

Proposed Guidelines for **Carcinogen** Risk Assessment (April 23, 1996)
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Proposed Guidelines for Carcinogen Risk Assessment

Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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**PROPOSED GUIDELINES FOR
CARCINOGEN RISK ASSESSMENT**

FRL-5460-3

AGENCY: U.S. Environmental Protection Agency

ACTION: Notice of Availability and Opportunity to Comment on Proposed Guidelines for Carcinogen Risk Assessment

SUMMARY: The U.S. Environmental Protection Agency (EPA) is today publishing a document entitled *Proposed Guidelines for Carcinogen Risk Assessment* (hereafter "Proposed Guidelines"). These Proposed Guidelines were developed as part of an interoffice guidelines development program by a Technical Panel of the Risk Assessment Forum within EPA's Office of Research and Development. These Proposed Guidelines are a revision of EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (hereafter "1986 cancer guidelines") published on September 24, 1986 (51 FR 33992). When final, these guidelines will replace the 1986 guidelines.

In a future *Federal Register* notice, the Agency intends to publish for comment how it will implement the Proposed Guidelines once they are finalized. The plans will propose and seek comment on how the Guidelines will be used for Agency carcinogen risk assessment and, in particular, will address the impact of the Guidelines on the Agency's existing assessments, and any mechanisms for handling reassessments under finalized Guidelines.

DATES: The Proposed Guidelines are being made available for a 120-day public review and comment period. Comments must be in writing and must be postmarked by August 21, 1996. See Addresses section for guidance on submitting comments.

FOR FURTHER INFORMATION, CONTACT: Technical Information Staff, Operations and Support Group, National Center for Environmental Assessment-Washington Office, telephone: 202-260-7345. Email inquiries may be sent to cancer-guidelines@epamail.epa.gov.

ADDRESSES:

The Proposed Guidelines will be made available in the following ways:

- 1) the electronic version will be accessible on EPA's Office of Research and Development home page on the Internet at <http://www.epa.gov/ORD>

1 2) 3½" high-density computer diskettes in Wordperfect 5.1 format will be available from ORD
2 Publications, Technology Transfer and Support Division, National Risk Management Research
3 Laboratory, Cincinnati, OH; telephone: 513-569-7562; fax: 513-569-7566. Please provide the EPA
4 No. (EPA/600/P-92/003Ca) when ordering.
5

6 3) This notice contains the full draft document. In addition, copies of the draft will be available for
7 inspection at EPA headquarters and regional libraries, through the U.S. Government Depository
8 Library program, and for purchase from the National Technical Information Service (NTIS),
9 Springfield, VA; telephone: 703-487-4650, fax: 703-321-8547. Please provide the NTIS PB No.
10 (PB96-157599) (\$35.00) when ordering.
11

12 **Submitting Comments**

13 Comments on the Proposed Guidelines may be mailed or delivered to the Technical
14 Information Staff (8623), NCEA-WA/OSG, U.S. Environmental Protection Agency, 401 M Street,
15 S.W., Washington, DC 20460. Comments should be in writing and must be postmarked by the date
16 indicated. Please submit one unbound original with pages numbered consecutively, and three copies.
17 For attachments, provide an index, number pages consecutively with the comment, and submit an
18 unbound original and three copies.

19 Please note that all technical comments received in response to this notice will be placed in a
20 public record. For that reason, commenters should not submit personal information (such as medical
21 data or home address), Confidential Business Information, or information protection by copyright. Due
22 to limited resources, acknowledgments will not be sent.
23

24 **SUPPLEMENTARY INFORMATION:** In 1983, the National Academy of Sciences
25 (NAS)/National Research Council (NRC) published its report entitled *Risk Assessment in the Federal*
26 *Government: Managing the Process* (NRC, 1983). In that report, the NRC recommended that
27 Federal regulatory agencies establish "inference guidelines" to ensure consistency and technical quality in
28 risk assessments and to ensure that the risk assessment process was maintained as a scientific effort
29 separate from risk management. The 1986 cancer guidelines were issued on September 24, 1986 (51
30 FR 33992). The Proposed Guidelines published today continue the guidelines development process.
31 These guidelines set forth principles and procedures to guide EPA scientists in the conduct of Agency
32 cancer risk assessments and to inform Agency decisionmakers and the public about these procedures.

33 Both the 1986 guidelines and the current proposal contain inference guidance in the form of
34 default inferences to bridge gaps in knowledge and data. Research conducted in the past decade has

1 elucidated much about the nature of carcinogenic processes and continues to provide new information.
2 The intent of this proposal is to take account of knowledge available now and to provide flexibility for
3 the future in assessing data and employing default inferences, recognizing that the guidelines cannot
4 always anticipate future research findings. Because methods and knowledge are expected to change
5 more rapidly than guidelines can practicably be revised, the Agency will update specific assessment
6 procedures with peer-reviewed supplementary, technical documents as needed. Further revision of the
7 guidelines themselves will take place when extensive changes are necessary.

