ELSEVIER

Mutation Research 464 (2000) 105–115

Genetic Toxicology and Environmental Mutagenesis

www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

U.S. Environmental Protection Agency's revised guidelines for carcinogen risk assessment: evaluating a postulated mode of carcinogenic action in guiding dose–response extrapolation

Jeanette A. Wiltse, Vicki L. Dellarco *

Office of Science and Technology, Office of Water (4304), U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460, USA

Received 3 February 1999; received in revised form 17 March 1999; accepted 2 April 1999

Abstract

There are new opportunities to using data from molecular and cellular studies in order to bring together a fuller biological understanding of how chemicals induce neoplasia. In 1996, the Environmental Protection Agency (EPA) published a proposal to replace its 1986 *Guidelines for Carcinogen Risk Assessment* to take advantage of these new scientific advances in cancer biology. The analytical framework within the new guidelines focuses on an understanding of the mode of carcinogenic action. Mode of action data come into play in a couple of ways in these new guidelines. For example, such information can inform the dose–response relationship below the experimental observable range of tumours. Thus, mode of action data can be useful in establishing more appropriate guidance levels for environmental contaminants. It is the understanding of the biological processes that lead to tumour development along with the response data derived from experimental studies that can help discern the shape of the dose–response at low doses (linear vs. nonlinear). Because it is experimentally difficult to establish ''true thresholds'' from others with a nonlinear dose–response relationship, the proposed guidelines take a practical approach to depart from low-dose linear extrapolation procedures when there is sufficient experimental support for a mode of action consistent with nonlinear biological processes (e.g., tumours resulting from the disruption of normal physiological processes).

Keywords: Cancer risk assessment; Carcinogenicity mechanisms; Dose–response assessment

1. Introduction

When establishing national standards for clean air and water, regulating products by approval of registration, or setting clean-up standard for hazardous site remediation, the Environmental Protection Agency (EPA) must quantify the amount of potential human risk that may be associated with exposure to environmental contaminants. Thus, dose–response assessment (i.e., how the frequency of adverse effects changes with decreasing dose) has been a long standing and critical issue to the USEPA. Given that extrapolations (such as from high to low doses, from a nonhuman species to human beings, from one route of exposure to another) must be performed, risk

* Corresponding author. Tel.: +1-703-305-1803; fax: +1-202-260-1036; e-mail: dellarco.vicki@epamail.epa.gov

1383-5718/00/$ - see front matter
PII: S1383-5718(99)00171-0

assessment is complex and often controversial. In assessing potential human cancer risk posed by environmental agents, the concept of low-dose linearity (no-threshold) vs. nonlinearity (or threshold) is one which has been surrounded by intense discussion and debate.

Cancer is a disease that develops through many cell and tissue changes over time. Traditional dose–response assessment procedures modelling tumour incidence have seldom taken into account key events integral to the carcinogenic process which precede the development of tumours, even though these events determine the shape of the overall dose–response curve. Such precursor responses may include changes in DNA, chromosomes, or other key macromolecules; effects on growth signal transduction; induction of hormonal changes; or physiological or toxic effects that affect cell proliferation. As more data become available on precursor events, and as our understanding improves about how chemicals cause these events and how the events relate to the cancer process, this information can help reduce the uncertainties attendant to the inferential process of projecting and estimating the magnitude of potential human risk.

In 1996, EPA published a proposal to update and replace its 1986 guidelines [1] to accommodate scientific advances in chemical carcinogenesis [2]. Information on mode of carcinogenic action is a central theme in the 1996 proposal. These new guidelines provide an analytical framework that brings in all relevant biological data in additional to tumour findings. In incorporating mode of action information, the new guidelines are intended to be both practical and flexible in addressing the variety of situations encounter in evaluating chemically induced cancer risk. This paper will briefly focus on the new dose–response approaches and the framework for incorporating mode of action data in cancer risk assessment. It should be emphasised that not all aspects of the new guidelines will be discussed, and thus the reader may wish to refer to the 1996 proposed guidelines themselves [2] or other discussions of these guidelines [3]. For example, although not address in this paper, evaluation of potential human carcinogenesis should be conducted for each exposure route of interest because a chemical may pose a cancer risk by one route but little or no risk by another route of exposure. When the new guidelines are final, EPA will make them available on the world wide Internet via its homepage (HTTP://www.epa.gov/ncea/raf).

