Effects

In summary, short-term exposure by inhalation resulted in cellular proliferation in nasal passages in rats and mice at concentrations as low as 2 ppm (9.8 mg/m^3), with ossification being observed at slightly higher concentrations following long-term exposure. In short-term studies, moderate hepatic changes were observed in mice at 10 ppm (49 mg/m^3); following both short- and long-term exposure to 25–30 ppm (123–147 mg/m^3), there were multiple adverse effects in the kidney and liver in both rats and mice in several studies. Following ingestion in drinking water, regenerative proliferation following short-term exposure of mice to doses as low as 17 mg/kg body weight has been observed. Following bolus dosing, increases in proliferation in the liver of rats have been observed following short-term exposure of rats at 10 mg/kg body weight per day and fatty cysts in the liver of dogs at 15 mg/kg body weight per day.

For oral exposure, therefore, lowest reported effect levels in various species for different endpoints are similar and occur following bolus dosing. One of the lowest dose levels at which effects on liver and kidney have been observed is that in dogs reported by Heywood et al. (1979). As a result, a PBPK model in dogs was developed for this assessment, since characterization of exposure–response for ingestion on the basis of this study is likely to be protective, although it should be considered in the context of an example, in view of the fact that effects on the liver of rodents have also been observed in a similar dose range.

Two dose metrics were investigated in exposure–response: the mean rate of metabolism per unit centrilobular region of the liver (VMRATEL) and the average concentration of chloroform in the nonmetabolizing centrilobular region of the liver (AVCL2). The two dose metrics were selected in order to evaluate the possibility of the fatty cyst formation in the dogs being the result of the solvent effects of chloroform or effects of a reactive metabolite.

The incidence of fatty cysts in this study (Table 4) versus VMRATEL and AVCL2 was fit to the model in the manner described for the assessment of exposure–response for cancer described above. Results of the model fitting are presented in Figure 2. The fit of the data on the incidence of fatty cysts was better for VMRATEL ($p = 1$) than for AVCL2 ($p = 0.45$).

Hence, fit supported the assumption that a metabolite rather than chloroform itself was responsible for the observed effects. The mean rate of metabolism per unit centrilobular region of the liver (VMRATEL) in humans associated with a 5% increase in fatty cysts estimated on the basis of the PBPK model is 3.8 mg/h/L (95% lower confidence limit = 1.3, chi-square = 0.00, degrees of freedom = 1, P-value = 1.00). This dose rate would result from continuous lifetime exposure to 37 mg/L in water or 2 ppm (9.8 mg/m^3) in air. Respective lower 95% confidence limits for these values were 12 mg/L and 0.7 ppm (3.4 mg/m^3).

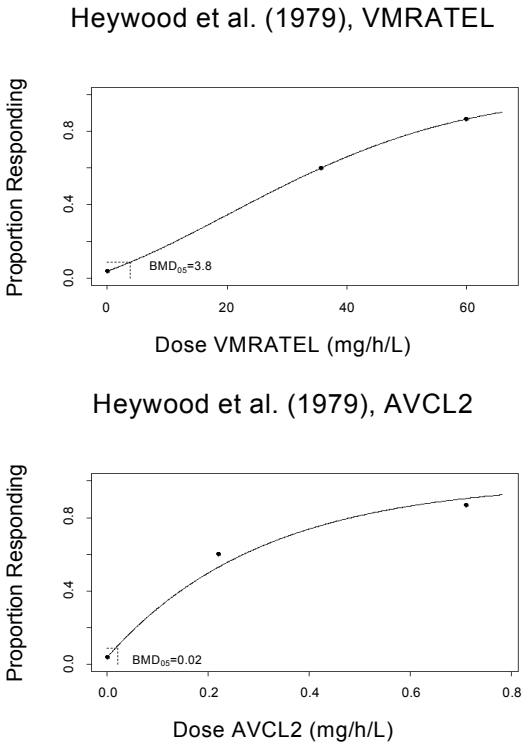

FIGURE 2. Benchmark Tissue Dose (Humans) for Incidence of Hepatic Fatty Cysts in Dogs (Heywood et al., 1979)

HUMAN HEALTH RISK CHARACTERIZATION

The exposure of Canadians was compared with the tissue dose measures described above through modeling of tissue doses resulting from a 24-h exposure scenario. This scenario included inhalation, ingestion, and dermal absorption from one 10-min shower, a brief washing-up period before retiring, discrete periods of food and water consumption, and inhalation of chloroform at various concentrations. The scenarios were based on midpoint and 95th percentiles of concentrations in outdoor air (background and commuting), indoor air, air in the shower compartment, air in the bathroom after showering, tap water, and food (Table 6). The greatest single contributor to chloroform exposure within the 24-h period results from inhalation during showering, which also includes dermal absorption. The human model was run with concentrations and durations in the multimedia exposure scenario. This resulted in an estimated tissue dose that was 1794 (lower 95% confidence limit, 570) times less than that associated with the TC for cancer. For noncancer, the comparable margin for the BMD_{05} was 591 (lower 95% confidence limit, 165).

TABLE 6. Recommended Concentrations in Media for Midpoint and Upper-Percentile Exposure Scenarios for PBPK Modelling

Medium	Midpoint estimate		Upper-percentile estimate	
	Concentration	Developed from	Concentration	Developed from
outdoor air (background)	0.14 μg/m^3 (29 ppt)	arithmetic mean from NAPS data (n = 5463) for 1993–1996[a]	0.31 μg/m^3 (63 ppt)	95th percentile from NAPS data (n = 5463) for 1993–1996[a]
outdoor air (commuting)	0.27 μg/m^3 (55 ppt)	arithmetic mean from NAPS data (n = 800) for 4 "road" sites for 1989–1996[a]	0.66 μg/m^3 (135 ppt)	95th percentile from NAPS data (n = 800) for 4 "road" sites for 1989–1996[a]
indoor air (all)	2.28 μg/m^3 (465 ppt)	arithmetic mean from Concord Environmental Corporation (1992) data (n = 754) following lognormal imputation[b]	8.0 μg/m^3 (1630 ppt)	95th percentile from Concord Environmental Corporation (1992) data (n = 754) following lognormal imputation[b]
air in shower compartment	833 μg/m^3 (170,000 ppt)	experimental data assessing the transfer efficiency of chloroform from tap water to shower air, assuming an average concentration[c]	1950 μg/m^3 (398 000 ppt)	experimental data assessing the transfer efficiency of chloroform from tap water to shower, assuming the 95th percentile of the distribution of concentrations[d]
air in bathroom after showering	5 μg/m^3 (1020 ppt)	estimated with the one-compartment model of Blancato & Chiu (1994),[e] assuming a bathroom volume of 13 m^3 and air exchange rate of 2.2 air changes per hour (ACH) from Wilkes et al. (1992)[f] and an average concentration of chloroform in tap water	18 μg/m^3 (3670 ppt)	estimated with the one-compartment model of Blancato & Chiu (1994),[e] assuming a bathroom volume of 13 m^3 and air exchange rate of 2.2 ACH from Wilkes et al. (1992)[f] and the 95th percentile of the distribution of concentrations of chloroform in tap water

Medium	Midpoint estimate		Upper-percentile estimate	
	Concentration	Developed from	Concentration	Developed from
tap water (cold)	47.3 µg/L	arithmetic mean from provincial/territorial data (n = 6607) for 1990–1997[g]	166 µg/L	95th percentile from provincial/territorial data (n = 6607) for 1990–1997[g]
food (all)	0.0035 µg/g	Canadian data for 24 food items[h]	0.0298 µg/g	Canadian and U.S. data for 131 food items[i]

[a] NAPS data from Dann (1998). Arithmetic mean concentrations were calculated for samples of 24-h duration. See Health Canada (1999) for further information.

[b] These data are from Concord Environmental Corporation (1992). Twenty-four-hour samples of indoor air were collected using passive sampling devices from 754 homes in nine provinces during 1991 and 1992. At a limit of detection of 3.5 µg/m^3, chloroform was detected in only 10.7% of these indoor air samples. The distribution of concentrations was assumed to be lognormal. Arithmetic mean (i.e., 2.28 µg/m^3) and geometric mean (i.e., 0.72 µg/m^3) concentrations were estimated by lognormal imputation, as described in Health Canada (1999). A 95th-percentile concentration (i.e., 8.0 µg/m^3) was also estimated.

[c] Estimates of the average concentrations of chloroform in the air of a shower compartment during a 10-min shower were developed in Health Canada (1999) for typical conditions of water temperature (i.e., approximately 40°C) and flow rates (i.e., 5 and 10 L/min), using the arithmetic mean and 95th percentiles of the distribution of concentrations of chloroform in tap water in Canada. A midpoint estimate of the average concentration was developed as follows. At an assumed concentration in water of 50 µg/L (compared with an arithmetic mean concentration of 46.4 µg/L; see Health Canada, 1999) and assuming minimum air exchange between the shower compartment and the adjacent (bathroom) area, estimates of the average concentration of chloroform in the air of the shower compartment during showering ranged from 300 to 1333 µg/m^3. An average concentration of 833 µg/m^3 was selected as the midpoint estimate, based on the assumptions of a water flow rate of 10 L/min and a transfer efficiency of 0.5 (i.e., 50% of the chloroform in the water passing through the shower head is assumed to be volatilized into the air of the shower compartment before the water passes through the shower drain).

[d] An upper-end estimate of the average concentration in the air of a shower compartment during a 10-min shower was developed in a similar manner. At an assumed concentration in water of 117 µg/L (the 95th percentile of the distribution of concentrations in tap water in Canada; see Health Canada, 1999) and assuming minimum air exchange between the shower compartment and the adjacent (bathroom) area, estimates of the average concentration of chloroform in the air of the shower compartment during showering ranged from 702 to 3120 µg/m^3. An average concentration of 1950 µg/m^3 was selected, also based on the assumptions of a water flow rate of 10 L/min and a transfer efficiency of 0.5.

[e] Blancato & Chiu (1994) indicate that the equilibrium relation of the concentration of chloroform in air to the concentration in tap water can be described according to $C_a = (f \times R_w \times C_w) \div (V_b \times R_b)$, where: C_a is the resulting average concentration (mg/m^3) of chloroform in the indoor air; f is the transfer efficiency (i.e., 0.5 assumed; see Health Canada, 1999); R_w is the rate of water use, expressed as L/shower, assuming a flow rate of 10 L/min and a duration of 15 min; C_w is the concentration (mg/L) of chloroform in the tap water (i.e., 0.0464 mg/L for the midpoint estimate, and 0.117 mg/L for the upper-percentile estimate); V_b is the volume (m^3) of the bathroom (a volume of 13 m^3 was assumed, based on Wilkes et al., 1992); and R_b is the bathroom ventilation rate (air exchanges/day).

[f] Wilkes et al. (1992) estimated a range of air exchange rates: 0.8 ACH (19.2/d) when the bathroom door is closed; 2.2 ACH (52.8/d) when the bathroom door is open; and 7.4 ACH (178/d) when the bathroom door is closed and an exhaust fan is operating. A bathroom ventilation rate of 2.2 ACH was assumed for both the midpoint and upper-percentile exposure scenarios.

[g] Data concerning the distribution of concentrations of chloroform in treated tap water in Canada in the 1990s are summarized in Health Canada (1999).

[h] Ranges of average intakes of chloroform from ingestion of foods for six age groups among the population were developed in Health Canada (1999) using average daily consumption rates (g/d) for 181 food items (EHD, 1998). The minimum intakes in the ranges were based on midpoint estimates of the concentrations of chloroform in 24 specific food items using data originating in Canada only.

[i] The maximum intakes in the ranges were based on midpoint estimates of the concentrations of chloroform in 131 specific food items using data originating in Canada or the United States. For the adult age group, the range of intakes (assuming an average body weight of 70.9 kg) was from 0.084 to 0.71 µg/kg body weight per day. Equivalent intakes in µg/d are 5.96–50.3. The total average daily consumption of the 181 food items by adults is 2353 g (EHD, 1998). Among these, there are two specific food items that are generally prepared using tap water. These are tea (at 317 g/d for adults) and coffee (at 348 g/d for adults). No intake estimates were developed for tea or coffee, as data indicating concentrations of chloroform were not available. The total average daily consumption of 179 food items (i.e., excluding tea and coffee) by adults is (2353 – 665 =) 1688 g. This amount was divided by the minimum (5.96 µg/d) and maximum (50.3 µg/d) of the range of daily intakes to estimate average concentrations of chloroform in the food consumed. The resulting range of average concentrations is 0.0035 µg/g (i.e., midpoint estimate) to 0.0298 µg/g (i.e., upper-percentile estimate).

Since the tumorigenic and benchmark doses for cancer and noncancer, respectively, are based on metabolized dose, they adjust for kinetic differences between animals and humans. An appropriate uncertainty factor for derivation of a Tolerable Intake for both cancer and noncancer effects would therefore be in the range of 25, i.e., 10 (for intraspecies variation in toxicokinetics and toxicodynamics) × 2.5 (for interspecies variation in toxicodynamics) (Health Canada, 1994). Hence, the margins between estimated exposure and tumorigenic and benchmark doses for cancer and noncancer, respectively, for chloroform are considerably greater than that considered as appropriate as a basis for development of a Tolerable Intake. As a result, exposure of the general population is considerably less than the level to which it is believed a person may be exposed daily over a lifetime without deleterious effect.

A PBPK model has not been developed for the nose. Therefore, the lowest concentrations reported to induce cellular proliferation in the nasal cavities of rats and mice in short-term studies (i.e., 2 ppm [9.8 mg/m^3]) were compared directly with the midpoint and 95th-percentile estimates of concentrations of chloroform in indoor air in Canada. These values were the same as those selected to run the human models for the kidney and liver. The midpoint and 95th-percentile estimates are 4298 and 1225 times less than the lowest value reported to induce a proliferative response in rats and mice (midpoint for indoor air = 2.28 µg/m^3, 95th percentile = 8.0 µg/m^3). Comparisons with midpoint and 95th-percentile estimates of concentrations during showering were considered unwarranted, since such exposures are intermittent and last for very limited periods of time during the day. Based on considerations similar to those mentioned above for cancer and noncancer effects associated with ingestion of chloroform, these margins are considerably greater than that considered appropriate as a basis for development of a Tolerable Concentration.

UNCERTAINTIES AND DEGREE OF CONFIDENCE IN EXPOSURE ESTIMATION, HAZARD CHARACTERIZATION, AND EXPOSURE–RESPONSE ANALYSIS

For the principal source of exposure of at least the older age groups of the general population to chloroform (i.e., showering), uncertainty is introduced by the assumption that concentrations in the water at the shower head are similar to those in the incoming cold tap water. Based on limited data, the average concentrations in

the warm water may be twice as high as that in the incoming cold water during the summer months and up to 4 times as high as that in the colder incoming water during winter months (Benoit et al., 1997). Additional uncertainty is introduced by the assumption that the concentrations measured in the water treatment plants and distribution systems are representative of the concentrations at the consumers' taps, to which the general population is exposed. Available data indicate that average concentrations may be 50% higher at the most remote locations than at the water treatment plants, depending on the specific treatment processes used and other factors.

For indoor air, confidence in characterization of concentrations is less that that for other media due primarily to the limited number of homes sampled and lack of sensitivity of analysis in the available survey (Concord Environmental Corporation, 1992). Concentrations measured were less than the limit of detection in approximately 90% of the samples from 754 homes, although the approach adopted for estimation of levels in these samples for characterization of exposure is not considered to be unrealistic or overly conservative.

There is a moderate degree of confidence in the quantitative estimates of the average intake of chloroform in drinking water for the general population. As indicated in relation to the estimates of exposure during showering, some uncertainty is introduced by the assumption that the concentrations measured in the water treatment plants and distribution systems are representative of the concentrations at the consumers' taps, to which the general population is exposed. This database included over 10,000 samples analyzed between 1985 and 1997. Although analyses were performed by a number of different laboratories, sampling and analytical methods were similar. Although similar dechlorinating preservatives were utilized, the pH of the preserved samples was not adjusted concomitantly, and hence there may have been some alteration in concentrations of chloroform during storage (Lebel & Williams, 1995). Uncertainty in the quantitative estimates of daily intakes of chloroform is also introduced by assuming daily rates of intake of total tap water, which includes tap water used to prepare beverages. Concentrations of chloroform in hot beverages (e.g., tea and coffee) are unlikely to be as high as the concentrations in the cold tap water used for their preparation, as chloroform rapidly volatilizes from tap water during heating and boiling.