8 Since 1986, the EPA has sponsored several workshops about revising the cancer guidelines
9 (U.S. EPA, 1989b, 1989c, 1994a). The Society for Risk Analysis conducted a workshop on the
10 subject in connection with its 1992 annual meeting (Anderson et al., 1993). Participants in the most
11 recent workshop in 1994 reviewed an earlier version of the guidelines proposed here and made
12 numerous recommendations about individual issues as well as broad recommendations about
13 explanations and perspectives that should be added. Most recently, the Committee on the Environment
14 and Natural Resources of the Office of Science and Technology Policy reviewed the guidelines at a
15 meeting held on August 15, 1995. The EPA appreciates the efforts of all participants in the process
16 and has tried to address their recommendations in this proposal.

17 In addition, the recommendations of the NRC (1994) in *Science and Judgment in Risk*
18 *Assessment* have been addressed. Responses to these recommendations are given generally in
19 Appendix B as well as being embodied in the Proposed Guidelines. Responses that explain the major
20 default assumptions adopted under these guidelines and the policy for using and departing from these
21 default assumptions appear in Section 1.3.

22 The Science Advisory Board also will review these Proposed Guidelines at a meeting to be
23 announced in a future *Federal Register* notice. Following these reviews Agency staff will prepare
24 summaries of the public and SAB comments. Appropriate comments will be incorporated, and the
25 revised Guidelines will be submitted to the Risk Assessment Forum for review. The Agency will
26 consider comments from the public, the SAB, and the Risk Assessment Forum in its recommendations
27 to the EPA Administrator.

28 29 **Major Changes from the 1986 Guidelines**

30 ***Characterizations***

31
32 Increased emphasis on providing characterization discussions for the hazard, dose response,
33 and exposure sections is part of the proposal. These discussions will summarize the assessments to
34 explain the extent and weight of evidence, major points of interpretation and rationale, and strengths

1 and weaknesses of the evidence and the analysis, and to discuss alternative conclusions and
2 uncertainties that deserve serious consideration (U.S. EPA, 1995). They serve as starting materials for
3 the risk characterization process which completes the risk assessment.
4

5 ***Weighing Evidence of Hazard***

6 A major change is in the way hazard evidence is weighed in reaching conclusions about the
7 human carcinogenic potential of agents. In the 1986 cancer guidelines, tumor findings in animals or
8 humans were the dominant components of decisions. Other information about an agent's properties, its
9 structure-activity relationships to other carcinogenic agents, and its activities in studies of carcinogenic
10 processes was often limited and played only a modulating role as compared with tumor findings. In this
11 proposal, decisions come from considering all of the evidence. This change recognizes the growing
12 sophistication of research methods, particularly in their ability to reveal the modes of action of
13 carcinogenic agents at cellular and subcellular levels as well as toxicokinetic and metabolic processes.
14 The effect of the change on the assessment of individual agents will depend greatly on the availability of
15 new kinds of data on them in keeping with the state of the art. If these new kinds of data are not
16 forthcoming from public and private research on agents, assessments under these guidelines will not
17 differ significantly from assessments under former guidelines.

18 Weighing of the evidence includes addressing the likelihood of human carcinogenic effects of
19 the agent and the conditions under which such effects may be expressed, as these are revealed in the
20 toxicological and other biologically important features of the agent. (Consideration of actual human
21 exposure and risk implications are done separately; they are not parts of the hazard characterization).
22 In this respect, the guidelines incorporate recommendations of the NRC (1994). In that report, the
23 NRC recommends expansion of the former concept of hazard identification, which rests on simply a
24 finding of carcinogenic potential, to a concept of characterization that includes dimensions of the
25 expression of this potential. For example, an agent might be observed to be carcinogenic via inhalation
26 exposure and not via oral exposure, or its carcinogenic activity might be secondary to another toxic
27 effect. In addition, the consideration of evidence includes the mode(s) of action of the agent apparent
28 from the available data as a basis for approaching dose response assessment.
29

30 ***Classification Descriptors***

31 To express the weight of evidence for carcinogenic hazard potential, the 1986 cancer guidelines
32 provided summary rankings for human and animal cancer studies. These summary rankings were
33 integrated to place the overall evidence in classification groups A through E, Group A being associated
34 with the greatest probability of human carcinogenicity and Group E with evidence of noncarcinogenicity

1 in humans. Data other than tumor findings played a modifying role after initial placement of an agent
2 into a group.