2. Mode of carcinogenic action

In the new guidelines, the approach to dose–response assessment for a particular agent is based on the conclusion reached as to its mode of carcinogenic action. The mode of action understanding is promoted in these guidelines not only to guide the most appropriate dose–response extrapolation procedure but also to help interpret the relevancy of the laboratory animal data. A mode of action is defined as a description of key events and processes starting with the interaction of an agent with a cell, through physiological and tissue/organ changes, resulting in tumour development. It is unlikely that complete knowledge of how an agent causes cancer will exist, certainly for the near term. Thus, ''mode'' of action is contrasted in the new guidelines with ''mechanism'', which implies a more detailed, molecular description of events than is meant by mode of action. Therefore, evidence is needed to draw a reasonable working conclusion of the agent's influence on key processes without having to establish the sequence of molecular processes in detail. There are many examples of possible modes of carcinogenic action including mutagenicity, inhibition of programmed cell death, cytotoxicity with reparative cell proliferation, physiological or hormonal disturbances, and immune suppression. Although an induced adverse effect may result from a complex and diverse process, a risk assessment must operationally dissect the presumed critical events, at least those that can be measured experimentally, to derive a reasonable approximation of human risk.

3. Framework for evaluating a postulated mode of action

When the proposed revisions to the EPA's guidelines for carcinogen assessment were published in 1996, the most frequent comment was more specific

guidance and direction was needed on how to evaluate an agent's mode of carcinogenic action. In response to this comment, the final guidelines will provide an analytical framework for judging whether available evidence supports a mode of carcinogenic action postulated for an agent. This framework is based on considerations for causality in epidemiologic investigations originally articulated by Hill [4] but later modified by others and extended to experimental studies [5,6]. There is an international effort to harmonised and come to consensus on how to look at mode of action information in risk assessment. The framework presented below is also being adopted by the World Health Organisation in its assessment of pesticides.

The framework for analysing mode of action begins with a summary description of the postulated mode(s) of action. This is followed by questions to be addressed to the available empirical data and experimental observations anticipated to be pertinent. The areas of inquiry in the framework are as follows.

- *Identification of Key Event(s):* A ''key event'' is defined as an empirically observable, precursor step that is a necessary element of the mode of action, or is a marker for such an element (e.g., increased cell growth and organ weight, hyperplasia, cellular proliferation, hormone or other protein perturbations, receptor-ligand changes, DNA or chromosome effects, cell cycle effects). To show that a postulated mode of action is operative, it is necessary to identify the key events and to outline the sequence of events leading to cancer. In order to judge how well data support involvement of an event in the carcinogenic processes and to support an association (i.e., a causal relationship between the key event(s) and tumour development), a body of experiments need to define and measure an event consistently. [It should be noted that an initial and prominent question to be examined when examining the key events is whether the chemical (or its metabolite) interacts directly with and mutates DNA to bring about changes in gene expression or does the agent bring about effects on gene expression via other processes. Carcinogenesis involves a complex series and interplay of events that alter the signals which a cell receives from its extracellular environment to promote growth. Neither all mutagens nor all agents that induce cell proliferation lead to tumour development. Thus, understanding the range of key influences that the chemical may have on the carcinogenic process is essential for evaluating mode of action.
- *Strength, Consistency, Specificity of Association:* Causality is supported by a significant statistical and biological association between key events and a tumour response in well-conducted studies. Consistent observations in a number of such studies, with differing experimental designs increases the support for the events being causally related to tumour development since different designs may reduce unknown biases or confounding. For example, studies showing ''recovery'' (i.e., absence or reduction of carcinogenicity when the event is blocked or diminished) are particularly important tests of the causal association, although not necessary. Specificity of the association, without evidence of other modes of action, strengthens a causal conclusion (i.e., the agent does not produce effects other than postulated).
- *Dose–Response Relationships:* A causal association can be strengthened if a key event(s) and tumour response increase correlatively with dose. Dose–response correlations of the key event with other precursor events can add further strength. Difficulty arises when an event is not causal, but accompanies the process generally. Dose–response studies may assist in clarifying these relationships.
- *Temporal Relationships:* If an event(s) is an essential element of tumorigenesis, it must precede tumour appearance (i.e., what is the ordering of events that underlie the carcinogenic process?). It may also be observed contemporaneously or after tumour appearance; these observations may add to the strength of association, but not to the temporal association
- *Biological Plausibility and Coherence:* The postulated mode of action and the event that are part of it need to be based on current understanding of cancer biology to be accepted (i.e., is the mode of action consistent with what is known about carcinogenesis in general and for the case specifically?). If the body of information under scrutiny is consistent with other examples (including structurally related agents) for which the postulated mode of action is accepted, the case is strengthened (i.e., are carcinogenic effects and events consistent across structural analogues?).