Although it contributes minimally to total exposure, there is a moderate degree of confidence in the characterization of the concentrations of chloroform in ambient air in Canada, due to the magnitude and sensitivity of the monitoring data. This was based on a large data set of 24-h average concentrations, measured across the country, throughout the 1990s (Dann, 1998). Samples were collected by a standardized protocol, on a cyclical basis, at a fixed network of atmospheric monitoring sites, for analysis by a single, specialized laboratory. Confidence in the data is increased by the observation that chloroform was detected with similar frequencies and at similar ranges of concentrations in the ambient air from rural areas situated in widely separated geographical locations of Canada. The observation that the frequencies of detection and concentrations of chloroform were higher at suburban and urban locations than in these rural areas is also consistent with what might be expected on the basis of proximity to sources. Some uncertainty is introduced by the locations of the monitors, which are not strictly representative of personal exposure.

Uncertainty is introduced into the estimates of the average daily intake of chloroform from ingestion of foods by the assumption that the limited Canadian data available for a specific food item are representative of the concentrations generally encountered by the general population when ingesting that food item. Additional uncertainty is introduced by the assumption that concentrations of chloroform measured in specific food items in the United States are similar to the concentrations in those food items in Canada. Also, the concentration of chloroform was assumed to be zero in all food items for which data are not available. Nevertheless, there is a high degree of certainty that chloroform is not highly concentrated in foods in Canada, since chloroform is only moderately lipophilic and does not significantly biomagnify in food chains.

Confidence in the quantitative estimates of daily intakes of chloroform by ingestion for infants is low. As no data were available concerning the presence or concentrations of chloroform in human breast milk in Canada, estimates of intake for exclusively breast-fed infants could not be developed. Uncertainty is introduced by the assumption that infants are exclusively formula fed, since data concerning the presence or concentrations of chloroform in concentrated (i.e., powdered or liquid) infant formula were not available. As a result, the concentration of chloroform in the reconstituted infant formula was assumed to be identical to the concentrations in the domestic water supply. Similar uncertainty is introduced when it is assumed that infants are fed table-ready foods, due to the limitations identified previously regarding the concentrations of chloroform in the majority of food items consumed daily in Canada.

With respect to the toxicity of chloroform, the degree of confidence that critical effects in animal species are well characterized in the available database is high. Indeed, in numerous investigations in experimental animals by various routes of exposure, effects on the kidney, liver, and nose have been consistently observed at lowest doses. The nature of the effects has been similar and generally consistent with a mode of action that involves cellular degeneration and death and regenerative proliferation induced by oxidative metabolites.

The degree of confidence in the database that supports an obligatory role of cytotoxicity in the carcinogenicity of chloroform is also high, although there are some uncertainties. Indeed, there are few compounds for which the supporting database in this regard is as complete, consistent, and cohesive as it is for chloroform. The weight of evidence in this regard is strongest for hepatic and renal tumors in mice. The evidence is more limited for renal tumors in rats, primarily due to the relative paucity of data, in strains where tumors have been observed, on metabolism and intermediate endpoints and the relationship between them. Uncertainty could be reduced, therefore, by acquisition of additional information on metabolism, cytotoxicity, and proliferative response in the strain in which tumors were observed (i.e., Osborne-Mendel rats) following long-term exposure to chloroform. Additional data on metabolism and chronic (e.g., 2-yr) cytotoxicity/proliferative response in the kidneys of F344 rats might also have contributed to greater confidence in the hypothesized mode of action.

While the overall weight of evidence for the genotoxicity of chloroform is negative, on the basis of available data, weak genotoxicity in the rat cannot be precluded. It would be desirable, therefore, to investigate further the possible nature of the interaction of chloroform with DNA in rats. Another area that could be clarified by further work is whether any of the metabolites of chloroform are DNA reactive.

For the PBPK model, among those parameters considered in the sensitivity analysis to have most impact on output, uncertainty was greatest for the metabolic parameters particularly in the kidney and for humans. Additional *in vitro* data on the metabolism of chloroform in the human kidney and liver would be useful not only to reduce uncertainty in these values, but, if performed on tissues from a number of individuals, potentially to address the issue of variability across the human population. In particular, it would be desirable to clarify whether the same pathways of metabolism contribute to the potential for cytotoxicity in rodents and humans, specifically with respect to CYP2E1 and other P450 isozymes. Determination of the kinetic constants for the CYP2E1 and CYP2B1 isoforms *in vivo* is also desirable and could be addressed through comparative kinetic analysis of gas uptake curves in phenobarbital-induced CYP2E1 knockout and normal mice. For the PBPK model for dogs, the blood/air partition coefficient in this species was considered to be similar to that for the rat, although these are normally higher in smaller species for small molecular weight chlorocarbons, possibly due to variations in binding to hemoglobin. Similarly, for this model, local rates of metabolism in the dog were based on hepatic and renal distribution of CYP2E1 in rats.

Characterization of exposure–response for both noncancer and cancer is based on increased incidence of the relevant endpoints (both fatty cysts in dogs and renal tumors in rats) for a small number of doses. However, the dose at which noncancer effects were observed in this study is similar to the lowest reported effect levels for proliferative response in target organs of other species.

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The health-related sections of the Assessment Report on which this paper is based are based in part on the deliberations of two expert groups in which staff of Health Canada participated. These were an International Programme on Chemical Safety Task Group on chloroform (WHO, 1994) and an ILSI Expert Panel convened to develop case studies for chloroform and dichloroacetic acid in the context of the revised cancer guidelines released in 1996 by the U.S. EPA (ILSI, 1997). The ILSI Expert Panel, which first met in September 1996, was composed of the following members: M. Andersen (ICF Kaiser International) (Chair), G. Boorman (National Institute of Environmental Health Sciences), D. Brusick (Covance Laboratories, Inc.), S. Cohen (University of Nebraska Medical Center), Y. Dragan (McArdle Laboratory for Cancer Research), C. Frederick (Rohm & Haas Company), J. Goodman (Michigan State University), G. Hard (American Health Foundation), M. E. Meek (Health Canada), and E. O'Flaherty (University of Cincinnati).

The final draft of the Expert Panel report on chloroform was reviewed externally by C. Klaassen (University of Kansas Medical Center), R. Melnick (National Institute of Environmental Health Sciences), and L. Rhomberg (Harvard Center for Risk Analysis).

The outcome of these assessments has been updated and considered in the context of the approach to assessment of "toxic" under CEPA. In addition, the PBPK model for animals included in ILSI (1997) was refined and a human component developed by the K.S. Crump Group (ICF Kaiser, 1999).

The section related to genotoxicity was reviewed by D. Blakey (Environmental and Occupational Toxicology Division, Health Canada). The PBPK model incorporated herein was reviewed externally by M. Gargas (ChemRisk, McLaren Hart Inc.).

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Chapter 11

Inhaled Formaldehyde: Exposure Estimation, Hazard Characterization and Exposure-Response Analysis

Liteplo, R.G. and Meek, M.E. (2003)

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ABSTRACT

Formaldehyde has been assessed as a Priority Substance under the Canadian Environmental Protection Act. Probabilistic estimates of exposure of the general population in Canada to formaldehyde in ambient and indoor are presented. Critical health effects include sensory irritation and the potential to induce tumours in the upper respiratory tract (the nasal region in rodents and potentially the lungs of humans). The majority of the general population is exposed to airborne concentrations of formaldehyde less than those typically associated with sensory irritation (i.e., 0.1 mg/m^3). Based primarily upon data derived from laboratory studies, the inhalation of formaldehyde under conditions which induce cytotoxicity and sustained regenerative proliferation within the respiratory tract is considered to present a carcinogenic hazard to humans. At airborne levels for which the prevalence of sensory irritation is minimal (i.e., 0.1 mg/m^3), risks of respiratory tract cancers for the general population estimated on the basis of a biologically motivated case-specific model at are exceedingly low. This biologically case specific model incorporates two-stage clonal expansion and is supported by dosimetry calculations from computational fluid dynamics (CFD) analyses of formaldehyde flux in various regions of the nose and single-path modelling for the lower respiratory tract.

The degree of confidence in the underlying database and uncertainties in estimates of exposure and in characterization of hazard and dose-response are delineated.

INTRODUCTION

Formaldehyde has been assessed as a Priority Substance under the *Canadian Environmental Protection Act* (CEPA). In Canada, formaldehyde is used primarily in the production of resins, with smaller amounts being used in fertilizers and as preservatives and disinfectants. Formaldehyde enters the Canadian environment from natural sources (including forest fires) and from direct human sources, such as automotive and other fuel combustion and industrial on-site uses. Secondary formation also occurs, by the oxidation of natural and anthropogenic organic compounds present in air.

Information that served as the basis for the exposure estimation, hazard characterization, and exposure–response analysis of inhaled formaldehyde is briefly summarized.[1] Additional information on the intake and effects associated with exposure to formaldehyde via this and other routes (i.e., primarily ingestion) is presented in the more extensive Supporting Documentation[2] and Assessment Report,[3] which served as the basis for preparation of this paper. These documents were externally reviewed by experts identified in the Acknowledgements.

[1] For a description of the approach to assessment of Priority Substances under CEPA, see Meek et al. (1994) and www.hc-sc.gc.ca/ehp/ehd/catalogue/bch.html.
[2] Available from Environmental Health Centre, Room 104, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2.
[3] Ibid.; also available at www.ec.gc.ca/cceb1/eng/final/index_e.html.

POPULATION EXPOSURE

Formaldehyde was detected (detection limit 0.05 μg/m^3) in 3810 of 3842 24-h ambient air samples collected at 16 sites in six provinces between August 1989 and August 1998 in Canada's National Air Pollution Surveillance (NAPS) program (Environment Canada, 1999). Concentrations ranged up to 27.5 μg/m^3 at eight urban sites, 12.03 μg/m^3 at two suburban sites, 9.1 μg/m^3 at two rural sites considered to be affected by urban and/or industrial influences, and 9.8 μg/m^3 at four rural sites considered to be regionally representative. Based on pooled monthly mean concentrations, levels of formaldehyde are highest between June and August (Health Canada, 2000).

Few recent data were identified concerning concentrations of formaldehyde in residential indoor air in Canada. The distributions of concentrations were similar in five of the seven studies for which methodology was highest quality conducted in Canada between 1989 and 1995, despite differences in sampling mode and duration (i.e., active sampling for 24 h or passive sampling for 7 d). The median, arithmetic mean, 95th percentile, and 99th percentile concentrations of the pooled data (n = 151 samples) from these five studies were 30, 36, 85, and 116 μg/m^3, respectively (Health Canada, 2000). Average concentrations of formaldehyde were an order of magnitude higher in indoor air than in outdoor air, indicating the presence of indoor sources of formaldehyde. There was no clear indication from these studies that concentrations of formaldehyde were greater in homes where environmental tobacco smoke was present.

Exposure of the general population in outdoor air was estimated based on a subset of data from the NAPS program considered representative of ranges and distributions of concentrations (Table 1). The range and distribution of concentrations for residential indoor air was based on the pooled data from the five studies mentioned above (Health Canada, 2000). Owing to lack of information to serve as the basis for probabilistic characterization of the proportion of time spent indoors, a mean time spent outdoors of 3 hours is assumed based on the point estimates of time spent indoors and outdoors. The distribution of the time spent outdoors (mean), is arbitrarily assumed to be normal in shape with an arithmetic standard deviation of 2 h. In the probabilistic simulation, this distribution is truncated at 0 h and 9 h. The time spent indoors is calculated as 24 h minus the time spent outdoors.

Estimates of the distribution of time-weighted 24-h concentrations of formaldehyde to which the general population is exposed were developed using simple random sampling with Crystal Ball™ Version 4.0 (Decisioneering, Inc., 1996) and simulations of 10 000 trials. The median, arithmetic mean, and upper percentiles of the distributions of 24-h time-weighted average concentrations of formaldehyde determined from these probabilistic simulations are summarized in Table 2. These estimates indicate that one of every two persons would be exposed to 24-h average concentrations of formaldehyde in air of 24–29 μg/m^3 or greater (i.e., median concentrations). Similarly, 1 in 20 persons (i.e., 95th percentile) would be exposed to 24-h average concentrations of formaldehyde in air of 80–94 μg/m^3 or greater.

TABLE 1. Concentrations of Formaldehyde in Outdoor Air and Residential Indoor Air in Canada

Medium of exposure	Number of samples	Mid-points of distributions ($\mu g/m^3$)		Upper percentiles of distributions of concentrations ($\mu g/m^3$)			
		Median	Mean[e]	75th	90th	95th	97.5th
Outdoor air – NAPS data[a]	2819	2.8	3.3	4.1	6.0	7.3	9.1
Outdoor air – reasonable worst-case site[b]	371	2.9	4.0	4.8	7.3	10.4	17.3
Indoor air – five studies[c]	151	29.8	35.9	46.2	64.8	84.6	104.8
Indoor air – lognormal distribution[d]	–	28.7	–	46.1	70.7	91.2	113.8

[a] Data are for selected suburban (n = 4) and urban (n = 4) sites of the NAPS Program (Dann, 1997, 1999) for the period 1990–1998. Concentrations are slightly lower for the subset of suburban sites and slightly higher for the subset of urban sites. Distributions are positively skewed.
[b] One of the four urban sites (i.e., NAPS site 060418 in Toronto) was selected for the reasonable worst-case purpose.
[c] Data were pooled from five studies of concentrations of formaldehyde in residential indoor air. These studies were conducted at various locations in Canada between 1989 and 1995.
[d] The geometric mean and standard deviation of the pooled data (n = 151) from the five Canadian studies were calculated. A lognormal distribution with the same geometric mean and standard deviation was generated and the upper percentiles of this distribution were estimated.
[e] These are the arithmetic mean concentrations. Since formaldehyde was detected in more than 99% of the samples, censoring of the data for limit of detection was not required.

TABLE 2. Probabilistic Estimates of 24-hour Time-weighted Average Concentrations of Formaldehyde in Air

	Mid-points of distributions ($\mu g/m^3$)		Upper percentiles of distributions of concentrations ($\mu g/m^3$) and relative standard deviations (%)			
	Median	Mean[c]	75th	90th	95th	97.5th
Simulation 1[a]	29	36	46 (± 0.5%)	62 (± 1.3%)	80 (± 1.9%)	97 (± 0.7%)
Simulation 2[b]	24	33	45 (± 1.2%)	75 (± 1.2%)	94 (± 1.6%)	109 (± 1.3%)

[a] In simulation 1, the distribution of concentrations of formaldehyde is represented by a frequency histogram of the pooled data from the five selected studies (n = 151 samples).
[b] For simulation 2, a lognormal distribution of concentrations, truncated at 150 $\mu g/m^3$, is assumed. This lognormal distribution has the same geometric mean (28.7 $\mu g/m^3$) and standard deviation (2.92) as the distribution of concentrations for the pooled data from the five selected studies.
[c] This is the arithmetic mean concentration.

HEALTH HAZARD CHARACTERIZATION

Sensory irritation of the eyes and respiratory tract by formaldehyde has been observed consistently in clinical studies and epidemiological (primarily cross-sectional) surveys in occupational and residential environments. The pattern of effects is consistent with increases in symptoms being reported at lowest concentrations, with the eye generally being most sensitive. At concentrations higher than those generally associated with sensory irritation, small, reversible effects on lung function have been noted, although evidence of cumulative decrement in pulmonary function is limited.

Results of cross-sectional studies are also consistent with observed increases in prevalence of histological changes in the nasal epithelium of workers being attributable to formaldehyde (Edling et al., 1988; Holmström et al., 1989c; Boysen et al., 1990; Ballarin et al., 1992).

Alterations in mucociliary clearance and histopathological changes within the nasal cavity are observed at lowest concentrations following acute exposure of rodents to formaldehyde (Monteiro-Riviere & Popp, 1986; Morgan et al., 1986a; Bhalla et al., 1991). Following repeated exposure, critical effects in rodents are histopathological effects in the nasal and respiratory tracts (e.g., hyperplasia, squamous metaplasia, basal hyperplasia, rhinitis, inflammation, erosion, ulceration, disarrangements) and increases in cell proliferation in the nasal cavity (Swenberg et al., 1980, 1983; Zwart et al., 1988; Swenberg et al., 1980; Kerns et al., 1983; Rusch et al., 1983; Appelman et al., 1988; Woutersen et al., 1989; Monticello et al., 1996).

Formaldehyde has also been carcinogenic in rats in a number of bioassays. Exposure–response in these investigations was similar and highly nonlinear, with sharp increases in tumor incidence in the nasal cavity occurring only at concentrations greater than 6 ppm (7.2 mg/m^3) formaldehyde.

Formaldehyde is not likely to affect reproduction or development at levels of exposure lower than those associated with adverse health effects at the site of contact. Based upon recent epidemiological studies of occupationally exposed individuals, there is no clear evidence indicating that either maternal or paternal inhalation exposure to formaldehyde is associated with an increased risk of spontaneous abortion (Hemminki et al., 1985; Lindbohm et al., 1991; John et al., 1994; Taskinen et al., 1994). In studies of laboratory animals exposed via inhalation (Saillenfait et al., 1989; Martin, 1990), formaldehyde had no effect on reproduction or fetal development at levels of exposure less than those causing notable adverse health effects at the site of contact. There is little convincing evidence that formaldehyde is neurotoxic in occupationally exposed populations.