3 These Proposed Guidelines take a different approach, consistent with the change in the basic
4 approach to weighing evidence. No interim classification of tumor findings followed by modifications
5 with other data takes place. Instead, the conclusion reflects the weighing of evidence in one step.
6 Moreover, standard descriptors of conclusions are employed rather than letter designations, and these
7 are incorporated into a brief narrative description of their informational basis. The narrative with
8 descriptors replaces the previous letter designation. The descriptors are in three categories:
9 "known/likely," "cannot be determined," or "not likely." For instance, using a descriptor in context, a
10 narrative could say that an agent is *likely* to be carcinogenic by inhalation exposure and *not likely* to be
11 carcinogenic by oral exposure. The narrative explains the kinds of evidence available and how they fit
12 together in drawing conclusions, and points out significant issues/strengths/limitations of the data and
13 conclusions. Subdescriptors are used to further refine the conclusion. The narrative also summarizes
14 the mode of action information underlying a recommended approach to dose response assessment.

15 In considering revision of the former classification method, the Agency has examined other
16 possibilities that would retain the use of letter and number designation of weights of evidence. The use
17 of standard descriptors within a narrative presentation is proposed for three primary reasons. First, the
18 proposed method permits inclusion of explanations of data and of their strengths and limitations. This is
19 more consistent with current policy emphasis on risk characterization. Second, it would take a large set
20 of individual number or letter codes to cover differences in the nature of contributing information
21 (animal, human, other), route of exposure, mode of action, and relative overall weight. When such a set
22 becomes large—10 to 30 codes—it is too large to be a good communication device, because people
23 cannot remember the definitions of the codes so they have to be explained in narrative. Third, it is
24 impossible to predefine the course of cancer research and the kinds of data that may become available.
25 A flexible system is needed to accommodate change in the underlying data and inferences, and a system
26 of codes might become out of date, as has the one in the 1986 cancer guidelines.

27 28 ***Dose Response Assessment***

29 The approach to dose response assessment calls for analysis that follows the conclusions
30 reached in the hazard assessment as to potential mode(s) of action. The assessment begins by
31 analyzing the empirical data in the range of observation. When animal studies are the basis of the
32 analysis, the estimation of a human equivalent dose utilizes toxicokinetic data, if appropriate and
33 adequate data are available. Otherwise, default procedures are applied. For oral dose, the default is
34 to scale daily applied doses experienced for a lifetime in proportion to body weight raised to the 0.75

1 power. For inhalation dose, the default methodology estimates respiratory deposition of particles and
2 gases and estimates internal doses of gases with different absorption characteristics. These two defaults
3 are a change from the 1986 cancer guidelines which provided a single scaling factor of body weight
4 raised to the 0.66 power. Another change from the 1986 guidelines is that response data on effects of
5 the agent on carcinogenic processes are analyzed (nontumor data) in addition to data on tumor
6 incidence. If appropriate, the analyses of data on tumor incidence and on precursor effects may be
7 combined, using precursor data to extend the dose response curve below the tumor data. Even if
8 combining data is not appropriate, study of the dose response for effects believed to be part of the
9 carcinogenic influence of the agent may assist in thinking about the relationship of exposure and
10 response in the range of observation and at exposure levels below the range of observation.

11 Whenever data are sufficient, a biologically based or case-specific dose response model is
12 developed to relate dose and response data in the range of empirical observation. Otherwise, as a
13 standard, default procedure, a model is used to curve-fit the data. The lower 95% confidence limit on
14 a dose associated with an estimated 10% increased tumor or relevant nontumor response (LED_{10}) is
15 identified. This generally serves as the point of departure for extrapolating the relationship to
16 environmental exposure levels of interest when the latter are outside the range of observed data. The
17 environmental exposures of interest may be measured ones or levels of risk management interest in
18 considering potential exposure control options. Other points of departure may be more appropriate for
19 certain data sets; as described in the guidance, these may be used instead of the LED_{10} . Additionally,
20 the LED_{10} is available for comparison with parallel analyses of other carcinogenic agents or of
21 noncancer effects of agents and for gauging and explaining the magnitude of subsequent extrapolation to
22 low-dose levels. The LED_{10} , rather than the ED_{10} (the estimate of a 10% increased response), is the
23 proposed standard point of departure for two reasons. One is to permit easier comparison with the
24 benchmark dose procedure for noncancer health assessment—also based on the lower limit on dose.
25 Another is that the lower limit, as opposed to the central estimate, accounts for uncertainty in the
26 experimental data. The issue of using a lower limit or central estimate was discussed at a workshop
27 held on the benchmark procedure for noncancer assessment (Barnes et al., 1995) and at a workshop
28 on a previous version of this proposal (U.S. EPA, 1994b). The latter workshop recommended a
29 central estimate; the benchmark workshop recommended a lower limit.