· *Other Modes of Action:* This discussion covers alternative modes of action for the tumour response considered and whether they are supported by the data. In addition, it provides a place to discuss other tumour observations that may be arising from a different mode of action than postulated.

It should be emphasised that the topics listed above for analysis should not be regarded as a checklist of necessary ''proofs''. The judgement whether a postulated mode of action is supported by available data takes account the weight of the evidence and the analysis as a whole. The framework is intended to provide a consistent structure for organising the facts upon which conclusions as to mode of action rest and to make analysis transparent.

4. Dose–response assessment

Dose–response analysis first covers the relationship of the dose to the degree of response in the dose range of observation in experiments or human studies. This evaluation is then followed by extrapolation to estimate response at lower environmental exposure levels. Biologically based models have been applied to extrapolate cancer risk, such as the two-stage models of initiation plus clonal expansion and progression [7,8]. These models continue to be improved, but are not yet standard methods. Furthermore, before applying such a model, extensive data to build its form as well as to estimate how well it conforms to the observed data are needed to support confidence in results. It is anticipated that such a rich data base will not be typically available for most chemicals. In the absence of data, theoretical estimates of critical parameters (such as for mutation and cell proliferation rates) should not be used because when a model is over parameterised (i.e., there are more parameters to be estimated than data points to be fitted) different models may differ substantially in their projections below the observed range despite providing equivalent fits to the observed data [9]. If data are extensive and sufficient to quantitatively relate specific key events in the cancer process to neoplasia, and the purpose of the assessment is such as to justify investing the necessary resources, a biologically based model should be considered.

4.1. The range of observation

Even though a biologically based model may not be feasible, the new guidelines provide an opportunity to use information about key events in the cancer process in the dose–response assessment in a variety ways (as discussed in Section 5). The principle underlying the new guidelines is to take a two-step approach to dose–response assessment so as to practically include as much information about precursor events as possible in extrapolating to lower exposures anticipated to occur (or actually measured) in humans. This two-step process distinguishes between what is known (i.e., the observed range of empirical data) and what is not known (i.e., the range of extrapolation). The first step in the process involves curve fitting the response data (i.e., tumour incidence or data on a key events) in the empirical range of observation. A point of departure is determined as the LED_{10} data — the 95% lower confidence limit on a dose associated with 10% extra risk adjusted for background. [1] The 10% level is selected because a 10% response is at or just below the limit of sensitivity for discerning a statistically significant tumour increase in most long-term rodent studies [10]. For tumour data, the LED_{10} is used as a matter of science policy to provide consistency among assessments. Other points of departure may be appropriate (e.g., if a response is observed below an increase in response at 10%). For some data sets (e.g., hyperplasia) or continuous data (e.g., tissue weight changes or blood levels of a hormone), estimating a lowest-observable-adverse-effect-level (LOAEL) or no observable-adverse-effect-level (NOAEL) may be more suitable rather than determining a point of departure from curve fitting in the observable range. The point of departure (whether a LED_{10} or NOAEL) is to mark the beginning of

[1] For incidence information, the Agency will apply a standard curve-fitting procedure to provide consistency among assessments. This procedure models incidence, adjusted for background, as an increasing function of dose; it will be available to the public on the US EPA's World Wide Web site (http\www.epa.ncea.raf) for use or for downloading when the new guidelines are finalized. The procedure will identify situations in which the standard algorithm fails to yield a reliable point of departure, signaling the need for additional judgment and an alternative analysis.

extrapolation to lower doses. Thus, the objective of deriving a point of departure is to determine the lowest reliable point on the dose–response curve for the beginning of the second step of the process — the extrapolation range.

4.2. The range of extrapolation

The second step involves extrapolation from the point of departure below the range of observation. As mentioned earlier, it will be unlikely that a biologically based model can be used for extrapolating low-dose risk in most cases. Therefore, the new guidelines will provide for several default extrapolation approaches (linear, nonlinear, or both), which begin with the point of departure. The extrapolation default approach that is taken should be based on the mode-of-action understanding about the agent. It is the understanding of the underlying biological mechanisms as they vary from species to species, from high dose to low dose, and from one route of exposure to another, that drives the choice of the most appropriate extrapolation approach. The EPA proposes to adopt these default procedures as a matter of science policy based on current hypotheses of the potential shapes of dose–response curves for differing modes of action at low doses. The choice of the procedure to be used in an individual case is a judgement based on the agent's mode of action.