Based upon the available limited data which are restricted to studies in animals, exposure to formaldehyde is unlikely to be associated with suppression of the immune response. Adverse effects on either cell- or humoral-mediated immune responses have not been consistently observed in studies conducted in laboratory animals (Dean et al., 1984; Adams et al., 1987; Holmström et al., 1989b; Jakab, 1992; Vargová et al., 1993). Studies with laboratory animals have revealed that formaldehyde may enhance their sensitization to inhaled allergens (Tarkowski & Gorski, 1995; Riedel et al., 1996). While there is suggestion based on sporadic reports that bronchial asthma following inhalation of formaldehyde may be due to immunological mechanisms, the potential mode of induction and role of predisposing

characteristics are unclear (Feinman, 1988; Bardana & Montanaro, 1991; Stenton & Hendrick, 1994).

Since sensory irritation and carcinogenicity are putatively the critical effects of long term exposure to formaldehyde, this section focuses primarily on information relevant to assessment of the weight of evidence for these effects (both that acquired in clinical and epidemiological investigations in human populations and in bioassays in experimental animals and *in vitro*). Areas addressed include genotoxicity and additional information relevant to assessment of the weight of evidence for the hypothesized mode of induction of tumours, including concordance with histopathological evidence of cytotoxicity and proliferative response.

Toxicokinetics

Most of the formaldehyde that is inhaled is deposited and absorbed in regions of the upper respiratory tract with which the substance comes into first contact (Heck et al., 1983; Swenberg et al., 1983; Patterson et al., 1986). In rodents, which are obligate nose-breathers, deposition and absorption occur primarily in the nasal passages, while in oronasal breathers (such as monkeys and humans), they occur in the nasal passages and oral cavity but also in the trachea and bronchus.

Upon absorption, formaldehyde forms intra- and intermolecular cross-links within proteins and nucleic acids at the site of contact (Swenberg et al., 1983). It is also rapidly metabolized to formate by a number of widely distributed cellular enzymes, the most important of which is NAD^+-dependent formaldehyde dehydrogenase. Metabolism by formaldehyde dehydrogenase occurs subsequent to formation of a formaldehyde–glutathione conjugate. Due to its deposition principally within the respiratory tract and rapid metabolism, inhalation exposure to formaldehyde does not result in an increase in levels of this substance in the blood of animals or humans (Heck et al., 1985; Casanova et al., 1988).

Sensory and Respiratory Irritation

Case Reports and Clinical Studies: In a number of clinical studies in volunteers, irritation of the eye, nose, and throat was reported following exposure for short periods to formaldehyde at levels ranging from 0.3 to 3.6 mg/m^3 (Andersen & Mølhave, 1983; Sauder et al., 1986, 1987; Schachter et al., 1986; Green et al., 1987, 1989; Witek et al., 1987; Kulle, 1993; Pazdrak et al., 1993). Mucociliary clearance in the anterior portion of the nasal cavity was reduced following exposure of volunteers to 0.3 mg/m^3 (Andersen & Mølhave, 1983). It appears that in healthy individuals as well as those with asthma, brief exposure (up to 3 h) to concentrations of formaldehyde up to 3.6 mg/m^3 had no significant clinically detrimental effect upon lung function (Day et al., 1984; Sauder et al., 1986, 1987; Schachter et al., 1986, 1987; Green et al., 1987; Witek et al., 1987; Harving et al., 1990).

Epidemiological Studies: In a number of studies in which individual exposure was monitored, there was a higher prevalence of symptoms of irritation of the eye and respiratory tract in workers exposed to formaldehyde in the production of resin-embedded fibreglass (Kilburn et al., 1985a), chemicals, and furniture and wood products (Alexandersson & Hedenstierna, 1988, 1989; Holmström & Wilhelmsson, 1988; Malaka & Kodama, 1990) or through employment in the funeral services

industry (Holness & Nethercott, 1989), compared with various unexposed control groups. Due to the small numbers of exposed workers (38–84), however, it was not possible to meaningfully examine exposure–response in most of these investigations. Workers in these studies were exposed to mean concentrations of formaldehyde ≥ 0.17 ppm (0.2 mg/m^3).

In a survey of residences in Minnesota, effects of formaldehyde were substantially greater at concentrations above 0.4 mg/m^3 (0.3 ppm) than for levels below 0.12 mg/m^3 (0.1 ppm) (Ritchie & Lehnen, 1987). Reports of eye irritation were most frequent, followed by nose and throat irritation, headaches, and skin rash. While proportions of the population reporting eye, nose and throat irritation or headaches at above 0.3 ppm (0.4 mg/m^3) were high (71–99%), those reporting effects at below 0.1 ppm (0.12 mg/m^3) were small (1–2% for eye irritation, 0–11% for nose or throat irritation, and 2–10% for headaches). The prevalence of skin rash was between 5% and 44% for >0.3 ppm (0.4 mg/m^3) and between 0% and 3% for <0.1 ppm (0.12 mg/m^3).

Results of investigations of effects on pulmonary function in occupationally exposed populations are somewhat conflicting. Pre-shift reductions (considered indicative of chronic occupational exposure) of up to 12% in parameters of lung function (e.g., forced vital capacity, forced expiratory volume, forced expiratory flow rate) were reported in a number of smaller studies of chemical, furniture, and plywood workers (Alexandersson & Hedenstierna, 1988, 1989; Holmström & Wilhelmsson, 1988; Malaka & Kodama, 1990; Herbert et al., 1994). In general, these effects were small and transient over a workshift, with a cumulative effect over several years that was reversible after relatively short periods without exposure (e.g., 4 wk). Workers in all but the Malaka & Kodama (1990) study were exposed to mean concentrations of formaldehyde ≥ 0.4 mg/m^3 (0.3 ppm). A dose–response relationship between exposure to formaldehyde and decrease in lung function was reported by Alexandersson & Hedenstierna (1989). However, evidence of diminished lung function was not observed in other studies of larger numbers of workers (84–254) exposed to formaldehyde through employment in wood product (Horvath et al., 1988) or resin (Nunn et al., 1990) manufacturing or in the funeral services industry (Holness & Nethercott, 1989). These groups of workers were exposed to mean concentrations of formaldehyde of up to >2 ppm (2.4 mg/m^3).

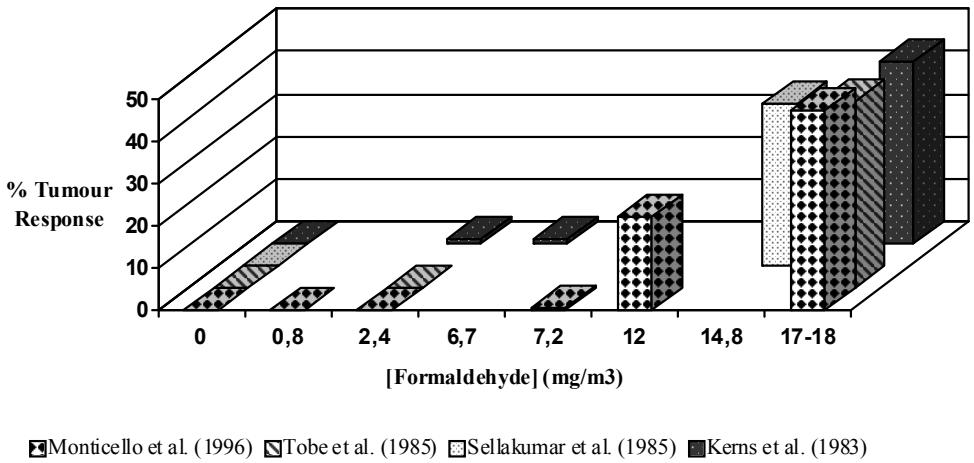
There has been a preliminary indication of effects on pulmonary function in children in the residential environment associated with relatively low concentrations of formaldehyde. The prevalence of physician-reported chronic bronchitis or asthma in 298 children aged 6–15 years exposed to concentrations between 60 and 140 ppb (72 and 168 µg/m^3) in their homes increased with exposure, especially among those also exposed to environmental tobacco smoke (Krzyzanowski et al., 1990). Levels of peak expiratory flow rates (PEFR) decreased linearly with exposure, with the decrease at 60 ppb (72 µg/m^3) equivalent to 22% of the level of PEFR in nonexposed children; this value was 10% at levels as low as 30 ppb (36 µg/m^3). Effects in a larger sample of 613 adults were less evident, with no increase in symptoms or respiratory disease and small transient decrements in PEFR only in the morning and mainly in smokers, the significance of which is unclear.

In a survey of 1726 occupants of homes containing UFFI and 720 residents of control homes, a series of objective tests of pulmonary function, nasal airway resistance, sense of smell, and nasal surface cytology were conducted (Broder et al., 1988). There were increases in prevalences of symptoms primarily at concentrations

> 0.12 ppm (0.14 mg/m^3) formaldehyde, although there was evidence of interaction between UFFI and formaldehyde associated with these effects. There were no effects on other parameters investigated, with the exception of a small increase in nasal epithelial squamous metaplasia in UFFI subjects intending to have their UFFI removed. The median concentration of formaldehyde in the UFFI homes was 0.038 ppm (0.046 mg/m^3) (maximum, 0.227 ppm [0.272 mg/m^3]); in the control homes, the comparable value was 0.031 ppm (0.037 mg/m^3) (maximum, 0.112 ppm [0.134 mg/m^3]).

Histopathological changes within the nasal epithelium have been examined in surveys of workers occupationally exposed to formaldehyde vapour (Berke, 1987; Edling et al., 1988; Holmström et al., 1989c; Boysen et al., 1990; Ballarin et al., 1992). The available data are consistent with the hypothesis that formaldehyde is primarily responsible for induction of these histopathological lesions in the nose. The weight of evidence of causality is weak, however, due primarily to the limited number of investigations of relatively small populations of workers that do not permit adequate investigation of, for example, exposure–response. Support for biological plausibility of these observed effects derives from the convincing evidence in monkeys (Rusch et al., 1983) and rodents of histopathological alterations (degenerative changes consistent with cytotoxicity) within the upper respiratory tract.

Figure 1. Formaldehyde Carcinogenicity



Carcinogenicity

Experimental Animals: The incidence of tumors in the nasal cavity has been increased in five investigations in which rats were exposed via inhalation to concentrations of formaldehyde greater than 7.2 mg/m^3 (Figure 1).

In a study in which F344 rats were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.4, 6.7, or 17.2 mg/m^3) formaldehyde for up to 24 mo, followed by a 6-mo observation period, the incidence of squamous cell carcinoma in the nasal cavity was markedly increased only in the high-concentration groups compared with the unexposed controls (0/118, 0/118, 1/119 [1%], and 51/117 [44%] in males and 0/118, 0/118, 1/116 [1%], and 52/119 [44%] in females in the control, low-, mid-, and high-concentration groups, respectively) (Kerns et al., 1983). Histopathological analysis revealed that more than half of the nasal squamous tumors in animals from the high-concentration group were located on the lateral side of the nasal turbinate and adjacent lateral wall at the front of the nose (Morgan et al., 1986c). Two nasal carcinomas (in male and female rats) and two undifferentiated carcinomas or sarcomas (in male rats) were also observed in animals from the high-concentration groups.

In a follow-up study, Monticello et al. (1996) exposed male F344 rats to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.8, 2.4, 7.2, 12, or 18 mg/m^3) formaldehyde for up to 24 mo and assessed tumor incidence within the nasal cavity. Epithelial cell proliferation at seven sites within the nasal cavity (e.g., anterior lateral meatus, posterior lateral meatus, anterior mid-septum, posterior mid-septum, anterior dorsal septum, medial maxilloturbinate, and maxillary sinus) was also determined after 3, 6, 12, and 18 months of exposure. The overall incidence of nasal squamous cell carcinoma in these animals was 0/90, 0/90, 0/90, 1/90 (1%), 20/90 (22%), and 69/147 (47%), respectively. Tumors were located primarily in the anterior lateral meatus, the posterior lateral meatus, as well as the mid-septum.

In a more limited study in which dose–response was not examined, Sellakumar et al. (1985) exposed male Sprague-Dawley rats to 0 or 14.8 ppm (0 or 17.8 mg/m^3) formaldehyde for approximately 2 yr. These authors reported a marked increase in the incidence of nasal squamous cell carcinoma (0/99 and 38/100 in the control and formaldehyde-exposed animals, respectively). These tumors were considered to have arisen primarily from the naso-maxillary turbinates and nasal septum. An increase in the incidence of nasal squamous cell carcinoma was also reported in a study by Tobe et al. (1985), in which groups of male F344 rats were exposed to formaldehyde at 0, 0.36, 2.4, or 17 mg/m^3 for 28 mo. Fourteen of 32 animals in the high-concentration group (i.e., 44%) developed nasal squamous cell carcinoma, compared with none in the unexposed (control), low-, or mid-concentration groups. In another study in which male F344 rats were exposed to 0, 0.3, 2.17, or 14.85 ppm (0, 0.36, 2.6, or 17.8 mg/m^3) formaldehyde for up to 28 mo, an increased incidence of nasal squamous cell carcinoma was observed in the high-concentration group (Kamata et al., 1997); the overall incidence of nasal tumors among these formaldehyde-exposed animals, dead or sacrificed after 12, 18, 24, and 28 months on study, was 13/32 (41%), compared with 0/32 and 0/32 in two groups of unexposed controls.

In other studies in rats, a small but not statistically significant increase in the incidence of tumors of the nasal cavity was observed in animals exposed daily to 20 ppm (24 mg/m^3) formaldehyde for 13 weeks and then observed until 130 weeks (Feron et al., 1988), but not in animals exposed to 9.4 ppm (11.3 mg/m^3) formaldehyde for 52 weeks (Appelman et al., 1988) or to 12.4 ppm (14.9 mg/m^3) formaldehyde for 104 weeks (in either the presence or absence of wood dust at a concentration of 25 mg/m^3) (Holmström et al., 1989a). The lack of observed

statistically significant increases in tumor incidence in these investigations may be a function of small group sizes and/or short periods of exposure.

In a study in which groups of $B6C3F_1$ mice were exposed to up to 14.3 ppm (17.2 mg/m^3) formaldehyde for up to 24 mo, followed by an observation period of 6 mo, there were no statistically significant increases in the incidence of tumours within the nasal cavity (Kerns et al., 1983), possibly due to the greater reduction in minute volume in mice exposed to formaldehyde than in rats (Chang et al., 1981; Barrow et al., 1983). The incidence of lung tumors was not increased in an early study in which groups of C3H mice were exposed to formaldehyde at concentrations of 0, 50, 100, or 200 mg/m^3 for three 1-h periods per week for 35 wk, although, due to high mortality, treatment in the high-dose group was discontinued in the 4th wk, and there was no evaluation of the nasal tissues (Horton et al., 1963). There was no increase in the incidence of respiratory tract tumors in male Syrian hamsters exposed to 12 mg/m^3 for their entire lives (Dalbey, 1982).

Epidemiological Studies: Possible associations between formaldehyde and cancers of various organs have been examined extensively in epidemiological cohort and case–control studies in occupationally exposed populations. In addition, several authors have conducted meta-analyses of the available data. The most extensive information is on cancers of the nasal cavity (addressed initially here) and lung (addressed subsequently).

In case–control studies, while sometimes no increase was observed overall (Vaughan et al., 1986a), significantly increased risks of nasopharyngeal cancer (up to 5.5-fold) were observed among workers with 10–25 years of exposure or in the highest exposure category in three out of four investigations (Vaughan et al., 1986a; Roush et al., 1987; West et al., 1993), although there were limitations of these studies. There was no increase in an additional investigation that is also considered to be limited (Olsen & Asnaes, 1986). In three studies in which the association between formaldehyde and nasal squamous cell carcinomas was examined, there were nonsignificant increases in two (Olsen & Asnaes, 1986; Hayes et al., 1990) and no increase in another (Luce et al., 1993), although there were limitations of these investigations. In the only investigation in which the association between exposure to formaldehyde and adenocarcinoma of the nasal cavity was examined, there was a nonsignificant increase that was enhanced in the presence of wood dust (Luce et al., 1993).

In a number of cohort studies of professionals or industrial workers, the potential association between exposure to formaldehyde and nasopharyngeal cancer has been investigated. Risks were not increased in smaller studies of anatomists or mortuary workers (Hayes et al., 1990) or in an investigation of proportionate incidence in industrial workers (Hansen & Olsen, 1995); in the latter study, however, the standardized proportionate incidence ratio for cancers of the "nasal cavity" was significantly increased (3-fold) in "more exposed" workers. In a cohort of 11 000 garment workers, the number of deaths due to cancer of the nasal cavity was considered too small to evaluate (Stayner et al., 1988). In a cohort of 14 000 workers employed in six chemical and plastic factories in the United Kingdom (35% of cohort exposed to >2 ppm [2.4 mg/m^3]), only one nasal cancer was observed versus 1.7 expected (Gardner et al., 1993). The results of the largest industrial cohort mortality study of 26 561 workers first employed before 1966 at 10 plants in the United States (4% of cohort exposed to >2 ppm [2.4 mg/m^3]) indicated an

approximately 3-fold excess of deaths due to nasopharyngeal cancer (Blair et al., 1986); however, subsequent analyses revealed that five of the seven observed deaths were among individuals who had also been exposed to particulates, and four of the seven observed deaths occurred at one specific industrial plant (Blair et al., 1987; Collins et al., 1988; Marsh et al., 1996). Three of the seven observed deaths due to nasopharyngeal cancer occurred in individuals with less than 1 year of employment (Collins et al., 1988); the four deaths at one specific plant were distributed equally amongst short-term and long-term workers (Marsh et al., 1996).