30 The second step of dose response assessment is extrapolation to lower dose levels, if needed.
31 This is based on a biologically based or case-specific model if supportable by substantial data.
32 Otherwise, default approaches are applied that accord with the view of mode(s) of action of the agent.
33 These include approaches that assume linearity or nonlinearity of the dose response relationship or
34 both. The default approach for linearity is to extend a straight line to zero dose, zero response. The

1 default approach for nonlinearity is to use a margin of exposure analysis rather than estimating the
2 probability of effects at low doses. A margin of exposure analysis explains the biological considerations
3 for comparing the observed data with the environmental exposure levels of interest and helps in
4 deciding on an acceptable level of exposure in accordance with applicable management factors.

5 The use of straight line extrapolation for a linear default is a change from the 1986 guidelines
6 which used the "linearized multistage" (LMS) procedure. This change is made because the former
7 modeling procedure gave an appearance of specific knowledge and sophistication unwarranted for a
8 default. The proposed approach is also more like that employed by the Food and Drug Administration
9 (U.S. FDA, 1987). The numerical results of the straight line and LMS procedures are not significantly
10 different (Krewski et al., 1984). The use of a margin of exposure approach is included as a new
11 default procedure to accommodate cases in which there is sufficient evidence of a nonlinear dose
12 response, but not enough evidence to construct a mathematical model for the relationship. (The
13 Agency will continue to seek a modeling method to apply in these cases. If a modeling approach is
14 developed, it will be subject to peer review and public notice in the context of a supplementary
15 document for these guidelines.)

16 The public is invited to provide comments to be considered in EPA decisions about the content
17 of the final guidelines. After the public comment period, the EPA Science Advisory Board will be
18 asked to review and provide advice on the guidelines and issues raised in comments. EPA asks those
19 who respond to this notice to include their views on the following:

20
21 (1) The proposed guidance for characterization of hazard, including the weight of evidence
22 descriptors and weight of evidence narrative which are major features of the proposal. There are three
23 categories of descriptors: "known/likely," "cannot be determined," and "not likely" which are further
24 refined by subdescriptors. It is felt that these three descriptors will satisfactorily delineate the types of
25 evidence bearing on carcinogenicity as they are used with subdescriptors in the context of a narrative of
26 data and rationale. However, an issue that has been discussed by external peer reviewers and by EPA
27 staff is whether the descriptor-subdescriptor called "cannot be determined—suggestive evidence"
28 should become a separate, fourth category called "suggestive." The EPA may choose this course in the
29 final guidelines and requests comment. In considering this issue, commenters may wish to refer not only
30 to Sections 2.6.2. and 2.7.2. which cover the descriptors and narrative, but also to case study example
31 #6 in Section 2.6.3. and example narrative #2 in Appendix A of the proposal. EPA asks commenters
32 on this question to address the rationale (science as well as policy) for leaving the categories of
33 descriptors as proposed or making the fourth category. How might the coverage of a "suggestive"
34 category be defined in order to be most useful?

1 (2) The use of mode of action information in hazard characterization and to guide dose
2 response assessment is a central part of the proposed approach to bringing new research on
3 carcinogenic processes to bear in assessments of environmental agents (Sections 1.3.2., 2.3.2., 2.5.,
4 3.1.). The appropriate use of this information now and in the future is important. EPA requests
5 comment on the treatment of such information in the proposal, including reliance on peer review as a
6 part of the judgmental process on its application.
7

8 (3) Uses of nontumor data in the dose response assessment and the methodological and
9 science policy issues posed are new to these guidelines (Sections 1.3.2., 3.1.2.). EPA requests
10 comment on both issues.
11

12 (4) Dose response assessment is proposed to be considered in two parts—range of observed
13 data and range of extrapolation (Section 3.1.). The lower 95% confidence limit on a dose associated
14 with a 10% response (tumor or nontumor response) is proposed as a default point of departure,
15 marking the beginning of extrapolation. This is a parallel to the benchmark procedure for evaluating
16 dose-response of noncancer health endpoints (Barnes et al.,1995). An alternative is to use the central
17 estimate of a 10% response. Another alternative is to use a 1%, instead of a 10%, response when the
18 observed data are tumor incidence data. Does the generally larger sample size of tumor effect studies
19 support using a 1% response as compared with using 10% for smaller studies? Are there other
20 approaches for the point of departure that might be considered?
21