4.2.1. Linear default

Application of the linearized multistage (LMS) model as called for in the 1986 EPA guidelines for extrapolating risk from upper-bound confidence intervals is no longer recommended as the linear default in the 1996 proposed guidelines. The linear default in the new guidelines is a straight-line extrapolation from the point of departure to the origin (i.e., zero incremental dose, zero incremental response) to give a probability of extra risk. The slope of the line expresses extra risk per dose unit, where risk is the product of the slope and anticipated or measured human exposure. The linear default approach would be considered for carcinogens that are DNA reactive and induce mutations. There might be modes of action other than DNA reactivity that are better supported by the assumption of linearity. When inadequate or no information exists to explain the carcinogenic mode of action of an agent, the linear default approach would be used as a science policy choice in the interest of public health. Likewise, a linear default would be used if evidence demonstrates the lack of direct DNA reactivity and mutagenicity, but there is an absence of sufficient information on another mode of action to explain the induced tumour response. The latter is also a public health protective policy choice. A linear default would also be supported in the situation where human exposure or body burden is high and near doses associated with key events in the carcinogenic process.

4.2.2. Nonlinear default

Although the understanding of the mechanisms of induced carcinogenesis will rarely be complete for chemical carcinogens, there are situations for which evidence is sufficient to support a presumption of nonlinearity. Because it is experimentally difficult to distinguish modes of actions with true ''thresholds'' from others with a nonlinear dose–response relationship, the proposed nonlinear default procedure is considered a practical approach to use without the necessity of distinguishing sources of nonlinearity. It is the current practice at EPA to speak of nonlinear dose–response relationships rather than thresholds (unless there is sufficient evidence defining a true threshold).

In cases of nonlinearity, risk is not extrapolated as probability of an effect at low doses. With modes of action consistent with nonlinearity, it is anticipated that the cancer response will fall more quickly than linearly with dose. A science policy default assumption of *nonlinearity* is appropriate when there is information supporting both a lack of linearity (e.g., absence of direct DNA effects) and sufficient mode of action evidence to support an assumption of nonlinearity (such as the mode of action may be a secondary effect of toxicity or of an induced physiological change which in itself is a nonlinear phenomenon). Nonlinear probability functions are not fitted to tumour response data to extrapolate quantitative low-dose risk estimates because different procedures can lead to a very wide range of results, and there is currently no basis, generally, to choose among them. [Sufficient information to choose a

model would likely lead to a biologically based model.

As a matter of science policy, a margin of exposure (MoE) analysis [2] will be used to evaluate concern for levels of exposure as the extrapolation procedure for the nonlinear default. The MoE is the point of departure divided by a measured or anticipated human environmental exposure situation. The risk manager decides whether a given MoE is acceptable within the context of a given regulatory program. The risk assessment provides an analysis with supporting information and guidance to assist decision makers in considering aspects of the exposure scenarios at issue in light of the mode of action understanding. A MoE analysis provides an integrative analysis of all of the important hazard and dose–response factors. The analysis may be based on information about key event(s), tumour incidence, or both. It is anticipated that many margins of exposure analyses for cancer will be for responses other than tumour incidence. The key objective of the MoE analysis is lower the dose from the LED_{10} to approach a zero to 1% effect level for key events, and to consider the interspecies and intraspecies variability in sensitivity. Several factors are to be considered in the analysis to evaluate a MoE for its protectiveness of the public health. For example, a shallow slope suggests less reduction than a steep one (i.e., how quickly does the response meet background). Information on factors such as the nature of response being used for point of departure (i.e., tumour data or a more sensitive precursor response) and biopersistence of the agent are important to consider in the MoE analysis. As a default assumption for two of these points, a numerical factor of no less than 10 each may be used to account for human variability and for interspecific differences in sensitivity when humans may be more sensitive than animals. When human are believed, based on the data, to be less sensitive than laboratory animals, a default factor of unity (i.e., no adjustment) may be employed to account for this, such as in the assessment of thyroid follicular cell tumours [11].

[2] These may be some changes in the approach to the margin of exposure analysis in the final guidelines as peer review comments are considered.

There may be situations for which it is appropriate to consider both linear and nonlinear default procedures. For example, an agent may produce tumours at multiple sites by different mechanisms. In another case, for example, when it is apparent that an agent is both DNA reactive and highly active as a promoter at higher doses, both linear and nonlinear default procedures may be used to distinguish between the events operative at different portions of the dose–response curve and to consider the contribution of both phenomena.