In most case–control studies, there have been no increases in lung cancer (Bond et al., 1986; Gérin et al., 1989; Brownson et al., 1993; Andjelkovich et al., 1994). In the single study where exposure–response was examined, there was no significant increase in adenocarcinoma of the lung for those with "long–high" occupational exposure; although the odds ratio was greater than for "lung cancer," the number of cases on which this observation was based was small (Gerin et al., 1989). There was no association of relative risks with latency period (Andjelkovich et al., 1994). In the most extensive investigation of exposure–response, there were no increases in lung cancer in workers subdivided by latency period, although there was a nonsignificant increase for those co-exposed to wood dust. There was no statistically significant increased risk for "all respiratory cancer" by level, duration, cumulative exposure, duration of repeated exposures to peak levels, or duration of exposure to dust-borne formaldehyde, except in one category (Partanen et al., 1990).

In cohort studies of professional and industrial workers, there have been no significant excesses of cancers of the trachea, bronchus, or lung (Stayner et al., 1988; Hayes et al., 1990; Gardner et al., 1993; Andjelkovich et al., 1995), the buccal cavity or pharynx (Stayner et al., 1988; Matanoski, 1989; Hayes et al., 1990; Gardner et al., 1993; Andjelkovich et al., 1995), the lung (Stroup et al., 1986; Bertazzi et al., 1989; Hansen & Olsen, 1995), or the respiratory system (Matanoski, 1989). The standardized mortality ratio for lung cancer was significantly increased in a "highly exposed" subgroup at one chemical and plastic factory in the United Kingdom, although there was no significant relationship with years of employment or cumulative exposure (Gardner et al., 1993). There was also a slight but significant (1.3-fold) excess of deaths due to lung cancer among the subcohort of white male industrial workers at 10 plants in the United States with ≥20 years since first exposure (Blair et al., 1986), although results of a number of follow-up studies have provided little additional evidence of exposure–response except in the presence of other substances (Blair et al., 1986, 1990a; Marsh et al., 1992, 1996; Blair & Stewart, 1994; Callas et al., 1996).

While it is the potential association between exposure to formaldehyde and cancer of the respiratory tract that has been examined in most studies, in some case–control and cohort studies, increased risks of various nonrespiratory tract cancers (e.g., multiple myeloma, non-Hodgkin's lymphoma, ocular melanoma, brain, connective tissue, pancreatic, leukemic, lymphoid and hematopoietic, colon) have occasionally been observed. However, such increases have been reported only sporadically, with little consistent pattern. Moreover, results of toxicokinetic and metabolic studies in laboratory animals and humans indicate that most inhaled formaldehyde is deposited within the upper respiratory tract. Available evidence for these tumors at sites other than the respiratory tract does not, therefore, fulfill

traditional criteria of causality (e.g., consistency, biological plausibility) for associations observed in epidemiological studies.

Cytotoxicity and Regenerative Proliferative Response

Increased cellular proliferation as a consequence of epithelial cell toxicity appears to be the most significant determinant of neoplastic progression associated with exposure to formaldehyde Kerns et al., 1983; Rusch et al., 1983; Appelman et al., 1988; Woutersen et al., 1989;). The effect of formaldehyde on cell proliferation within the respiratory epithelium of rats has been examined in a number of short-term, subchronic, and chronic studies (Swenberg et al., 1983; Wilmer et al., 1987, 1989; Zwart et al., 1988; Reuzel et al., 1990; Monticello et al., 1991, 1996; Casanova et al., 1994). Histopathological effects and a sustained increase in proliferation of nasal epithelial cells has not been observed following the exposure of rats to concentrations of formaldehyde of 2.4 mg/m^3 (2 ppm) or less, irrespective of the exposure period. In rats, histopathological effects and associated increased respiratory epithelial cell proliferation in the nasal cavity appear to be more closely related to the concentration of formaldehyde to which the animals are exposed, than to the total cumulative exposure (Swenberg et al., 1983; Wilmer et al., 1987, 1989). The relative magnitude of the increase in the proliferative response is dependent upon the specific site within the nasal cavity being examined (Swenberg et al., 1986; Monticello et al., 1991, 1996; Monticello & Morgan, 1994). The extent of the carcinogenic response following exposure to formaldehyde is also dependent upon the size of the target cell population within specific regions of the nasal cavity (Monticello et al., 1996).

Owing to the reactivity of formaldehyde as well as differences in breathing patterns, adverse effects following short-term inhalation exposure of formaldehyde in rodents are generally restricted to the nasal cavity, while effects in primates may be observed deeper within the respiratory tract. Although direct evidence in humans is lacking, increased epithelial cell proliferation (respiratory and olfactory epithelia) and DNA–protein cross-link formation (middle turbinates, lateral wall and septum, and nasopharynx) within the upper respiratory tract have been observed in monkeys exposed to formaldehyde by inhalation (Monticello et al., 1989; Casanova et al., 1991). At similar levels of exposure, concentrations of DNA–protein cross-links were approximately an order of magnitude less in monkeys than in rats. In rats, the cumulative yield of DNA–protein cross-links was similar after acute and subchronic exposure, suggesting rapid repair (Casanova et al., 1994).

Genotoxicity

Experimental Animals: The results of a wide variety of *in vitro* assays (see IARC, 1995, for a review) have indicated that formaldehyde is genotoxic at high concentrations (i.e., weakly genotoxic) in both bacterial and mammalian cells *in vitro* (inducing both point and large-scale mutations). Formaldehyde induces mutations in *Salmonella typhimurium* and *Escherichia coli*, with positive results obtained in the presence or absence of metabolic activation systems. Formaldehyde increases the frequency of chromatid/chromosome aberrations, sister chromatid exchange, as well as gene mutations in a variety of rodent and human cell types. Exposure to formaldehyde increased DNA damage (strand breaks) in human fibroblasts and rat

tracheal epithelial cells and increased unscheduled DNA synthesis in rat nasoturbinate and maxilloturbinate cells.

In *in vivo* studies, exposure to formaldehyde had no effect on the proportion of bone marrow cells with cytogenetic anomalies (e.g., chromatid or chromosome breaks, centric fusions) (Dallas et al., 1992) in male Sprague-Dawley rats or the frequency of sister chromatid exchange or chromosomal aberrations and mitotic index in blood lymphocytes in male and female F344 rats (Kligerman et al., 1984). On the other hand, exposure led to a modest (1.7- to 1.8-fold), statistically significant (i.e., $p < 0.05$) increase in the proportion of pulmonary macrophages with chromosomal aberrations in male Sprague-Dawley rats at 18 mg/m^3 (Dallas et al., 1992). A statistically significant increase in the proportion of bone marrow cells with chromosomal aberrations (chromatid or chromosome breaks) was observed in one study with female Wistar rats exposed to low concentrations (up to 1.5 mg/m^3) of formaldehyde (Kitaeva et al., 1990).

The mutational profile for formaldehyde varies with cell type and formaldehyde concentration. In human lymphoblasts, about half of the mutants at the X-linked *hprt* locus had deletions of some or all of the *hprt* gene bands; the other half were assumed to have point mutations (Crosby et al., 1988). In a study of the mutational spectra induced by formaldehyde at the *gpt* gene in *E. coli* (Crosby et al., 1988), a 1-h exposure to 4 mmol/L induced a spectrum of mutants that included large insertions (41%), large deletions (18%), and point mutations (41%), the majority of which were transversions occurring at GC base pairs; a 40 mmol/L exposure resulted in a more homogeneous spectrum, with 92% of the mutants being produced by a point mutation, 62% of which were transitions at a single AT base pair. In 5 of 11 squamous cell carcinomas from rats exposed to 15 ppm (18 mg/m^3) for up to 2 years, there were point mutations at the GC base pairs in the p53 cDNA sequence (Recio et al., 1992).

Formaldehyde-induced DNA–protein cross-linking has been observed in the nasal epithelium of rats (Casanova & Heck, 1987; Heck & Casanova, 1987; Casanova et al., 1989, 1994) as well as in epithelia lining the respiratory tract of monkeys (Casanova et al., 1991) exposed via inhalation. DNA–protein cross-links are considered a marker of mutagenic potential, since they may initiate DNA replication errors, resulting in mutation. The exposure–response relationship is highly nonlinear, with a sharp increase in DNA–protein cross-linking at concentrations above 4 ppm (4.8 mg/m^3) without accumulation on repeated exposure (Casanova et al., 1994). Formaldehyde has also induced the formation of DNA–protein cross-links in a variety of human and rat cell types (Saladino et al., 1985; Bermudez & Delehanty, 1986; Snyder & van Houten, 1986; Craft et al., 1987; Heck & Casanova, 1987; Cosma et al., 1988; Olin et al., 1996).

Epidemiological Studies: Evidence has been most consistent for effects at site of first contact with an increased incidence of micronucleated buccal or nasal mucosal cells being reported in some surveys of individuals occupationally exposed to formaldehyde (Ballarin et al., 1992; Suruda et al., 1993; Kitaeva et al., 1996; Titenko-Holland et al., 1996). Evidence of genetic effects (i.e., chromosomal aberrations, sister chromatid exchanges) in peripheral lymphocytes from individuals exposed to formaldehyde vapour has also been reported in some studies (Suskov & Sazonova, 1982; Bauchinger & Schmid, 1985; Yager et al., 1986; Dobiás et al.,

1988, 1989; Kitaeva et al., 1996), but not others (Fleig et al., 1982; Thomson et al., 1984; Vasudeva & Anand, 1996; Zhitkovich et al., 1996).

Assessment of Weight of Evidence including Mode of Action - Cancer

In epidemiological studies of occupationally exposed populations, there has been little evidence of a causal association between exposure to formaldehyde and lung cancer. Indeed, results of studies in a rather extensive database of cohort and case–control studies do not fulfill traditional criteria of causality in this regard, such as consistency, strength, and exposure–response. Increases in mortality or incidence have not been observed consistently, and, where examined, there has consistently been no evidence of exposure–response. The data for nasal and nasopharyngeal cancer are less clear. In case–control studies, there have been increases in cancers of the nasal or nasopharyngeal cavities that fulfill, at least in part, traditional criteria of causality, with tumors having been observed in workers with highest levels or duration of exposure. It should be noted, though, that measures of exposure in these population-based investigations are rather less reliable than those in the larger, most extensive cohort studies of occupationally exposed populations; moreover, methodological limitations complicate interpretation of several of the case–control studies. Excesses of cancers of the nasal or nasopharyngeal cavities have not been observed consistently in cohort studies. Where there have been excesses, there has been little evidence of exposure–response, although the total number of observed tumors was small.

Five carcinogenicity bioassays have provided consistent evidence that formaldehyde is carcinogenic in rats exposed via inhalation (Kerns et al., 1983; Sellakumar et al., 1985; Tobe et al., 1985; Monticello et al., 1996; Kamata et al., 1997). The incidence of nasal tumors was not significantly increased in mice exposed to formaldehyde by inhalation (Kerns et al., 1983). This has been attributed, at least in part, to the greater reduction in minute volume in mice exposed to formaldehyde than in rats (Chang et al., 1981; Barrow et al., 1983), resulting in lower exposures in mice than in rats (Barrow et al., 1983).

Observation of tumors at the site of contact is consistent with toxicokinetic considerations. Formaldehyde is a highly water-soluble, highly reactive gas that is absorbed quickly at the site of contact. It is also rapidly metabolized, such that exposure to even high concentrations of atmospheric formaldehyde does not result in an increase in blood concentrations.

Results of epidemiological studies in occupationally exposed populations are consistent with a pattern of weak positive responses for the genotoxicity of formaldehyde, with good evidence of an effect at site of contact (e.g., micronucleated buccal or nasal mucosal cells). Evidence for distal (i.e., systemic) effects is equivocal (chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes). The contribution of co-exposures to observed effects cannot be precluded.

The results of a large number of *in vitro* assays of a variety of endpoints indicate that formaldehyde is weakly genotoxic in both bacterial and mammalian cells. The spectrum of mutation induced by formaldehyde *in vitro* varies among cell types and concentrations to which cells were exposed but includes both point and large-scale changes. The results of *in vivo* studies in animals are similar to those in humans, with effects at site of contact being observed (e.g., modest increase in the

proportion of pulmonary macrophages with chromosomal aberrations in rats following inhalation and cytogenetic alterations in the gastrointestinal epithelium of rats following oral exposure). Evidence of distal (systemic) effects is less convincing. Indeed, in the majority of studies of rats exposed to formaldehyde via inhalation, genetic effects within peripheral lymphocytes or bone marrow cells have not been observed. Overall, formaldehyde is weakly genotoxic, with effects most likely to be observed *in vivo* in cells from tissues or organs with which the aldehyde comes into first contact.

The mechanisms by which formaldehyde induces nasal tumors in rats are not understood. However, it has been hypothesized that a sustained increase in epithelial cell regenerative proliferation resulting from cytotoxicity is a requisite precursor in the mode of induction of tumors. Mutation, for which the formation of DNA–protein cross-links serves as a marker of potential, may also contribute to the carcinogenicity of the compound in the nasal cavity of rats. Studies relevant to assessment of the mode of action include a cancer bioassay (Monticello et al., 1996) in which intermediate endpoints (proliferative response in various regions of the nasal epithelium) have been investigated. The relevant database also includes numerous shorter-term studies in which proliferative response and the formation of DNA–protein cross-links in the nasal epithelium of rats and other species have been examined following exposure via regimens often similar to those in the cancer bioassays (Swenberg et al., 1983; Casanova & Heck, 1987; Heck & Casanova, 1987; Casanova et al., 1989, 1991, 1994; Monticello et al., 1989, 1991). It should be noted, though, that due to the limited data on intermediate endpoints in most of the cancer bioassays, information available as a basis for direct comparison of the incidence of intermediate lesions (i.e., proliferative response as a measure of cytotoxicity and DNA–protein cross-linking) and tumors is limited.

In all cases where examined (Table 3), without exception, sustained cytotoxicity and cellular proliferation were observed in the nasal cavities of the same strain of rats exposed in a similar manner in short-term studies to concentrations or doses that induced nasal tumors in the cancer bioassays (Monticello et al., 1991, 1996). However, the converse is not always true. Similarly, tumors have been observed only at concentrations at which increases in DNA–protein cross-links have been observed in shorter-term studies in the same strain (Casanova & Heck, 1987; Heck & Casanova, 1987; Casanova et al., 1989, 1994).

In addition, where proliferative response (Monticello et al., 1991, 1996) and DNA–protein cross-linking (Casanova et al., 1994) have been examined in various regions of the nasal passages, sites at which there are increases are similar to those where tumors have been observed. The concentration–response relationships for DNA–protein cross-linking, cytotoxicity, proliferative response, and tumors are highly nonlinear, with significant increases in all endpoints being observed at concentrations of 4 ppm (4.8 mg/m^3). This correlates well with the concentration at which mucociliary clearance is inhibited (>greater than 2.4 mg/m^3 (Morgan et al., 1986a) and glutathione-mediated metabolism saturated (i.e., 4 ppm [4.8 mg/m^3]) (Casanova & Heck, 1987). Histological changes, increased epithelial cell proliferation, and DNA–protein cross-linking are more closely related to the exposure concentration, than to total cumulative exposure (Swenberg et al., 1983; Casanova et al., 1994).

TABLE 3. Comparative Effects of Formaldehyde Exposure upon Cell Proliferation, DNA-protein Crosslinking and Tumour Incidence

Formaldehyde Concentration mg/m^3 (ppm)	Cell Proliferation[1]			DNA-Protein Crosslink Formation[2]		Incidence of Nasal Carcinoma[3]			
	Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	High tumour region	Low tumor region	All sites	Anterior Lateral meatus	Posterior lateral meatus	Anterior mid-septum
0 (0)	10.11	7.69	6.58	0	0	0/90	0/90	0/90	0/90
0.8 (0.7)	10.53	7.82	8.04	5	5	0/90	0/90	0/90	0/90
2.4 (2)	9.83	11.24	12.74	8	8	0/96	0/96	0/96	0/96
7.2 (6)	15.68	9.96	4.15	30	10	1/90	1/90	0/90	0/90
12 (10)	76.79	15.29	30.01	-	-	20/90	12/90	2/90	0/90
18 (15)	93.22	59.52	75.71	150	60	69/147	17/147	9/147	8/147

[1] Cell proliferation (i.e., [^{3}H]thymidine-labelled cells/mm basement membrane) measured in three locations of the nasal epithelium in male F-344 rats exposed to the indicated concentrations of formaldehyde, 6 hours/day, 5 days/week for three months (Monticello et al. , 1996).