22 (5) Discussions of default assumptions and other responses to the 1994 NRC report *Science*
23 *and Judgment in Risk Assessment* appear in Section 1.3.1. and Appendix B of the proposal,
24 respectively. Comments are requested on responses to the NRC recommendations and how the
25 guidelines as a whole address them.
26
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28

29 April 10, 1996
30 Date

Carol M. Browner
Administrator

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1
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3 **1. INTRODUCTION**

4 **1.1. PURPOSE AND SCOPE OF THE GUIDELINES**

5 These guidelines revise and replace United States Environmental Protection Agency (EPA)
6 Guidelines for Carcinogen Risk Assessment published in 51 FR 33992, September 24, 1986. The
7 guidelines provide EPA staff and decisionmakers with guidance and perspectives to develop and use
8 risk assessments. They also provide basic information to the public about the Agency's risk assessment
9 methods.

10 The guidelines encourage both regularity in procedures to support consistency in scientific
11 components of Agency decisionmaking and innovation to remain up-to-date in scientific thinking. In
12 balancing these goals, the Agency relies on input from the general scientific community through
13 established scientific peer review processes. The guidelines incorporate basic principles and science
14 policies based on evaluation of the currently available information. As more is discovered about
15 carcinogenesis, the need will arise to make appropriate changes in risk assessment guidance. The
16 Agency will revise these guidelines when extensive changes are due. In the interim, the Agency will
17 issue special reports, after appropriate peer review, to supplement and update guidance on single
18 topics, (e.g., U.S. EPA, 1991b)

19 **1.2. ORGANIZATION AND APPLICATION OF THE GUIDELINES**

20 **1.2.1. Organization**

21 Publications of the Office of Science and Technology Policy (OSTP, 1985) and the National
22 Research Council (NRC, 1983, 1994) provide information and general principles about risk
23 assessment. Risk assessment uses available scientific information on the properties of an agent¹ and its
24 effects in biological systems to provide an evaluation of the potential for harm as a consequence of
25 environmental exposure to the agent. Risk assessment is one of the scientific analyses available for
26 consideration, with other analyses, in decisionmaking on environmental protection. The 1983 and 1994
27 NRC documents organize risk assessment information into four areas: hazard identification, dose
28 response assessment, exposure assessment, and risk characterization. This structure appears in these
29 guidelines, which additionally emphasize characterization of evidence and conclusions in each part of the
30 assessment. In particular, the guidelines adopt the approach of the NRC's 1994 report in adding a
31 dimension of characterization to the hazard identification step. Added to the identification of hazard is

The term "agent" refers generally to any chemical substance, mixture, or physical or biological entity being assessed, unless otherwise noted.

1 an evaluation of the conditions under which its expression is anticipated. The risk assessment questions
2 addressed in these guidelines are:

- 3 C For hazard--Can the agent present a carcinogenic hazard to humans, and if so, under what
4 circumstances?
- 5 C For dose response--At what levels of exposure might effects occur?
- 6 C For exposure--What are the conditions of human exposure?
- 7 C For risk--What is the character of the risk? How well do data support conclusions about
8 the nature and extent of the risk?

10 **1.2.2. Application**

11 The guidelines apply within the framework of policies provided by applicable EPA statutes and
12 do not alter such policies. The guidelines cover assessment of available data. They do not imply that
13 one kind of data or another is prerequisite for regulatory action concerning any agent. Risk
14 management applies directives of regulatory legislation, which may require consideration of potential
15 risk, or solely hazard or exposure potential, along with social, economic, technical, and other factors in
16 decisionmaking. Risk assessments support decisions, but to maintain their integrity as decisionmaking
17 tools, they are not influenced by consideration of the social or economic consequences of regulatory
18 action.

19 Not every EPA assessment has the same scope or depth. Agency staff often conduct
20 screening-level assessments for priority-setting or separate assessments of hazard or exposure for
21 ranking purposes or to decide whether to invest resources in collecting data for a full assessment.
22 Moreover, a given assessment of hazard and dose response may be used with more than one exposure
23 assessment that may be conducted separately and at different times as the need arises in studying
24 environmental problems in various media. The guidelines apply to these various situations in
25 appropriate detail given the scope and depth of the particular assessment. For example, a screening
26 assessment may be based almost entirely on structure-activity relationships and default assumptions.
27 As more data become available, assessments can replace or modify default assumptions accordingly.
28 These guidelines do not require that all of the kinds of data covered here be available for either
29 assessment or decisionmaking. The level of detail of an assessment is a matter of Agency management
30 policy regarding the applicable decisionmaking framework.