There may be situations for which there is insufficient data to provide high confidence in a conclusion about any single mode of action of a given agent and for which different mechanisms may be operating at the different sites of tumour induction. Although the available data generally supports nonlinearity, a linear mechanism (e.g., a mutagenic metabolite for one of the tumour sites) cannot be dismissed. Both defaults are conducted and a discussion of the degree of confidence in each is provided to the risk manager. The linear default may be viewed as conservative (i.e., likely to overestimate the risk at low exposures), and it might be more appropriate for screening analyses. The nonlinear default may be viewed as more representative of the risk given the growth-promoting potential and toxicity of the given agent.

5. Role of data on key events in dose–response assessment

Information about the key events proceeding tumour development should be used in the dose–response assessment without having to apply a biologically based model. The principle underlying the new guidelines is to use as much information about these events as possible. When such information is available, it may be used in a variety of ways.

- If a key event(s) is quantitatively described and considered key to cancer development, its dose–response assessment in the range of observation can be used in conjunction with, or in lieu of, the dose–response for tumour incidence to establish the point of departure for extrapolation. [Rates of molecular events such as mutation or cell proliferation or of signal transduction may be difficult to relate to cell

or tissue changes overall. The timing of observations of these phenomena, as well as the cell type involved, need to be linked to other precursor events to ensure the measurement is truly a ''key'' event. In many cases such rates are more appropriately used as described below.]

· Quantitative description of a key event(s) can be used to test whether the dose–response for tumour incidence can be confidently extended to support a lower point of departure for linear extrapolation than the tumour data alone would support (e.g., a dose associated with 1% extra risk from one associated with 10% extra risk).

· Quantitative information on a key event(s) can be used to address the question of how quickly risk decreases as dose decreases in a MoE analysis.

6. An example of the dose–response analysis under the new guidelines — thyroid disruption

Below is an example of how the new guidelines' mode of action framework is used to evaluate a postulated mode of carcinogenic action of a given agent, and how a MoE analysis is approached. This illustration is based on a carcinogen that induces thyroid tumours. Although this example is derived from actual data, it is meant to be an illustration thus the name of the chemical is labelled as *ChemT*.

Summary Description of Postulated Mode of Action: Thyroid hormone production is regulated by actions of the hypothalamus, pituitary and thyroid gland. Homeostasis of thyroid hormone is maintained by a feedback loop between the hypothalamus and pituitary and the thyroid gland. The hypothalamus produces thyrotrophin reducing hormone (TRH) which stimulates the pituitary to produce thyroid stimulating hormone (TSH) which, in turn, stimulates the thyroid to produce thyroid hormone. The hypothalamus and pituitary respond to high level of circulating thyroid hormone by suppressing TRH and TSH production, and to a low level by increasing them. The mode of action considered is continuous elevation of TSH levels that stimulates the thyroid gland to deplete its stores of thyroid hormone and continues to push production resulting in hypertrophy of the production cells (follicular cells) leading to hyperplasia, nodular hyperplasia, and, eventually, tumours of these cells. In rats, the chain of events may be induced by direct effects on hormone synthesis or by metabolic removal of circulating hormone.

Key Events: The key events considered with respect to *ChemT*-induced tumorigenesis in male rats include hormone changes in TSH, T_4, T_3 and changes in hepatic T_4-UDPGT, indicators of liver microsomal enzyme induction, enhanced liver metabolism, increased biliary excretion of T_4, increase in thyroid weight and liver weight, and thyroid follicular cell hypertrophy/hyperplasia. These events have been well defined and measured in male rats in subchronic studies augmenting observations at interim and terminal sacrifice in a chronic study.

Strength, Consistency, Specificity of Association of Tumour Response with Key Events: The thyroid tumour response in the chronic study at the highest dose was associated with hypertrophy/hyperplasia in the thyroid and increase in weight of the thyroid. In subchronic studies, the organ weight and hypertrophy/hyperplasia were shown to appear and reverse under the same conditions of dose and time as the appearance and reversal of changes in thyroid hormone levels and thyroid hormone metabolism in statistically significant degree. Stop/recovery studies showed that cessation of dosing was followed in turn by return of hormone levels to control levels, reduction in liver and thyroid weights, and reversal of hyperplasia in thyroid follicular cells. The only sign slow to reverse was thyroid weight after the longest dosing period. Strength, consistency and specificity of association were well established in the studies.