[2] Extent of DNA-protein crosslink formation (i.e., pmole [^{14}C]formaldehyde bound/mg DNA) measured in two regions of the nasal cavity (respiratory mucosa) in male F-344 rats exposed to the indicated concentrations of formaldehyde, 6 hours/day, 5 days/week for about 12 weeks; the complete lateral meatus was designated the high tumour region; the low tumour region comprised the medial aspects of naso- and maxilloturbinates, posterior lateral wall, posterior dorsal septum excluding olfactory region, and nasopharyngeal meatuses (Casanova et al., 1994). Data were derived from graphical representations in the reference cited.

[3] Incidence of nasal tumors within the entire nasal cavity or the anterior lateral meatus, posterior lateral meatus or anterior mid-septum in male F-344 rats exposed to the indicated concentrations of formaldehyde, 6 hours/day, 5 days/week for 24 months (Monticello et al., 1996).

While the respective roles of DNA–protein cross-linking, mutation, and cellular proliferation in the induction of tumors in the rat nose are not fully delineated, the hypothesized mode of carcinogenesis is in keeping with the growing body of evidence supporting the biological plausibility that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis. Regenerative cell proliferation following formaldehyde-induced cytotoxicity increases the number of DNA replications and thus increases the probability of a DNA–protein cross-link initiating a DNA replication error, resulting in a mutation. This proposed mode of action is consistent with the observed inhibition of DNA replication in the rat nose at elevated concentrations (Heck & Casanova, 1994) and point mutations in the p53 tumor suppressor gene in tumors from the noses of rats exposed to formaldehyde (Recio et al., 1992).

The hypothesized mode of induction of formaldehyde-induced tumors that satisfies several criteria for weight of evidence, including consistency, concordance of exposure–response relationships across intermediate endpoints, and biological plausibility and coherence of the database, is likely relevant to humans, at least qualitatively. Increased cell proliferation (Monticello et al., 1989) and DNA–protein cross-link formation (Casanova et al., 1991) within epithelia of the upper respiratory tract have been observed in monkeys exposed to formaldehyde vapor. Although not sufficient in itself as a basis for inferring causality, direct evidence on histopathological lesions in the nose of humans exposed primarily to formaldehyde in the occupational environment is consistent with a qualitatively similar response of the upper respiratory tract in humans and experimental animals to formaldehyde.

Because formaldehyde is highly reactive at the site of contact, dosimetry is of critical importance when extrapolating across species that have significantly different anatomical features of the nasal and respiratory passages and patterns of flow of inhaled air. Since humans as well as other primates are oronasal breathers, whereas rats are obligate nose breathers, effects in humans associated with the inhalation of formaldehyde are likely to be observed in a wider area deeper within the respiratory tract. Indeed, in rats exposed to moderate levels of formaldehyde, histopathological changes, increased epithelial cell proliferation, as well as DNA–protein cross-link formation are restricted to the nasal cavity; in formaldehyde-exposed monkeys (as surrogates for humans), on the other hand, effects have been observed deeper within the respiratory tract. While the epidemiological studies taken as a whole do not provide strong evidence for a causal association between formaldehyde exposure and human cancer, the possibility of increased risk of respiratory cancers, particularly those of the upper respiratory tract, cannot be excluded on the basis of available data.

Based primarily upon data derived from laboratory studies, therefore, the inhalation of formaldehyde under conditions that induce cytotoxicity and sustained regenerative proliferation is considered to present a carcinogenic hazard to humans.

EXPOSURE–RESPONSE ANALYSIS

Sensory and Respiratory Irritation

There are sufficient data from clinical studies and cross-sectional surveys of human populations, as well as supporting observations from experimental studies conducted with laboratory animals, to indicate that the irritant effects of formaldehyde on the

eyes, nose, and throat occur at lowest concentration. Although individual sensitivity and exposure conditions such as temperature, humidity, duration, and co-exposure to other irritants are likely to influence response levels, in well-conducted studies, only a very small proportion of the population experiences symptoms of irritation following exposure to ≤0.1 ppm (0.12 mg/m^3) formaldehyde. This is less than the levels that induce mucociliary clearance in the anterior portion of the nasal cavity in available clinical studies in human volunteers (0.3 mg/m^3) and histopathological effects in the nasal epithelium in cross-sectional studies of formaldehyde-exposed workers (0.3 mg/m^3).

Additional investigation of preliminary indication of effects on pulmonary function in children in the residential environment associated with lower concentrations of formaldehyde (40–60 ppb [48–72 µg/m^3]) (Krzyzanowski et al., 1990) may be warranted.

Carcinogenicity

There is indisputable evidence that formaldehyde is carcinogenic in rats following inhalation, with the carcinogenic response being limited to the site of contact (e.g., the nasal passages of rodents). While the mechanism of action is not well understood, based primarily upon data derived from laboratory studies, regenerative proliferation associated with cytotoxicity appears to be an obligatory intermediate step in the induction of cancer by formaldehyde. Interaction with genetic material, the potential for which is indicated by DPX, likely also contributes, although the probability of mutation resulting from DPX is unknown.

Available data are also consistent with the hypothesis that humans would respond qualitatively similarly to experimental animals. Owing to limited sensitivity of the available epidemiological studies, insufficiency of evidence for a causal association in epidemiological studies is not inconsistent with this hypothesis. However, since formaldehyde is highly reactive at the site of contact, dosimetry is of critical importance in predicting interspecies variations in response, as a function of flux to the tissue and regional tissue susceptibility, due to the significantly different anatomical features of the nasal and respiratory passages, ventilation and breathing patterns between experimental animals and humans.

The outcomes of two approaches to dose-response modelling are presented here – a biologically based case specific model and default, curve-fitting methodology (CIIT, 1999; Health Canada, 1998). It is the biologically motivated case-specific model that is considered to provide the most defensible estimates of cancer risk. While this model entails simplification of cancer biology, which requires selection of a number of parameters and use of simplifying assumptions, it is considered to offer improvement over default methodology due to incorporation of as many biological data as possible. Moreover, in view of the clear preference herein for the biologically motivated case-specific model, there has been no attempt to incorporate more of the biological data in the calculation of tumorigenic concentrations by default methodology (e.g., dose and time dependence to derive an empirical dose metric for rats).

Sensitivity analysis conducted to determine which of the model parameters has greatest impact on risk estimates or to identify which parameters are known with the highest degree of certainty for this biologically motivated case specific model was limited to a few parameters of the clonal growth (i.e., time delay, division rate at

maximum flux into the nose of the rat, the relationship between DPX concentration and the probability of mutation per cell generation) and dosimetry (number of flux bins) components. However, output of the model is considered adequate as a basis to ensure that measures taken to prevent sensory irritation[4] in human populations are sufficiently protective with respect to carcinogenic potential.

The biologically motivated case specific model incorporates regenerative cell proliferation as a required step in the induction of tumours by formaldehyde and a contribution from mutagenicity (not defined specifically by DPX) that has greatest impact at low exposures through modelling of complex functional relationships for cancer due to actions of formaldehyde on mutation, cell replication and exponential clonal expansion. The incorporated clonal growth modelling is identical to other biologically based, two-stage clonal growth models [also known as MVK models], incorporating information on normal growth, cell cycle time and cells at risk (in various regions of the respiratory tract). Species variations in dosimetry are taken into account by CFD modelling of formaldehyde flux in various regions of the nose and a single-path model for the lower respiratory tract of humans (CIIT, 1999).

Based upon the biologically motivated case specific model, the predicted additional risk of upper respiratory tract cancer for non-smoking workers with an 80-year lifetime continuous exposure to 0.004 ppm (0.0048 mg/m^3) formaldehyde, and having 40 years occupational exposure (8 hours/day, 5 days/week) to 1 ppm (1.2 mg/m^3) formaldehyde was 8.8×10^{-6} (CIIT, 1999). For the general population, the predicted additional risks of upper respiratory tract cancer for non-smokers, associated with an 80-year continuous exposure to levels of formaldehyde between 0.001 and 0.1 ppm (1.2 and 120 µg/m^3), range from 2.3×10^{-10} to 2.7×10^{-8}, respectively (CIIT, 1999).

No excess risk was predicted by the human clonal growth model in a cohort exposed to formaldehyde at a specific plant examined in two epidemiological studies (Blair et al., 1986; Marsh et al., 1996). This was consistent with the observed number of cases of respiratory tract cancer (113 observed deaths; 120 expected) in the cohort. Thus, the outcome of the model was consistent with the results of the epidemiological studies.

For comparison, based upon the approach typically employed in the assessment of Priority Substances, a Tumorigenic Concentration$_{05}$ (TC_{05}) (i.e., the concentration associated with a 5% increase in tumour incidence over background) of 7.9 ppm (9.5 mg/m^3) (95% lower confidence limit [LCL] = 6.6 ppm [7.9 mg/m^3]) formaldehyde was derived from data on the incidence of nasal squamous tumours in rats exposed to this substance in the single study (i.e., Monticello *et al*., 1996) in which exposure–response was best characterized.[5] The TC_{05} is calculated by first fitting a multistage model to the exposure–response data.

[4] Occurs at lower concentrations than effects on mucociliary clearance or histopathological damage to the nose of humans.

[5] Based upon the incidence of nasal tumours in rats exposed to formaldehyde, combined from the studies conducted by Kerns *et al*. (1983) and Monticello *et al*. (1996), the concentration of formaldehyde associated with a 5% increase in tumour incidence (maximum likelihood estimate) was approximately 6.1 ppm (7.3 mg/m^3) (CIIT, 1999).

RISK CHARACTERIZATION

In humans (as well as laboratory animals), signs of ocular and upper respiratory tract sensory irritation have been observed at exposures typically greater than 0.1 ppm [120 μg/m^3]). The estimated median and mean 24-hour time-weighted average exposures to formaldehyde in air in Canada, are, at most, one-third of this value. This value is also greater than the estimated time-weighted average exposure to which 95% of the population is exposed. In some indoor locations, however, concentrations may approach the level associated with signs of eye and respiratory tract sensory irritation in humans.

The risks of upper respiratory tract cancer predicted by the biologically motivated case-specific model to be associated with exposure to the median, mean and 95th percentile concentrations of formaldehyde in air in Canada are also exceedingly low (i.e., $<2.7 \times 10^{-8}$). Comparison of the output of the biologically motivated case-specific model with that for the comparable value for default methodology (i.e., estimation of tumorigenic concentrations close to the experimental range), indicates that values for the former are at least three orders of magnitude less than that for the latter.

UNCERTAINTIES AND DEGREE OF CONFIDENCE IN EXPOSURE ESTIMATION, HAZARD CHARACTERIZATION, AND EXPOSURE–RESPONSE ANALYSIS

There is a moderate degree of confidence in the characterization of the principal source of exposure of the general population (i.e., residential indoor air). In the two studies where there was active sampling for a 24-h duration, the analytical and sampling methodologies were optimum, all of the samples were analyzed by a single specialized laboratory, and the effects of diurnal variation were minimized by the 24-h sampling duration. The data are also reasonably current (i.e., 1991–1993) and the measured values consistent with those determined in surveys in other countries. While some uncertainty is introduced by pooling of these data with those from the remaining three studies, which involved passive sampling, the ranges and distributions of concentrations in these subsets of data were similar. Some uncertainty is introduced by the limited size and representation of the data set (n = 151 homes in Windsor, Hamilton, Trois-Rivières, Quebec City, Saskatoon, and various locations in the Northwest Territories), lack of random sampling of the homes, and involvement of volunteers.

Although it contributes less to total exposure, there is a high degree of confidence in the characterization of the concentrations of formaldehyde in ambient air in Canada, due to the magnitude and sensitivity of the relevant monitoring data. Uncertainty concerning the time spent indoors by Canadians is judged to be low, since the estimate is based on the most current Canadian data, the time–activity data were obtained based on a random sampling scheme, and analysis of the data involved population weighting. The probabilistic estimates of exposure are limited by lack of information on time activity data and concentrations to which individuals are exposed.

With respect to toxicity, the degree of confidence that critical effects are well characterized is high. A relatively extensive database in both humans and animals indicates that critical effects occur at the initial site of exposure to this substance. The database in humans is also sufficiently robust to serve as a basis for confident

conclusion concerning the consistently lowest levels at which effects (i.e., sensory irritation) occur, although additional investigation of an unconfirmed report of effects on respiratory function in children exposed to lower levels of formaldehyde may be desirable.

The degree of confidence in the database that supports an obligatory role of regenerative proliferation in the induction of nasal tumours in rats is moderate to high, although the mechanism of carcinogenicity of formaldehyde is unclear. Although the biologically motivated case-specific model for estimation of cancer risks is clearly preferred due to incorporation of as many biological data as possible, there are a number of uncertainties described in more detail in CIIT (1999) and summarized briefly here, although sensitivity analyses were not conducted. For dosimetry, sources of uncertainty for which sensitivity analyses would have been appropriate include the use of individual rat, primate and human nasal anatomies as representative of the general population, the use of a typical-path human lung structure to represent people with compromised lungs, the sizes of specific airways, the use of a symmetric Weibel model for the lung, the estimation of the location and extent of squamous and olfactory epithelium and of mucus- and non-mucus-coated nasal regions in the human, and the values of mass transfer and dispersion coefficients. The lack of human data on formaldehyde-related changes in the values of key parameters of the clonal growth component accounts for much of the uncertainty in the biologically motivated case specific model.

In order to better define the mode of action of induction of tumours, elaboration of the quantitative relationship between DPX and mutation and the time course of loss of DNA–protein crosslinks is desirable. Additional characterization of the shape of the concentration–response relationship for regenerative proliferative response would also be informative.

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To ensure transparency and defensibility of the health assessments, a cut-off date for consideration of new data is specified so as not to compromise the integrity of several stages of internal and external review. Data obtained after January 1999 were not considered for inclusion in the assessment.

M. Walker and J. Zielenski, Division of Biostatistics and Research Coordination, Health Canada, and D. Blakey and G. Douglas, Environmental and Occupational Toxicology Division, Health Canada, contributed to the preparation of sections on dose–response analyses for cancer and genotoxicity, respectively.

In 1996, a government–private Steering Committee was formed in the United States to develop a model for dose–response analyses for formaldehyde that takes into account as much of the biological database on formaldehyde as possible. This partnership involved primarily the CIIT and the U.S. EPA. Toxicology Excellence for Risk Assessment, commissioned by the Formaldehyde Epidemiology, Toxicology, and Environmental Group, Inc., also participated, preparing sections of draft documentation related to hazard assessment. Health Canada joined this partnership later, contributing by organizing, in collaboration with the U.S. EPA, an external peer review workshop and revising some sections of the draft documentation related to hazard assessment (in particular, those addressing epidemiological data).

The product of this joint effort was a draft document entitled "Formaldehyde: Hazard Characterization and Dose–Response Assessment for Carcinogenicity by the

Route of Inhalation" (CIIT, 1999). This report, which was developed primarily by CIIT (with input from J. Overton, U.S. EPA), was reviewed at an external peer review workshop of the following invitees, convened by Health Canada and the U.S. EPA on March 18–20, 1998, in Ottawa, Ontario (Health Canada, 1998): B. Allen (RAS Associates), M. Andersen (ICF Kaiser Engineering, Chair), D. Blakey (Health Canada), A. Dahl (Lovelace Respiratory Research Institute), D. Gaylor (U.S. Food and Drug Administration), J. Harkema (Michigan State University), D. Jacobson-Kram (MA BioServices), D. Krewski (Health Canada), R. Maronpot (National Institute of Environmental Health Sciences), G. Marsh (University of Pittsburgh), J. Siemiatycki (Institut Armand-Frappier), and J. Ultman (Pennsylvania State University). Written comments were also provided by S. Moolgavkar (Fred Hutchinson Cancer Research Center).

Following the workshop, the report was revised to reflect comments of the external reviewers and recirculated; written comments on the subsequently revised draft were submitted by all members of the external review panel. The final draft (dated September 28, 1999) (CIIT, 1999) was reviewed by the Chair of the workshop (Andersen, 1999).

Background sections of the supporting documentation pertaining to human health were reviewed primarily to address adequacy of coverage. Written comments were provided by J. Acquavella (Monsanto Company), S. Felter (Toxicology Excellence for Risk Assessment), O. Hernandez (U.S. Environmental Protection Agency [EPA]), R. Keefe (Imperial Oil Limited), N. Krivanek (Dupont Haskell Laboratory), J. Martin (consultant), and F. Miller (Chemical Industry Institute of Toxicology [CIIT]).