32 **1.3. USE OF DEFAULT ASSUMPTIONS**

33 The National Research Council, in its 1983 report on the science of risk assessment (NRC,
34 1983), recognized that default assumptions are necessarily made in risk assessments where gaps exist in

1 general knowledge or in available data for a particular agent . These default assumptions are inferences
2 based on general scientific knowledge of the phenomena in question and are also matters of policy
3 concerning the appropriate way to bridge uncertainties that concern potential risk to human health (or,
4 more generally, to environmental systems) from the agent under assessment.

5 EPA's 1986 guidelines for cancer risk assessment (EPA, 1986) were developed in response to
6 the 1983 NRC report. The guidelines contained a number of default assumptions. They also
7 encouraged research and analysis that would lead to new risk assessment methods and data and
8 anticipated that these would replace defaults. The 1986 guidelines did not explicitly discuss how to
9 depart from defaults. In practice, the agency's assessments routinely have employed defaults and, until
10 recently, only occasionally departed from them.

11 In its 1994 report on risk assessment, the NRC supported continued use of default assumptions
12 (NRC, 1994). The NRC report thus validated a central premise of the approach to risk assessment
13 that EPA had evolved in preceding years--the making of science policy inferences to bridge gaps in
14 knowledge--while at the same time recommending that EPA develop more systematic and transparent
15 guidelines to inform the public of the default inferences EPA uses in practice. It recommended that the
16 EPA review and update the 1986 guidelines in light of evolving scientific information and experience in
17 practice in applying those guidelines, and that the EPA explain the science and policy considerations
18 underlying current views as to the appropriate defaults and provide general criteria to guide preparers
19 and reviewers of risks assessments in deciding when to depart from a default . Pursuant to this
20 recommendation, the following discussion presents descriptions of the major defaults and their
21 rationales. In addition, it presents general policy guidance on using and departing from defaults in
22 specific risk assessments.

23 24 **1.3.1. Default Assumptions**

25 The 1994 NRC report contains several recommendations regarding flexibility and the use of
26 default options:

- 27 C EPA should continue to regard the use of default options as a reasonable way to deal with
28 uncertainty about underlying mechanisms in selecting methods and models for use in risk
29 assessment.
- 30 C EPA should explicitly identify each use of a default option in risk assessments.
- 31 C EPA should clearly state the scientific and policy basis for each default option.
- 32 C The Agency should consider attempting to give greater formality to its criteria for a
33 departure from default options in order to give greater guidance to the public and to lessen
34 the possibility of ad hoc, undocumented departures from default options that would

1 undercut the scientific credibility of the Agency's risk assessments. At the same time, the
2 Agency should be aware of the undesirability of having its guidelines evolve into inflexible
3 rules.

- 4 C EPA should continue to use the Science Advisory Board and other expert bodies. In
5 particular, the Agency should continue to make the greatest possible use of peer review,
6 workshops, and other devices to ensure broad peer and scientific participation to guarantee
7 that its risk assessment decisions will be based on the best science available through a
8 process that allows full public discussion and peer participation by the scientific community.

9 In the 1983 report (p. 28), NAS defined the use of "inference options" (default options) as a
10 means to bridge inherent uncertainties in risk assessment. These options exist when the assessment
11 encounters either "missing or ambiguous information on a particular substance" or "gaps in current
12 scientific theory." Since there is no instance in which a set of data on an agent or exposure is complete,
13 all risk assessments must use general knowledge and policy guidance to bridge data gaps. Animal
14 toxicity data are used, for example, to substitute for human data because we do not test human beings.
15 The report described the components of risk assessment in terms of questions encountered during
16 analysis for which inferences must be made. The report noted (p. 36) that many components ". . . lack
17 definitive scientific answers, that the degree of scientific consensus concerning the best answer varies
18 (some are more controversial than others), and that the inference options available for each component
19 differ in their degree of conservatism. The choices encountered in risk assessment rest, to various
20 degrees, on a mixture of scientific fact and consensus, on informed scientific judgment, and on policy
21 determinations (the appropriate degree of conservatism). . . ." The report did not note that the mix
22 varies significantly from case to case. For instance, a question that arises in hazard identification is how
23 to use experimental animal data when the routes of exposure differ between animals and humans. A
24 spectrum of inferences could be made, ranging from the most conservative, or risk adverse one that
25 effects in animals from one route may be seen in humans by another route, to an intermediate,
26 conditional inference that such translation of effects will be assumed if the agent is absorbed by humans
27 through the second route, to a nonconservative view that no inference is possible and the agent's effects
28 in animals must be tested by the second route. The choice of an inference, as the report observed,
29 comes from more than scientific thinking alone. While the report focused mainly on the idea of
30 conservatism of public health as a science policy rationale for making the choice, it did not evaluate
31 other considerations. These include such things as the matters of time and resources and whether the
32 analysis is for an important decision required to be made soon or is simply a screening or ranking effort.
33 For a screening analysis, one might make several "worst case" inferences to determine if, even under
34 those conditions, risk is low enough that a problem can be eliminated from further consideration. In the

1 above discussion concerning inferences about route-to-route extrapolation, one might use the most
2 conservative one for screening.