Dose–Response (D / R) Relationship: Dose correlations exist for parameters in the chronic and subchronic studies or all of the relevant parameters. Thyroid follicular cell tumours, thyroid hypertrophy/hyperplasia and increased thyroid and liver weight are noted at similar doses, usually at dietary levels of 1000 and 3000 ppm *ChemT*. Correspondingly in the subchronic study, at 3000 ppm T_4 is depressed while TSH is elevated. At 1000 and 3000 ppm, hepatic T_4-UDPGT activity is statistically significantly elevated, ant there is an increase in biliary excretion of T_4 at 3000 ppm. The only parameter showing significant effect at a dose below 1000 ppm *ChemT* was liver weight increase in a subchronic study at 300 ppm.

Temporal Association: The chronic study together with the three subchronic studies of key events observing effects after different durations at one dose, at multiple doses, and after recovery, show events occurring in the following sequence: (1) increase in hepatic glucuronidation, de-iodination and excretion of T_4, as well as its elimination from the blood; (2) a rise in circulating TSH; (3) an increase in thyroid weight, and thyroid follicular cell hypertrophy; (4) thyroid follicular cell hyperplasia; (5) thyroid follicular cell tumours. The stop experiments indicate reversal of the thyroid and liver weight increases as well as reversal of hormone and other protein measures. While reversal of thyroid weight increases in the recovery study was less after a longer duration of treatment, hypertrophy/hyperplasia did reverse after the longer duration.

Biological Plausibility and Coherence of the Database: Under EPA science policy [11], determination of the antithyroid activity of a chemical requires empirical demonstration of five items: (1) increases in thyroid growth, (2) changes in thyroid and pituitary hormones, (3) location of the site(s) of antithyroid action, (4) dose–response correlations among various key precursor events and tumour incidence, and (5) reversibility of effects following treatment cessation. The database on *ChemT* ably documents all such information.

Thyroid tumorigenesis, particularly in the male rat, has been observed to be associated with exposure to a number of industrial chemicals, pesticides and pharmaceuticals. A significant number of these appear to work in a manner similar to *ChemT* by enhancing thyroid hormone metabolism and excretion by the liver.

Thyroid tumours did not appear in the female rats in the 2-year study, Thyroid hypertrophy and hyperplasia were observed in the females 6 months after their appearance in males. As is noted with other chemicals, the female rat is less sensitive to the effect of antithyroid chemicals regarding key events and tumour development. It should be noted that

Table 1
ChemT case study: consideration of point of departure

Toxicity study	Endpoint	NOAEL[a] mg/kg/day (ppm)		LOAEL[a] mg/kg/day (ppm)	
		Male	Female	Male	Female
Rat					
24 Months	thyroid tumours	4 (100)	177 (3000)	44 (1000)	–
	thyroid hypertrophy/hyperplasia	44 (1000)	5 (100)	136 (3000)	56 (1000)
	↑ thyroid weight	4 (100)	5 (100)	44 (1000)	56 (1000)
	liver hypertrophy	4 (100)	5 (100)	44 (1000)	56 (1000)
	↑ liver weight	4 (100)	5 (100)	44 (1000)	56 (1000)
Special subchronics	↑ thyroid weight	15 (300)		50 (1000)	
	↑ liver weight	5 (100)		15 (300)	
	↓ T_4	50 (1000)		150 (3000)	
	↓ T_3	50 (1000)		150 (3000)	
	↑ TSH	50 (1000)		150 (3000)	
	thyroid hypertrophy/hyperplasia	50 (1000)		150 (3000)	
	↑ T_4 UDPGT activity	15 (300)		50 (1000)	
Mouse					
18 Months	↑ liver weight	17 (400)	27 (400)	66 (800)	108 (800)
	liver hypertrophy	2 (100)	27 (400)	17 (400)	108 (800)
Dog					
Subchronic	thyroid hyperplasia	35 (1000)	35 (1000)	175 (5000)	160 (5000)
	↑ relative thyroid weight	35 (1000)	35 (1000)	175 (5000)	160 (5000)
	liver hypertrophy	6 (100)	3 (100)	35 (1000)	35 (1000)
12 Months	liver hypertrophy/hyperplasia	1 (20)	1 (20)	8 (200)	9 (200)
	↑ liver weight	8 (200)	9 (200)	86 (2000)	78 (2000)

[a]Rounded to nearest integer.

dogs receiving high doses of *ChemT* show enlargement of the thyroid gland.