R. Vincent, Environmental Toxicology Division, Health Canada, provided comments on the Assessment Report. Accuracy of reporting, adequacy of coverage, and defensibility of conclusions with respect to hazard characterization and dose–response analyses were considered in written review by M. Andersen (Colorado State University), V. Feron (TNO-Nutrition and Food Research Institute), and J. Swenberg (University of North Carolina).

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Chapter 12

Discussion

As stated in the introduction, the goal of the research described in this thesis is the additional development and application of analytical frameworks in chemical risk assessment. Specifically addressed is the human relevance of mode of action (MOA/HR) framework and implications for dose-response assessment and toxicity testing. The development, evolution and current and potential (likely essential) additional impact of this framework is considered here.

Framework analysis contributes to better coordination of the research and regulatory communities. It impacts both the design and translation of relevant mechanistic studies and interpretation of results as a basis for quantitative characterization of risk. It contributes principally through increasing transparency and collaboration in identifying relevant critical data gaps in a risk assessment context and ultimately, testing strategies.

Evolution of Analytical Frameworks for Hazard Characterization and Dose-Response Analysis:

The MOA/HR framework emphasizes the importance of mechanistic data in predicting risk. It additionally brings rigor to analysis through requirement to state explicitly an initial hypothesis for mode(s) of action with delineated key events. Also, the weight of evidence for hypothesized mode(s) of action is considered in the context of stated and well accepted criteria. This rigor necessarily requires early and continuing relevant interdisciplinary input.

In addition to increasing communication between the relevant communities, these analyses have considerable potential to increase consistency in the output of hazard and risk characterization. This in turn may promote consistency and potentially (over the longer term), convergence in decision-making. Framework analysis also facilitates peer engagement owing to increased transparency of documentation. Critical research needs are clearly identified, as are uncertainties in the analysis.

This rigor and transparency provides a basis for integration of information from evolving technologies and data sources which are more mechanistically based. These analyses have considerable potential, then, to increase understanding and evolution of more relevant and predictive chemical testing strategies. This and related frameworks for the use of mechanistic data in dose-response assessment will be important, then, in transitioning the risk assessment community from reliance on relatively uninformed default approaches to more progressive and predictive mode of action based hazard and risk characterization. Increased understanding and experience in the use of such information in regulatory risk assessment is likely to contribute immeasurably to the subsequent refocus of current toxicity testing from hazard identification to hazard characterization as a basis for more predictive and protective public health protection. These developments are essential as a basis to address current regulatory challenges which require priority setting and assessment with limited resources, for large numbers of substances currently in commerce (Chapter 2 and 3).

The MOA/HR framework was conceived due to the collaborative efforts of a number of principally senior regulatory scientists from the U.S. EPA, Health Canada and agencies within Europe, coordinated within several international initiatives (Chapter 4). The work was initiated based on observation that a major impediment to harmonization of hazard characterization in risk assessment was inconsistency in the evaluation of mode of action. Initially in 2001, then, as part of its efforts to harmonize and advance risk assessment practice, the International Programme on Chemical Safety (IPCS)

(WHO/ILO/UNEP) published a framework for assessment of the weight of evidence for hypothesized MOAs for carcinogenesis in laboratory animals (animal MOA) (Sonich-Mullin et al., 2001).

This was based on consideration of specific aspects of data analysis developed much earlier by Bradford Hill as a basis for evaluation of causality of observed associations in epidemiological studies (Hill, 1964). It also derived from the leading contribution of Faustman *et al.* (1997) who proposed application of these criteria in the consideration of weight of evidence for hazard (developmental toxicity) which were subsequently refined for cancer (USEPA 2005). Critical aspects of the proposed framework included clear delineation of key events in an hypothesized mode(s) of action. Weight of evidence for key events is considered in the context of the "Hill criteria" including dose response and temporal concordance between key and end events, consistency, specificity, biological plausibility and coherence. In its initial development, the framework for the weight of evidence of an MOA in animals was illustrated through application to principally non DNA reactive carcinogens.

The framework also encourages drawing much more robustly on the totality of the toxicological data, as a basis to inform hypotheses concerning modes of action. It requires, for example, consideration particularly of key events that may be occurring at lower doses or earlier time points, based on data which are often not emphasized in assessment currently. Lack of robust assimilation of these data can be attributed, often, to (premature) focus on effects at lowest doses in longer term studies as a basis to identify points of departure (such as effect levels or benchmark doses or concentrations), in default approaches to dose-response assessment.

More recently, the IPCS framework for mode of action in animals was expanded to address human relevance (HRF) in a project of the International Life Sciences Risk Sciences Institute (ILSI RSI). Case studies to address both cancer (Chapter 5) and non-cancer effects (Seed et al., 2005) were developed. The framework has been additionally further refined through application to additional case studies in related subsequent initiatives of IPCS (Boobis et al, 2006, 2008).

In this framework, human relevance of an hypothesized MOA in animals, which is supported by the weight of evidence is considered in the context of the key events along the causal pathway (Chapter 5). This involves consideration of not only chemical specific data but more generic information, such as anatomical, physiological and biochemical variations among species, human disease models and/or patterns of effects for chemicals with related modes of action. In this manner, the framework encourages early assimilation of information on mode of action. It also encourages maximum use of both chemical-specific and more generic information (Figure 2).

Though developed and refined initially through application in case studies for principally non DNA reactive carcinogens in the context of hazard characterization, more recently, the framework has been extended to all types of toxic effects. This includes DNA reactive carcinogens, non-cancer endpoints and different life stages. Additional to its use in hazard characterization, the implications of consideration of key events in dose-response assessment are being taken into account. This includes the pre-existing framework for chemical specific adjustment factors (IPCS, 2005). It additionally includes a series of related ongoing international projects addressing PBPK modeling, mutagenic modes of action, combined exposures and a dose-response framework relevant not only for chemicals but also pathogenic organisms, allergens, and nutrients (Loizou et al., 2008; Meek et al., 2009a,b; Olin, personal communication, 2009).

Development of this framework for the human relevance of mode of action has now involved engagement of more than 150 scientists internationally (Meek et al., 2008). In the period since its inception, the framework has been widely adopted in regulatory risk assessment, being incorporated into national, international and supra-national guidance as a basis to increase transparency concerning uncertainty, promote consistency in decision-making, facilitate peer engagement and identify critical research needs (EFSA, 2006; European Commission, 2003; IPCS, 2006; JMPR, 2006; OECD, 2002; UNECE, 2007). It has also been extensively, even routinely, adopted in risk assessments by the U.S. Environmental Protection Agency (USEPA, 2005a, 2007; SAB 1999; SAP, 2000; Dellarco and Baetcke, 2005), the UK (COC, 2004), Health Canada (see, for example, Chapters 10 and 11) and other governmental organizations. Peer recognition of the contribution of the framework is evidenced by the Society of Toxicology's 2006 awards for Best Paper in *Fundamental and Applied Toxicology* and *Toxicological Sciences* (Green et al., 2005 and Pastoor et al., 2005).

This framework sets the scene for systematic incorporation of mode of action data in dose-response assessment (Chapters 6 and 7) and dovetails well with earlier international work on chemical specific adjustment factors (Chapters 8 and 9). This earlier project involved the preparation of guidance under the auspices of IPCS concerning the quantity and nature of mode of action data considered appropriate for replacement of default uncertainty factors with chemical -specific quantitative values. This guidance was developed based on consideration of defined data sets in case studies which were selected to include a representative range of the nature of data that may be informative in this context. They were also chosen as a basis to consider priorities for data acquisition to decrease uncertainty and the resulting reliance on defaults.

The guidance was developed and refined through a series of planning and technical meetings and larger workshops of a broad range of participants from academia, government agencies and the private sector. Its principal objectives were:

1) to increase common understanding and to encourage the generation and incorporation of relevant quantitative mode of action (kinetic and dynamic) data in a context consistent with traditional approaches to development of measures of dose/concentration–response, and
2) to more fully delineate appropriate avenues of research to enable more predictive estimates of risk.

As such, the guidance is relevant not only to risk assessors, but also to those who commission, design or conduct relevant studies as a basis for informing risk assessment (IPCS, 2001; IPCS, 2005; Meek et al., 2001; Meek et al., 2002a; Meek et al., 2002b; Gundert Remy and Sonich-Mullin, 2002).

Chemical specific adjustment factors (CSAF) can replace the default factors for interspecies differences (i.e., the variation in response between animals and a representative healthy human population) and human variability (the variation in response between a representative healthy human population and sensitive subgroups). This is based on subdivision of these two default factors into components which address kinetics and dynamics as a basis for replacement with partial data in one or both of these areas.

Subdivision of the default factors to address kinetics and dynamics was suggested in seminal work by Renwick (1993) and Renwick & Lazarus (1998).

Quantitative values of the default components were proposed based on kinetic parameters and PKPD modelling for a range of pharmacological and therapeutic responses to pharmaceutical agents in animals and humans. The default for kinetics was also consistent with an approximately four fold difference in basic physiological parameters based on allometric scaling between rats (the most commonly used test species) and humans. The originally proposed values for these components were refined marginally within the IPCS project (Figure 3).

The subdivision of the existing default values of ten-fold for interspecies differences and human variability does not reflect confidence in these pre-existing values, *per se.* Rather, it is justified on the basis that in the absence of data, the approach collapses to the currently well accepted (though not necessarily well justified) defaults. Reliance on the previous values in the absence of data is anticipated to facilitate understanding in the regulatory risk assessment community of the nature of more relevant and predictive data in a context that is familiar.

It is additionally anticipated that the availability of relevant information will increase with a better common understanding of its appropriate nature, based on application of this framework, even where data are sparse. In fact, and while there must be good understanding of the adequacy of data for appropriate application, the nature of required information is not necessarily complex but does require some understanding of mode of action.

For example, for kinetic parameters, replacement of default can involve comparison between mean values in humans and animals (interspecies differences) of simple dose metrics (kinetic parameters) such as area under the plasma concentration time curve or clearance for the active entity (i.e., parent compound or metabolite). For human variability, data requirements are more demanding, since population distributions for the relevant parameters must be characterized.

Data as a basis for replacement for dynamic components are derived generally from *in vitro* studies in tissues from animals and humans. For interspecies differences, they involve quantitative interspecies comparison of the concentrations which cause effect of defined magnitude in human and animal tissues. For human variability, data could be derived from *in vitro* studies in tissues of the critical effect from average versus sensitive humans (Chapter 8).

Based on experience in mode of action-based replacement of defaults, it is clear that the relevant quantitative values for kinetics and dynamics are chemical specific and vary widely. As a result, depending upon the case at hand, the 10 fold default values for interspecies differences and human variability may be either overly conservative or not sufficiently protective. As it is, taking into account just kinetic differences, the value of 10 is insufficient for extrapolations between mice and humans, where allometric scaling would indicate about a 12 fold variation in basic physiological parameters between the two species.

The CSAF framework is presented specifically in the context of default approaches in which "safe" levels of exposure are developed. It is important to recognize, however, that the replacement of defaults with more certain chemical specific data is equally applicable to other default approaches to dose response assessment (such as exposure potency indices or linear low dose extrapolation).

The application of MOA/HR framework analyses and their extension to dose-response based on pre-existing guidance such as that for chemical specific adjustment factors is an important step in increasing common understanding of the appropriate application of data on mode of action in regulatory risk assessment. It serves as a

basis to increase communication among the risk assessment, modelling and toxicological communities and to meaningfully impact testing strategies (Chapter 9). Examples of application in hazard characterization and dose-response assessment in regulatory risk assessments are provided in Chapters 10 (chloroform) and 11 (formaldehyde).

Expansion of the Frameworks: Addressing Combined Exposures and Physiologically Based Pharmacokinetic Modelling

Frameworks for mode of action/human relevance and their implications for dose-response analysis are also relevant to systematic consideration of the weight of evidence for and communication of risks associated with combined exposures to multiple chemicals. This is another of the areas which presents a continuing challenge to toxicity testing and chemical risk assessment which are designed currently to address principally individual substances.

Additional development to consider risk associated with combined exposures to multiple chemicals based on MOA/HR framework analyses is being addressed in an ongoing initiative of the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. In a workshop on this project held in 2007, it was recommended that terminology describing various aspects of exposure to and effects of multiple chemicals be revised to be more precisely descriptive. In fact, this has been a source of continuing confusion, with terminology such as "aggregate" and "cumulative" being used in different contexts in various agencies and even among offices within the same agency. In particular, it was recommended that combined exposure to multiple chemicals be defined in the context of whether or not the components act by similar or different modes of action (i.e., "**Single Mode of Action**" or "**Multiple Modes of Action**") rather than by the numbers and nature of components (Meek et al., 2009a)

Subsequently to the workshop, a draft framework for consideration of risk from combined exposures to multiple chemicals has been proposed, which is now available for public comment. It is decision-based and iterative in nature, involving stepwise consideration of both exposure and mode of action informed hazard in several tiers of increasingly data-informed analyses. This hierarchical approach is essential as a basis to efficiently scope the need (or not) for additional assessment and/or recommend required data generation (Meek et al., 2009b).

The extent of assessment and nature of recommendations for generation of additional data are dependent upon the extent of the knowledge base, the magnitude of public health concern (i.e., taking into account margins between exposure and effect), and the objective of the risk assessment (e.g., implications of potential risk management decisions). For mode of action considerations, approaches range from predictive methodologies and conservative assumptions in early tiers to more data intensive options (including probabilistic approaches) in later tiers with accompanying rigorous descriptions of decreasing relative uncertainty. Consistent with MOA/HR analyses, the proposed framework is hypothesis driven, involving analysis of available information followed by a conclusion based on weight of evidence and subsequent refinement (as necessary).

An additional initiative of the WHO/IPCS project on harmonization, is focused on advancement of characterization and communication of the weight of evidence for selection of relevant dose metric(s) for PBPK models consistent with MOA/HR analysis.

This involves development of clearly articulated hypotheses for the relevant dose metric through consideration of the weight of evidence in the context of mode of action based on criteria included in framework analyses such as consistency, specificity and biological plausibility. This is critical as a basis for increasing understanding between the modeling and regulatory risk assessment communities to better tailor the development of the models for and facilitate their uptake. Indeed, despite the availability of PBPK models for a number of chemicals incorporating significant additional biological data over default and the potential of such models to contribute more broadly to the development of additionally informative testing strategies, their adoption in regulatory risk assessment has been limited.

The initiative includes preparation of guidance and case studies on the characterization, documentation, evaluation and communication of PBPK models for risk assessment. Aspects also being addressed include the need for early and continuing communication between risk assessors and modelers, greater consistency in consideration of mode of action as a basis for relevant PBPK models and sufficiently transparent documentation of model development to support potential application. More consistent and transparent consideration of the basis for and output of PBPK models relative to default approaches in risk assessment is also being addressed (IPCS, 2009).

Implications for Testing

Traditionally, toxicity tests on laboratory animals are conducted separately for various endpoints for individual chemicals – for example, cancer, developmental and reproductive effects, repeated dose toxicity, etc.. Current test methods in laboratory animals, such as rats and mice were developed incrementally over the past 50 to 60 years and their continued evolution has focused principally on standardization to permit direct comparison among chemicals with diverse structural and/or biological properties. However, this standardization necessarily detracts somewhat from prediction in the context of risk (e.g., selection of appropriate test species that most resemble man based on mechanistic inference).

Indeed, the nature of current toxicity testing does not lend itself well to assimilation and interpretation in a public health context and requires interpretation based on a number of assumptions and extrapolations that remain controversial. Test animals are generally exposed to much higher doses than would be expected for typical human exposures. They are also generally observed for overt signs of adverse health effects, which provide little information about early biological changes as a basis to inform mode of action. Finally, the use of animals in long term testing (particularly) is expensive and time consuming, necessarily limiting the throughput of chemicals and the extent of the dose-response relationship which can be examined. There is also increasing pressure worldwide to reduce animal testing, due to ethical concerns.

In addition to generating data of less than optimum relevance from the perspective of predicting public health risk, only a relatively small subset of the resource intensive data that is generated in current toxicological testing informs the final risk assessment. To increase efficiency and accuracy, then, in public health protection, there is a need for much more iterative and integrated testing strategies which include early consideration of mode of action. In fact, a *paradigm shift* is required to move in a scientifically credible and transparent manner from that which requires extensive hazard (animal) testing at high doses to one in which the most

relevant *in vivo* information is generated in a hypothesis- and risk-driven approach (see, e.g., Van Leeuwen et al., 2007). Efficient and credible prediction of toxicity drawing upon available information is essential, then, as a basis to facilitate design of testing strategies. The underlying rationale is to:

- Minimise animal testing through introduction of alternative methods
- Apply shorter term and less expensive methods before labour intensive ones.
- Design to address hazard characterization relevant to risk assessment
- Enable early consideration of potential for exposure as a key determinant of testing strategies and risk assessment.
- Maximise the use of up-to-date information from different sources in an integrated manner (i.e., framework analyses).
- Allow more robust and focussed regulatory decisions using testing and non-testing approaches
- Allow greater flexibility in introducing new tools and scientific knowledge.