3 These revised guidelines retain the use of default assumptions as recommended in the 1994
4 report. Generally, these defaults remain public health conservative, but in some instances, they have
5 been modified to reflect the evolution of scientific knowledge since 1986.

6 In addition, the guidelines reflect evaluation of experience in practice in applying defaults and
7 departing from them in individual risk assessments conducted under the 1986 guidelines. The
8 application and departure from defaults and the principles to be used in these judgments have been
9 matters of debate among practitioners and reviewers of risk assessments. Some observers believe that
10 in practice EPA risk assessors have been too resistant to considering departures; others question
11 whether proposed departures have been adequately supported. Some cases in which departures have
12 been considered have been generally accepted, while others have been controversial. The guidelines
13 here are intended to be both explicit and more flexible than in the past concerning the basis for making
14 departures from defaults, recognizing that expert judgment and peer review are essential elements of the
15 process.

16 In response to the recommendations of the 1994 report, these guidelines call for identification of
17 the default assumptions used within assessments and for highlighting significant issues about defaults
18 within characterization summaries of component analyses in assessment documents. As to the use of
19 peer review to aid in making judgments about applying or departing from defaults, we agree with the
20 NRC recommendation. The Agency has long made use of workshops, peer review of documents and
21 guidelines, and consultations as well as formal peer review by the Science Advisory Board (SAB). In
22 1994, the Administrator of EPA published formal guidance for peer review of EPA scientific work
23 products that increases the amount of peer review for risk assessments as well as other work, as a
24 response to the NRC report and to SAB recommendations (U.S. EPA, 1994b).

25 The 1994 NRC report recommended that EPA should consider adopting principles or criteria
26 that would give greater formality and transparency to decisions to depart from defaults. The report
27 named several possible criteria for such principles (p. 7): ". . . [P]rotecting the public health, ensuring
28 scientific validity, minimizing serious errors in estimating risks, maximizing incentives for research,
29 creating an orderly and predictable process, and fostering openness and trustworthiness. There might
30 be additional relevant criteria. . . ." The report indicated, however, that the committee members had
31 not reached consensus on a single criterion to address the key issue of how much certainty or proof a
32 risk assessor must have in order to justify departing from a default. Appendix N of the report contains
33 two presentations of alternative views held by some committee members on this issue. One view,
34 known as "plausible conservatism," suggested that departures from defaults should not be made unless

1 new information improves the understanding of a biological process to the point that relevant experts
2 reach consensus that the conservative default assumption concerning that process is no longer plausible.
3 The same criterion was recommended where the underlying scientific mechanism is well understood,
4 but where a default is used to address missing data. In this case, the default should not be replaced
5 with case-specific data unless it is the consensus of relevant experts that the proffered data make the
6 default assumption no longer plausible. Another view, known as the "maximum use of scientific
7 information" approach, acknowledged that the initial choice of defaults should be conservative but
8 argued that conservatism should not be a factor in determining whether to depart from the default in
9 favor of an alternate biological theory or alternate data. According to this view, it should not be
10 necessary to reach expert consensus that the default assumption had been rendered implausible; it
11 should be sufficient that risk assessors find the alternate approach more plausible than the default.

12 The EPA is not adopting a list of formal decision criteria in the sense of a checklist based on
13 either view. It would not be helpful to generate a checklist of uniform criteria for several reasons. First,
14 risk assessments are highly variable in content and purpose. Screening assessments may be purposely
15 "worst case" in their default assumptions to eliminate problems from further investigation. Subsequent
16 risk assessments based on a fuller data set can discard worst-case default assumptions in favor of
17 plausibly conservative assumptions and progressively replace or modify the latter with data. No
18 uniform checklist will fit all cases. Second, a checklist would likely become more a source of rote
19 discussion than of enlightenment about the process.

20 Instead, these guidelines use a combination of principles and process in the application of and
21 departure from default assumptions. The guidelines provide a framework of default assumptions to
22 allow risk assessment to proceed when current scientific theory or available case-specific data do not
23 provide firm answers in a particular case, as the 1983 report outlined. Some of the default assumptions
24 bridge large gaps in fundamental knowledge which will be filled by basic research on the causes of
25 cancer and on other biological processes, rather than by agent-specific testing. Other default
26 assumptions bridge smaller data gaps that can feasibly be filled for a single agent, such as whether a
27 metabolic pathway in test animals is like (default) or unlike that in humans.