Other Modes of Action: ChemT does not belong to a class of chemicals that is expected to generate reactive metabolites, and no related chemicals have been tested for carcinogenicity. Short-term studies demonstrate that the chemical does not increase gene mutations in Salmonella (Ames test) or cultured mammalian cells (maximal dosage may not have been reached), micronuclei in bone marrow cells, and unscheduled DNA synthesis in cultured cells. No other modes of action, apart from thyroid disruption are in evidence to account for the thyroid tumours.

Several sites of action were investigated as being the source of the antithyroid effects of *ChemT*. The chemical does not inhibit the entry of inorganic iodide into the thyroid (iodide pump) or block the organification and incorporation of iodide into thyroid hormone (thyroid peroxidase); likewise, it does not inhibit monodeiodinase which blocks the conversion of T_4 to T_3.

ChemT administration leads to renal adenomas in male and female rats; the response lacked statistical significance. The mode of action for the thyroid tumours does not account for the renal tumours. Assessment of the significance and mode of action of the renal tumours requires separate analysis.

Conclusion: The weight of evidence supports a conclusion that the pesticide *ChemT* acts to cause tumours in the male Sprague–Dawley rat by inducing hepatic metabolism and biliary elimination of thyroid hormone prompting increased production of TSH which ultimately results in thyroid follicular cell neoplasia. In addition, there is no indication that *ChemT* is mutagenic. The thyroid response in the rat is the sole, significant tumour response observed in animal studies. Under the EPA's policy for assessment of thyroid follicular cell tumours, when an agent causes this antithyroid mode of action in the male rat and is not mutagenic, dose–response assessment proceeds by MoE analysis.

6.1. Selecting a point of departure

MoE analysis begins with selection of a point of departure considered to represent the lowest reliable endpoint in the range of observation, being either tumour incidence or data on a key event(s) that is an integral part of the carcinogenic process. Table 1 shows NOAEL/LOAEL data for multiple endpoints in various studies in the rat as well as the mouse and dog. The NOAEL data are used as point of departure data as opposed to modelling, because the NOAEL's can be compared among the many studies which include several data sets of continuous data about hormone levels and tissue weights in addition to incidence data on tumour response. Doses are shown in the table as parts per million (ppm) in the diet and as mg/kg/day. The sensitivity of the animals for several parameters results in a NOAEL of 4 mg/kg/day (100 ppm) as the point of departure. The male rat data are used because studies in other species did not show tumours in conjunction with other effects of thyroid disruption; as typical in such cases, the male rat is more sensitive than other test animals to carcinogenic effects of thyroid disruption. As a human equivalent dose, the point of departure is 1.0 mg/kg/day) after application of an oral, interspecies scaling factor of $BW^{0.75}$ [2]. This is selected as the most sensitive point of departure for the data set, and is applicable to evaluations of the 24-month rat study for thyroid weight, and liver weight and hepatocellular hypertrophy. The data show that protection against these key events protects against tumour development. The dose–response for these key events and tumours is virtually the same, the NOAEL for tumours also being 4 mg/kg/day (100 ppm).

6.2. Margin of exposure analysis

The initial goal in the MoE analysis is to identify the dose at which the key events just begin to occur in a heterogeneous, human population and to judge how the animal dose should be adjusted to approximate this as dose of a 0 to 1% key event response.

· First, one considers whether the response assessment is for tumour or key events with a default of using a 10-fold factor to reduce dose if tumour is the response. In this case, key events of liver and thyroid weight increase and liver hypertrophy have the same NOAEL as the tumour response in the 2-year study. The course of events of the mode of action revealed by the combination of all of the chronic and subchronic studies is that the tumour response is secondary to the disruption of thyroid hormone homeostasis which is, in turn, secondary to effects on the liver. The key event data on liver and subsequent hormone and tissue effects are appropri-

ately the focus of the analysis and the 10-fold adjustment is not needed in this case because it is clear that the tumour response will not occur below the NOAEL for liver effects.

· Second, one examines whether the dose–response is shallow or steep. When a NOAEL for a key event is used, there is a 10-fold downward adjustment dose unless examination of the whole array of data supports a conclusion that the NOAEL is probably a no effect level or very close. In this case, the NOAEL is probably a no effect level for the thyroid disruption and the subsequent tumour response. Both of the latter are entirely dependent on the liver effects. Moreover, the hormone and liver enzyme effects occur in subchronic studies with a higher NOAEL than liver weight increase. Overall, one can be confident that the identified NOAEL does measure where the key events appear above background.