Increasing emphasis on the mode of action of substances and evolution of focus to hazard characterization rather than hazard identification in testing is anticipated to better inform the most important inferences that we make in chemical risk assessment—that is, extrapolation between and within species and from high to low doses. The analytical frameworks for human relevance of mode of action and implications for dose-response analysis frame critical data gaps in a risk assessment context. They are also helpful in transitioning the risk assessment community to refocus attention on earlier, less adverse key events at lower doses as a basis for public health protection. This results in more fulsome early integration of all available (and arguably more relevant) data as a basis to inform hazard and risk characterization.

These frameworks, then, are an essential step in transition to more mode of action based testing strategies, since it the regulatory community that will largely determine the future of toxicity testing. The human relevance of mode of action framework and implications for dose-response analysis provides the relevant "bridge" for the risk assessment/research communities in this transition.

For example, the focus of experimental studies on carcinogenicity in animals has been the long term combined chronic/cancer bioassay in rats and mice. This bioassay has been designed principally as a basis to identify hazard for tumours at relatively elevated dose with limited attention and resources dedicated to understanding the nature of early key events at lower doses. Indeed, the highest dose is selected to result in signs of toxicity (often in the range of a 10% decrease in body weight gain).

Results of such bioassays are generally combined in a weight of evidence approach with those of short-term principally *in vitro* screening assays which identify potential for interaction with DNA, including mutation. Unfortunately, most of these assays have also been designed to identify hazard at relatively high doses. They provide limited or no information in a mode of action context (i.e., determination of early, rate limiting key events in a mode of action involving interaction with DNA). Neither testing strategy nor a combination, thereof, provides relevant and robust dose-response information. Neither do they provide even a minimum amount of the kinetic and dynamic data in a mode of action context which would most meaningfully contribute to estimation of human risk. As a result, considerable investment of

resources in these assays provides only limited information directly relevant to public health protection.

As discussed in the Introduction and presented in Chapter 2, the requirement of mandates worldwide to systematically assess much larger numbers of chemicals necessitates more efficient and effective toxicity testing. This includes intelligent testing strategies to focus early on endpoints of interest and consideration of "chemical space" in targeted investigation (i.e., to determine priorities for testing from among large numbers of substances based on the extent to which chemicals with similar properties have already been characterized). Targeted investigation will include focus on shorter term *in vivo* assays of a range of intermediate endpoints based on consideration of mode of action of the specific or like chemical(s) and acquisition of additional mechanistic data at interim time points in any longer term *in vivo* studies prioritized for conduct.

These pragmatic intermediate approaches to better tailor and target testing as a basis for more informative characterization of risk in humans are essential prerequisites to meeting broader, longer term strategies to revamp toxicity testing. These strategies include, for example, that outlining greater reliance on computational modelling and *in vitro* data in humans envisaged by the NRC committee on Toxicity Testing in the 21st Century (NRC, 2007).

This Committee identified four major objectives for toxicity testing in future:

"*depth*, providing the most accurate, relevant information possible for hazard *identification* and dose-response assessment;
breadth, providing data on the broadest possible universe of chemicals, end points, and life stages;
animal welfare, causing the least animal suffering possible and using the fewest animals possible;
and *conservation*, minimizing the expenditure of money and time on testing and regulatory review.

These considerations and increasing regulatory pressures require more efficient consideration of potential risks associated with much larger numbers of substances. As a result toxicity must continue to evolve from the use of prescribed protocols of whole animal bioassays to greater emphasis on understanding the underlying pathways that lead to toxicity (i.e., perturbations that lead to key events in a mode of action).

Much of the testing envisioned in this report entails *in vitro* studies (particularly, using tests based on high-throughput assays) in human cells or tissues which has potential to eliminate the need for interspecies extrapolation, to increase efficiencies in testing and to reduce the use of animals. These assays aim to characterize cellular processes and toxicity pathways more accurately by testing different levels of cellular function, including:

genomics, the study of genes and their function as a whole;
proteomics, the large scale study of proteins and their function; and
metabolomics, the study of all metabolites in a biological system that are being used to describe toxicant responses.

Computational biology techniques can be applied to this "Omics" data to link toxicity pathways and to identify patterns characteristic of specific toxicants.

The goal of the strategy proposed in this report is to use high-throughput testing to detect early pathway perturbations that disrupt normal function in dynamic pathways. However, adaptation can reverse such changes if they do not exceed the homeostatic limits; thus these initial perturbations are not necessarily adverse. Most agents exhibit more than one effect with increasing exposure. These effects generally have different mechanisms or modes of action and would be expected to cause perturbations in several different pathways. Agents will produce multiple perturbations of dynamic pathways and the proposed testing strategy needs a clearly defined approach to categorize these effects as beneficial, adverse or irrelevant (normal variation) in the context of existing approaches in order to achieve credibility as a risk assessment tool with the regulatory community (Meek and Doull, 2009).

A pragmatic and seemingly essential first step in addressing this re-evaluation of adversity would be a recommendation to relate early perturbations in pathways to apical endpoints. Frameworks designed to systematically address key events in modes of action and their subsequent implications for dose-response in risk assessment are ideally suitable for this purpose. Such framework analyses are instrumental, then, in advancing common understanding in both the research and risk assessment communities in potential appropriate application of data on early events in a toxicity pathway. Increasing experience in this context could provide the necessary basis for revisiting guidelines for toxicity testing.

Next Steps (Conclusions and Recommendations)

The human relevance of mode of action frameworks such as those developed by the International Programme on Chemical Safety/International Life Sciences Institute will continue to play a critical role in hypothesis generation and the systematic consideration of the weight of evidence supporting the use of mechanistic data in regulatory risk assessment. Application of framework analyses increases the transparency of delineation of the relative degrees of uncertainty associated with various options for consideration in assessment of risk for impacted populations. Framework analyses are also instrumental in acquiring transparency on critical data gaps that will further reduce uncertainty. As such, they force distinction of choices made on the basis of science policy versus those that are science judgment related, including reliance on default, based on erroneous premise that it is always health protective.

The potential of these frameworks to increase consistency and transparency in decision making contributes to increase common understanding among communities and jurisdictions. They are an important tool for coordination and communication between the research and regulatory risk assessment communities. They are also an essential "bridge" in the evolution of toxicity testing to be more predictive, relevant and risk-based, through relation of early perturbations to apical endpoints in a context relevant to current application in regulatory risk assessment.

As we move forward to develop more integrative test strategies to meet evolving and demanding regulatory mandates to deal efficiently with significantly larger numbers of chemicals including groups and combined exposures, early assimilation of the information in a mode of action context as envisaged by application of these frameworks is essential.

Figures

Figure 2. The IPCS/ILSI Human Relevance of Mode of Action Framework

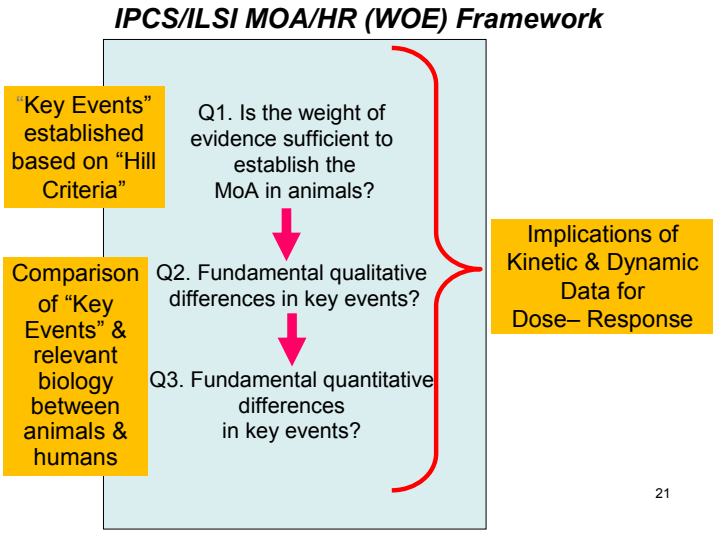
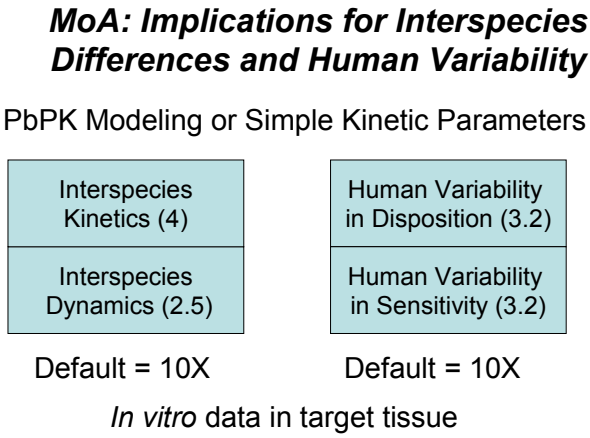


Figure 3. Subdivision of Default Uncertainty Factors for Interspecies Differences and Human Variability to Address Kinetic and Dynamic Components



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Nederlandse samenvatting

Het gebruik van analytische kaders gebaseerd op de werking van stoffen bij het testen van toxiciteit en de risicobeoordeling

Inleiding:

Recente wijzigingen in de internationale regelgeving hebben er toe geleid, dat een groot aantal chemicaliën nader op hun veiligheid voor mens en milieu geëvalueerd moeten worden. Hiervoor is het nodig dat toxiciteittesten efficiënter en effectiever moeten worden uitgevoerd, om als basis te dienen voor het doen van voorspellingen op het gebied van de risicobeoordeling. Dit maakt het noodzakelijk om veel meer nadruk te leggen op het ontwerp van testmethodologieën die zowel rekening houden met de potentiële blootstelling als ook een gerichte focus hebben op het relevante werkingsmechanisme, met een daaruit voortvloeiende verschuiving in aandacht voor de identificatie van een potentiëel schadelijk effect ("hazard") naar een karakterisering hiervan.

Bovengenoemde veranderingen vereisen ook een beter begrip bij het risicomanagement omtrent de aard van de wetenschappelijke informatie, welke geschikt is voor het leveren van kennis op het gebied van werkingsmechanisme en de daarmee samenhangende dosis-effect relaties, en de uiteindelijke risico karakterisering. Om dit in goede banen te leiden is het noodzakelijk om de oorspronkelijk kwalitatieve benaderingen in de toxicologie om te buigen naar een meer voorspellende en kwantitatieve benadering in de risicobeoordeling. Een dergelijke verandering heeft ook gevolg voor de juiste communicatie en training van wetenschappers, die zich bezighouden met de risicobeoordeling van stoffen.

De ontwikkeling van analytische kaders op het gebied van (hypothetisch) humaan relevante werkingsmechanismen (Mode of Action/Human Relevant (MOA/HR)) of chemisch specifieke onzekerheidsfactoren (Chemical Specific Adjustment Factors (CSAF)) zijn belangrijke facetten. Deze kunnen dienen als een basis om de input van de onderzoekers en regelgevende autoriteiten beter te integreren. Duidelijk mag zijn dat deze kaders ook belangrijk zijn om de vertaling van mechanistische informatie naar kwantitatieve risico karakterisering te maken. Als zodanig leveren deze kaders een bijdrage aan een verbeterd begrip bij zowel wetenschappers als regelgevende autoriteiten voor het adequaat evalueren van op dat moment beschikbare informatie en de consequenties voor het vervolgen van de teststrategie.

De kritische rol van deze analytische kaders op het gebied van MOA en CSAF worden in dit proefschrift besproken. Daarnaast wordt het onderzoek dat tot hun ontwikkeling geleid heeft beschreven, alsmede de belangrijke rol die zij spelen bij de ontwikkeling van meer gerichte, efficiënte en effectieve beschermingsmethoden voor het publiek bij de risicobeoordeling van stoffen. Aan de hand hiervan worden een aantal belangrijke verbeteringen voorgesteld.

Regulering van stoffen: de historische context

In het begin van de jaren zeventig lag de nadruk van regulering van chemicaliën in Europa en Noord Amerika hoofdzakelijk op nieuw te introduceren chemische stoffen ("nieuwe stoffen"). Als gevolg van deze regelgeving werd de chemische industrie direct gedwongen tot de ontwikkeling van commerciële chemicaliën, waarvan verwacht kon worden dat deze de minst schadelijke effecten voor mens en milieu zouden hebben. Aan de andere kant werden echter bestaande commerciële chemicaliën ("bestaande stoffen") over het algemeen niet gelijkwaardig geëvalueerd ten opzichte van de nieuwe stoffen. Hierbij werd een systematische prioritering voor risicomanagement van alle bestaande stoffen niet verlangd, hoewel een beperkt aantal bestaande stoffen in een vroeg stadium geïdentificeerd werd voor verdere beoordeling.

De laatste tijd is meer aandacht ontstaan voor de noodzaak om op een systematische wijze prioriteiten voor het risicomanagement stellen voor de honderdduizenden bestaande stoffen. Een dergelijke prioritering is gerechtvaardigd vanuit het idee dat bepaalde reeds bestaande chemicaliën wel eens een belangrijker bedreiging voor mens en milieu zouden kunnen vormen, dan degene die als nieuwe stoffen bestempeld werden op basis van de introductie van de betreffende wetgeving.

Het belang van de werkingswijze van stoffen

Een toenemend gemeenschappelijk inzicht in de concepten werkingswijze (mode of action) en werkingsmechanisme (mechanism of action) is een belangrijke stap voorwaarts geweest in de risicobeoordeling van stoffen. Werkingswijze is een beschrijving van cruciale metabole, cytologische, genetische en biochemisch processen, die leiden tot het ontstaan van een relevant toxicologisch eindpunt waarvoor biologische plausibiliteit aan te geven is. Het werkingsmechanisme van een stof heeft betrekking op gedetailleerde moleculaire procesbeschrijvingen. De samenhang tussen deze twee begrippen is een voorgestelde werkingswijze van een stof, ondersteund door degelijke informatie op het gebied van werkingsmechanisme, hetgeen als biologisch plausibel beschouwd moet worden. Het goed beschrijven en herkennen van essentiële effecten is dus een kritische component in het vroeg herkennen van een werkingswijze van een stof in de risicobeoordeling. Hierbij is vaak een interdisciplinaire aanpak bij de interpretatie en ontwikkeling van data noodzakelijk. Dit proces kan beschouwd worden als de cruciale stap in de risicobeoordeling, waarbij argumenten omtrent humane relevantie en bijbehorende dosis-effect relatie eveneens van groot belang zijn.

Informatie over de werkingswijze van stoffen is essentieel voor de voorspelling van risico's van stoffen, nl. als hulpmiddel bij het vaststellen van de relevantie van effecten in het proefdier voor de mens, bij het vaststellen van overgangen van het ene effect in het andere bij verschillende doseringen, en het identificeren van mogelijk extra kwetsbare bevolkingsgroepen. Daarnaast is het van belang bij de vraag of de specifieke effecten bij het proefdier ook inderdaad kunnen voorkomen bij de mens. Deze overeenkomst kan dan verder dienen voor de ontwikkeling van stof specifieke biomarkers in epidemiologische studies. Het begrip werkingswijze zoals dat gehanteerd wordt in de betreffende analytische kaders is samengesteld uit biokinetiek[1] (opname,

[1] *Opgemerkt wordt hier dat het begrip farmacokinetiek, toxicokinetiek en biokinetiek uitwisselbaar in het proefschrift gebruikt kunnen worden. De betreffende terminologie heeft in de afgelopen decennia*

lichaamsdistributie, metabolisme en uitscheiding) en toxicodynamiek (interactie met receptoren en daaropvolgende nadelige effecten voor het organisme). Biokinetiek is in dit proefschrift opgenomen als een deel van de werkingswijze van een stof, omdat metabole activatie vaak een relevante stap is bij het optreden van een toxisch effect.

Historisch gezien richtte het toxicologisch onderzoek zich grotendeels op het kwalitatief identificeren van schadelijke effecten van een stof, of anders gezegd, de intrinsieke potentie van een stof om een toxisch effect te induceren. In de toekomst zullen meer integrale testmethoden gebruikt moeten worden om zo snel mogelijk de werkingswijze van een stof te identificeren. Dit moet de basis zijn voor een duidelijke karakterisering van een schadelijke effect, zodat het publiek dan voldoende beschermd kan worden.