The second goal of the analysis is to consider interspecies and intraspecies variability with the goal of reaching a MoE that is protective of the population overall, including sensitive subpopulations.

· For interspecies variability, a default uncertainty factor of 10-fold is appropriate on an assumption that humans may be more sensitive than test animals. In this case, the policy about male rat thyroid disruption is that a factor of unity is used instead. A factor of unity addresses the fact that male rats are more sensitive to the effect than other test species, the substantial question whether this mode of action is relevant to humans, and the uncertainty whether humans get thyroid cancer as a result of thyroid hormone disruption.

· Human variability in sensitivity is a difficult issue to assess without specific data. A default factor of 10-fold is appropriate to account for variability in toxicokinetics/toxicodynamics. There is no indication that children are at special risk to the thyroid cancer-inducing potential of antithyroid chemicals [11]. Dividing the point of departure by 10 yields a chronic value of 0.1 mg/kg/day for comparison with chronic exposures of interest.

6.3. MoE for brief exposure

Thyroid hormone disruption is a concentration-dependent effect that is anticipated to cause toxicity only with sustained exposure, since normal homeostasis will resume after cessation of exposure. This reversibility was observed for *ChemT* in the male rat. Therefore, the MoE for brief duration and occasional use of *ChemT* can be 1 to 10, depending on the actual duration and frequency.

7. Conclusions

Compared with the traditional approaches used to assess cancer risk, the EPA's new guidelines for carcinogen assessment include a more complete discussion of the issues and an evaluation of all relevant information, promoting the use of mode-of-action information to reduce the uncertainties associated with using experimental data to characterise and project how human beings will respond to certain exposure conditions. This emphasis on mechanisms is to promote research and testing to improve the scientific basis of health risk assessment and stimulate thinking on how such information can be applied. As the science continues to evolve the practice and policies of EPA risk assessment guidelines will reflect these advances.

Acknowledgements

We would like to acknowledge the other authors of the EPA's 1996 *Proposed Guidelines for Carcinogen Risk Assessment*, Drs. Richard Hill of the Office of Pollution Prevention, Vanessa Vu and V. James Cogliano of the National Center for Environmental Assessment, Karl Baetcke of the Office of Pesticide Programs, and Arnold Kuzmack of the Office of Water, who have contributed substantially to the development of these new guidelines.

References

[1] U.S. Environmental Protection Agency, Guidelines for carcinogen risk assessment, Fed. Regul. 51 (185) (1986) 33992–34003.

[2] U.S. Environmental Protection Agency, Proposed guidelines for carcinogen risk assessment, Fed. Regul. 61 (79) (1996) 17960–18011.

[3] J.A. Wiltse, V.L. Dellarco, The U.S. Environmental Protection Agency guidelines for carcinogen risk assessment: past and future, Mutat. Res. 365 (1996) 3–16.
[4] A.B. Hill, The environment and disease: association or causation?, Proc. R. Soc. Med. 58 (1965) 295–300.
[5] Department of Health and Human Services, The health consequences of smoking — cancer, a report of the Surgeon General, Washington, 1982, pp. 17–20, 29 et seq.
[6] E. Faustman, et al., Experimental approaches to evaluate mechanisms of developmental toxicity, in: R.D. Hood (Ed.), Handbook of Developmental Biology, CRC Press, Boca Raton, FL, 1997, pp. 13–41.
[7] S.H. Moolgavkar, A.G. Knudson, Mutation and cancer: a model for human Carcinogenesis, J. Natl. Cancer Inst. 66 (1981) 1037–1052.
[8] C. Chen, W. Farland, Incorporating cell proliferation in quantitative cancer risk assessment: approaches, issues, and uncertainties, in: B. Butterworth, T. Slaga, W. Farland, M. McClain (Eds.), Chemical Induced Cell Proliferation: Implications for Risk Assessment, Wiley-Liss, New York, 1991, pp. 481–499.
[9] C. Portier, Statistical properties of a two-stage model of carcinogenesis, Environ. Health Perspect. 76 (1987) 125–131.
[10] J.K. Haseman, Issues: a reexamination of false-positive rates for carcinogenesis studies, Fundam. Appl. Toxicol. 3 (1983) 334–339.
[11] U.S. Environmental Protection Agency, Assessment of thyroid follicular cell tumours, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, 1998, EPA/630/R-97/002.