Vaststellen van een dosis- of blootstellings-respons relatie is de kwantificering van de waarschijnlijkheid dat een bepaalde blootstelling een negatief effect op de gezondheid van een populatie kan hebben. Hierbij wordt uitgegaan van nadelige effecten die als kritisch en relevant voor de mens beschouwd kunnen, waarbij kritische effecten gedefinieerd zijn als biologisch relevante effecten bij de laagste dosis. Een verbetering in het algemene begrip omtrent het onderscheid tussen de werkingswijze (minder gedetailleerd met nadruk op de bepalende biologische veranderingen) en het werkingsmechanisme (moleculaire basis), en het onderkennen van de essentiële rol die de bepalende biologische veranderingen spelen, leidt tot het inzetten van additionele informatie voor het onderbouwen van de dosis-respons relatie. Dit gebeurt door rekening te houden met de vorm van de dosis-effect relatie van de bepalende biologische veranderingen (en niet alleen van het uiteindelijke nadelige effect). Daarnaast moet in overweging genomen worden welke van deze effecten een snelheidsbeperkende stap kunnen zijn bij verschillende doseringen.

De biokinetische en toxicodynamische aspecten die behoren bij de werkingswijze van een stof kunnen eveneens informatie verstrekken over de kwantitatieve verschillen tussen soorten, inclusief de mens, en de verschillen tussen mensen. Al naar gelang de hoeveelheid beschikbare gegevens kan een continuüm van benaderingen gedefinieerd worden, van default ("beschermend bij veronderstelling") tot biologisch onderbouwd ("voorspellend"). In de optie waarbij gegevens minimaal aanwezig zijn worden default-waarden toegepast zonder rekening te houden met stof- of soort-specifieke aspekten. De achtergrond voor de afleiding van standaard onzekerheidsfactoren is weinig onderbouwd en de wetenschappelijke ondersteuning voor hun toepassing blijft vaag. De toepassing van deze standaard onzekerheidsfactoren wordt soms echter wel verantwoord door retrospectief gebruik te maken van beschikbare informatie uit empirisch onderzoek.

Het is mogelijk om categorische default factoren te ontwikkelen door rekening te houden met fysisch-chemische eigenschappen van stoffen of de eigenschappen van het proefdier waarin het kritische effect gemeten is. Hierbij kan gedacht worden aan allometrische schaling voor verschillende diersoorten of de ontwikkeling van referentie waarden voor de inhalatie van gassen en deeltjes zoals reeds toegepast door de U.S. EPA. De aanwezigheid van aanvullende stofspecifieke informatie over kinetische en dynamische componenten in de inter- of intraspecies variatie kan leiden tot

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meerdere veranderingen ondergaan. Afhankelijk van de auteur of het tijdschrift heeft hierbij één van deze termen over de afgelopen jaren de voorkeur genoten.

stofspecifieke onzekerheidsfactoren. Hierbij kan o.a. gedacht worden aan vergelijkende biokinetische en toxicodynamische parameters tussen mens en dier of individuen. Wanneer er meer kwantitatieve biokinetisch informatie beschikbaar is voor een stof kan mogelijk een "physiological based biokinetic model" (PBBK) ontwikkeld worden. Met behulp van een dergelijk model kan dan een biologisch effectieve (interne) dosis berekend worden, die gebaseerd is op werkingswijze van de stof terwijl tegelijkertijd fysiologisch schaling, relevante fysisch-chemische eigenschappen en biologische waarden worden verwerkt. Hoewel het zelden gebeurt, zou voor een stof waarbij voldoende biokinetische en toxicodynamische informatie beschikbaar is, een PBBK model dat specifiek voor die stof geldt, opgesteld kunnen worden.

De overbrugging tussen ontwikkelingen in de regelgeving en huidige aanpak voor risicobeoordeling.

Er is een behoorlijk hiaat tussen enzerzijds de huidige benaderingen voor toxiciteittesten en risicobeoordeling, en anderzijds de behoefte aan voorspellende mogelijkheden in verband met het vaststellen van prioriteiten binnen het grote aantal stoffen dat geëvalueerd moet worden. Dit komt hoofdzakelijk door de traditionele nadruk op de identificatie van potentieel gezondheidschadelijke effecten en standaard (default) benaderingen in de karakterisering van dosis-effect relaties. Hierbij dient opgemerkt te worden dat de algemene standaard benaderingen (bijvoorbeeld het delen van doseringen in dierstudies met een factor 100 of meer, of lineaire extrapolatie over een nog grotere afstand) in het algemeen als beschermend worden beschouwd, maar dat hiervoor meestal geen adequate experimentele onderbouwing beschikbaar is. Dit uitgangspunt is tevens in tegenspraak met verkregen resultaten uit onderzoek dat gericht was op de werkingswijze van stoffen bedoeld om standaard (default) waarden te vervangen. Stof-specifieke biokinetische en toxicodynamische gegevens gaven hierbij aan dat de default waarden soms te groot en soms te klein waren.
Deze tekortkoming in voorspellende mogelijkheden ten aanzien van humane gezondheidsrisico's is vooral een gevolg van het feit dat het testen van toxiciteit hoofdzakelijk gericht is op het identificeren van gezondheidsschadelijke effecten en op de weliswaar simpele maar niet bijzonder voorspellende default benaderingen zoals gewoonlijk toegepast in de risicobeoordeling. Dit vormt een aanzienlijke drempel bij de huidige uitdaging van het prioriteren en evalueren met beperkte financiële middelen van grote aantallen stoffen. Als voorbeeld hierbij kan de beperkte bruikbaarheid van kwantitatieve structuur activiteitsrelaties (QSARs) voor de humane risicobeoordeling genoemd worden, alsmede die van de "Threshold of Toxicological Concern". De beperkte bruikbaarheid van deze methoden is een gevolg van de afwezigheid van een onderbouwing in een werkingswijze-context.

Daarnaast is het gebrek aan voldoende aandacht voor de werkingswijze van stoffen in de risicobeoordeling ook terug te voeren naar de onvoldoende bruikbare gegevens over het werkingsmechanisme, vanwege de gegevens soms ongecoördineerde en ongerichte gegenereerd worden. Bovendien heeft het vastzitten aan de default benadering niet bijgedragen aan het transparant maken van het type gegevens dat meer informatief zou zijn (d.w.z. het specificeren van kritische hiaten in de gegevens welke zouden bijdragen aan inzicht in de werkingswijze).

Zelfs in die gevallen waarin een aanzienlijk hoeveelheid informatie beschikbaar is op het gebied van het werkingsmechanisme, wordt deze vaak niet wordt gebruikt bij de risicobeoordeling. Soms is de reden hiervan dat er domweg onvoldoende inzicht is

binnen de uitvoerende instanties en/of dat de risicobeoordeling onder druk van de regelgeving in een kort tijdsbestek moet worden uitgevoerd. Daarnaast kan geconstateerd worden dat een interdisciplinaire samenwerking tussen de risicobeoordelaars (meestal toxicologen), modeleurs, en degenen die mechanistisch onderzoek doen vaak veel te wensen overlaat. Door een dergelijk gebrek aan samenwerking wordt het ontwikkelen van hypotheses voor de werkingswijze van de stof, en de daarbij behorende bewijslast ("Weight of Evidence") als ook het vaststellen van de dosis-respons, duidelijk gehinderd. Op dit gebied lijkt verbetering in de communicatie tussen de verschillende betrokken wetenschappers en uitvoerende autoriteiten, maar ook interdisciplinaire training een kritische voorwaarde. Tot slot speelt een rol dat er vaak een gebrek aan transparantie is bij de scheiding tussen wetenschappelijke beoordeling en op wetenschap gebaseerde beleidskeuzes (de standaard onzekerheidsfactor wordt als meer beschermend gezien voor gezondheidseffecten).

In dit proefschrift wordt de ontwikkeling van analytische kaders die zich richten op de humane relevantie van de werkingswijze van een stof nader beschouwd. Ook de implicaties hiervan op het gebied van relevante dosis-effect relaties worden hierbij betrokken. Beide aspecten kunnen een essentiële bijdrage leveren aan een verbeterde communicatie tussen risicobeoordelaars en onderzoekers. Bovendien wordt de zich evoluërende inhoud en de groeiende toepassing van deze kaders bezien in de context van een meer progressieve strategie van het testen van toxiciteit, om daarmee te kunnen voldoen aan de uitgebreidere vereisten vanuit de regelgeving.

<u>De rol van analytische kaders in het onderzoek en de risicobeoordeling</u>

In deze kaders voor humaan relevante werkingswijzen van stoffen is de identificatie van de cruciale (mechanistische) processen een eerste stap. Vervolgens wordt het onderliggende bewijs ("weight of evidence") bepaald met de zgn. Hill criteria: de dosis-respons, temporele overeenstemming tussen de cruciale processen en het uiteindelijke effect, consistentie, specificiteit, biologische plausibiliteit en coherentie. De humane relevantie wordt vervolgens beoordeeld in de context van de cruciale processen in causaal verband. Hierbij moet niet alleen gekeken worden naar chemisch specifieke eigenschappen, maar ook meer algemene informatie zoals anatomische, fysiologische en biochemisch variatie tussen soorten, modellen voor humane ziektes, en effectpatronen van stoffen met een vergelijkbare werkingswijze. Op deze manier dwingen deze analytische kaders voor de werkingswijze van stoffen een tijdig verwerken van toxicologische informatie naast het optimale gebruik van zowel stof-specifieke als algemeen-biologische informatie.

Zowel de strictheid als de transparantie waarmee deze analytische kaders leiden tot het beschouwen van de werkingswijze en de humane relevantie daarvan, alsmede de implicaties voor het vaststellen van de dosis-respons, werkt als katalysator bij het verbeteren van het onderlinge begrip tussen risicobeoordelaars en onderzoekers. Die strictheid vraagt, m.n. bij het identificeren van vroege cruciale processen, vanaf het eerste stadium om relevante interdisciplinaire input.

Deze analytische kaders zullen een belangrijke rol gaan spelen bij de overgang van standaard (default) benaderingen naar een meer progressieve en voorspellende benadering gebaseerd op de werkingswijze van de stof. Een toenemend begrip en ervaring bij het gebruik van dit soort informatie in de risicobeoordeling zal een aanzienlijke bijdrage leveren bij het verschuiven van de aandacht voor identificatie

naar aandacht voor de karakterisering van een schadelijk effect, en daarmee leiden tot een meer voorspellende bescherming van de samenleving. Bovenstaande ontwikkelingen zijn essentieel in het aangaan van de uitdaging om met beperkte financiële middelen de grote aantal stoffen die in gebruik zijn te prioriteren en te evalueren.

De gevolgen voor het testen van toxiciteit

De huidige wijze van het testen van toxiciteit leent zich niet goed voor assimilatie en interpretatie in een context van volksgezondheid, en vereist interpretaties die gebaseerd zijn op controversiële aannames en extrapolaties. Proefdieren worden meestal blootgesteld aan doseringen die veel hoger zijn dan die van de mens. Ook worden zij meestal onderzocht op duidelijke tekenen van schadelijke effecten. Deze leveren echter weinig kennis omtrent vroege biologische veranderingen, welke zouden kunnen dienen als basis voor de werkingswijze van een stof. Tot slot is het gebruik van proefdieren voor chronische toxiciteittesten duur en tijdsintensief. Door deze aspecten blijft het aantal stoffen dat getest kan worden en de vastgestelde dosis-respons relatie(s) beperkt. Daarnaast is er een groeiende publieke aversie tegen het gebruik van proefdieren op ethische gronden.

Wanneer in de toekomst de nadruk meer komt te liggen op de werkingswijze van een stof en de karakterisering i.p.v. niet kwantitatieve identificatie van het schadelijk effect kan een beter onderbouwde risicobeoordeling verwacht worden. Hierbij moet met name gedacht worden aan extrapolatie tussen en binnen soorten en van hoge naar lage doseringen. De in dit proefschrift beschreven analytische kaders voor werkingswijze van stoffen met humane relevantie brengen de kritische hiaten in de gegevens in kaart. Tevens kunnen deze er toe bijdragen dat de risicobeoordelaars hun aandacht gaan verleggen naar vroegtijdig optredende en minder gezondheidschadelijke effecten bij lagere doseringen als basis voor bescherming voor onze samenleving.

De in dit proefschrift gepresenteerde analytische kaders voor de werkingswijze van stoffen kunnen daarom gezien worden als een essentiële stap in de overgang naar meer op werkingswijze gebaseerde teststrategieën. Het zijn vooral de wetgevende instanties die de toekomst van het testen van toxiciteit zullen bepalen. De relevantie voor de mens van het werkingswijze-kader en de consequenties voor de dosis-respons analyse vormen daarbij een overbrugging tussen de risicobeoordelaars en experimentele onderzoekers.

Conclusies en aanbevelingen

Het gebruik van deze analytische kaders voor de werkingswijze van stoffen zal naar verwachting de eenduidigheid en helderheid bij de besluitvorming verbeteren. Deze kunnen daarmee als een belangrijk handvat dienen voor een verbeterde communicatie tussen de wetenschappelijk gemeenschap en de regelgevende en uitvoerende autoriteiten. Verder kunnen deze kaders een essentiële brug vormen bij de evolutie van het testen van toxiciteit naar een meer voorspellende, relevante en op risico's gebaseerde aanpak.

Het verder ontwikkelen van meer geïntegreerde teststrategieën is nodig om de veranderende vragen van regelgevers aan te kunnen, m.n. om de grote aantallen stoffen (inclusief groepen, en gecombineerde blootstellingen) op een efficiënte manier

te evalueren. Daarbij is het vroegtijdig assimileren van de beschikbare gegevens in de context van de werkingswijze zoals aangegeven in deze analytische kaders, van essentieel belang.

Major Conclusions

Evolving regulatory mandates to more efficiently assess much larger numbers of chemicals require a shift from current toxicity testing that focuses on hazard identification to mode of action based strategies for more predictive hazard characterization *(Chapter 2).*

These evolving expanding mandates necessitate early acquisition of multidisciplinary input as a basis for increasing predictive efficiency of assessment and testing strategies, while maintaining defensibility *(Chapter 3).*

Analytical frameworks for consideration of the weight of evidence of mode of action and human relevance and implications for dose-response analyses bring rigor to relevant evaluations and make essential contribution as a basis to increase common understanding between the risk assessment and research communities. They have potential to increase consistency in the outcomes of assessment, provide a basis for reasonable integration of information from evolving technologies and data sources and contribute to more relevant and predictive chemical testing strategies *(Chapter 4).*

Mode of action and human relevance analysis draws broadly on available data, including much that is not chemical specific. *(Chapter 5)*

Wherever possible, highly uncertain "default" approaches to dose-response extrapolation should be replaced with more predictive, mode of action data informed options. Relative uncertainty of various options (including default) should be delineated as a basis to differentiate science judgment determinations from those related to policy regarding public health protection *(Chapters 6 -8).*

Integration of analytical frameworks for mode of action/human relevance and implications for subsequent dose-response analysis encourage early assimilation of critically important predictive data on mode of action and conduct of relevant studies (*Chapter 9).*

Good understanding of the potential contribution of framework analyses for mode of action/human relevance and implications for dose-response is best acquired through experience in their conduct *(Chapters 10 and 11).*

The potential of MOA/HR frameworks to increase consistency and transparency in decision making contributes to increase common understanding among communities and jurisdictions. Moving forward to develop more integrative test strategies to meet evolving, demanding regulatory mandates to deal efficiently with significantly larger numbers of chemicals including groups and combined exposures, early assimilation of the information in a mode of action context as envisaged by application of these frameworks is essential *(Chapter 12).*

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Curriculum Vitae

Mary Elizabeth (Bette) Meek was born on 4 November 1954, in Kingston, Ont., Canada. She received her B.Sc. (Honours) in Biology from Queen's University, Kingston, Ont., in May 1976 and her M.Sc. in Toxicology (with Distinction) from the University of Surrey in Guildford, U.K. in September, 1981.

Bette joined Health Canada in May, 1976, where she has worked in a number of positions of increasing responsibility in regulatory risk assessment. These included as an evaluator, senior evaluator and manager of programmes on contaminants of drinking water and air. Subsequently, she managed regulatory mandates under the Canadian Environmental Protection Act including the Priority Substances Programme and subsequently, the Existing Substances Division. Her responsibilities in this capacity included development of process and methodology and managing conduct of in depth evaluations and setting priorities for assessment from among all 23, 000 commercial chemicals used in Canada (i.e., categorization).

Bette is currently the Associate Director of Chemical Risk Assessment with the McLaughlin Centre of the Institute of Population Health of the University of Ottawa on interchange from Health Canada.

Bette has considerable experience in the development of methodology for and evaluation of health-related data on environmental contaminants. She has and continues to act as an advisor to several international organizations and national Governments, has and continues to lead several international initiatives to advance the science base of regulatory risk assessment and authored over 150 scientific publications in this area.

Specific areas of experience include development of frameworks to increase transparency in the assessment of human relevance of animal modes of action, increasing incorporation of biological data in dose-response as a replacement for default, development of predictive exposure and hazard modelling and increasing efficiency in assessment through effective problem formulation and early and continuing peer engagement.

Bette and her teams have also been two time recipients of the highest awards in the Public Service of Canada, namely those of the Deputy Minister and Public Service for Team Excellence, in 1998 (for the second Priority Substances List) and 2007 (for categorization).