28 The decision to use a default, or not, is a choice considering available information on an
29 underlying scientific process and agent-specific data, depending on which kind of default it is.
30 Generally, if a gap in basic understanding exists, or if agent-specific data are missing, the default is used
31 without pause. If data are present, their evaluation may reveal inadequacies that also lead to use of the
32 default. If data support a plausible alternative to the default, but no more strongly than they support the
33 default, both the default and its alternative are carried through the assessment and characterized for the
34 risk manager. If data support an alternative to the default as the more reasonable judgment, the data

1 are used. (This framework of choices is not wholly applicable to screening assessments. As mentioned
2 above, screening assessments may appropriately use "worst case" inferences to determine if, even
3 under those conditions, risk is low enough that a problem can be eliminated from further consideration.)
4

5 Scientific peer review, peer consultative workshops and similar processes are the principal
6 ways determining the strength of thinking and generally accepted views within the scientific community
7 about the application of and departure from defaults and about judgments concerning the plausibility
8 and persuasiveness of data in a particular case. The choices made are explicitly discussed in the
9 assessment, and if a particular choice raises a significant issue, it is highlighted in the risk
10 characterization.

11 The discussion of major defaults in these guidelines together with the explicit discussion of the
12 choice of inferences within the assessment and the processes of peer review and peer consultation will
13 serve the several goals stated in the 1994 report. One is to encourage research, since results of
14 research efforts will be considered. Another is to allow timely decisionmaking, when time is a
15 constraint, by supporting completion of the risk assessment using defaults as needed. Another is to be
16 flexible, using new science as it develops. Finally, the use of public processes of peer consultation and
17 peer review will ensure that discipline of thought is maintained to support trust in assessment results.

18 Experience has shown that the most difficult part of the framework of choices is the judgment of
19 whether a data analysis is both biologically plausible and persuasive as applied to the case at hand.
20 There is no set of rules for making this judgment in all cases. Two criteria that apply in these guidelines
21 are that the underlying scientific principle has been generally accepted within the scientific community
22 and that supportive experiments are available that test the application of the principle to the agent under
23 review. For example, mutagenicity through reactivity with DNA has been generally accepted as a
24 carcinogenic influence for many years. This acceptance, together with evidence of such mutagenicity in
25 experiments on an agent, provides plausible and persuasive support for the inference that mutagenicity
26 is a mode of action for the agent.

27 Judgments about plausibility and persuasiveness of analyses vary according to the scientific
28 nature of the default. An analysis of data may replace a default or modify it. An illustration of the
29 former is development of EPA science policy on the issue of the relevance for humans of male rat
30 kidney neoplasia involving alpha 2u globulin (U.S. EPA, 1991b). The 1991 EPA policy gives guidance
31 on the kind of experimental findings that demonstrate whether the alpha 2u globulin mechanism is
32 present and responsible for carcinogenicity in a particular case. Before this policy guidance was issued,
33 the default assumption was that neoplasia in question was relevant to humans and indicated the potential
34 for hazard to humans. A substantial body of data was developed by public and private research groups

1 as a foundation for the view that the alpha 2u globulin-induced response was not relevant to humans.
2 These studies first addressed the alpha 2u globulin mechanism in the rat and whether this mechanism
3 has a counterpart in the human being, both were large research efforts. The resulting data presented
4 difficulties; some reviewers were concerned that the mechanism in the rat appeared to be understood
5 only in outline, not in detail, and felt that the data were insufficient to show the lack of a counterpart
6 mechanism in humans. It was particularly difficult to support a negative such as the nonexistence of a
7 mechanism in humans because so little is known about what the mechanisms are in humans. Despite
8 these concerns, in its 1991 policy guidance, EPA concluded that the alpha 2u globulin-induced
9 response in rats should be regarded as not relevant to humans (i.e., as not indicating human hazard).

10 One lesson in the development and peer review of this policy is that if the default concerns an
11 inherently complex biological question, large amounts of work will be required to replace the default. A
12 second is that addressing a negative is difficult. A third is that "proof" in the strict sense of having laid all
13 reasonable doubt to rest is not required. Instead, an alternative may displace a default when it is
14 generally accepted in peer review as the most reasonable judgment. The issue of relevance may not
15 always be so difficult. It would be an experimentally easier task, for example, to determine whether
16 carcinogenesis in an animal species is due to a metabolite of the agent in question that is not produced in
17 humans.

18 When scientific processes are understood but case-specific data are missing, defaults can be
19 constructed to be modified by experimental data, even if data do not suffice to replace them entirely.
20 For example, the approaches adopted in these guidelines for scaling dose from experimental animals to
21 humans are constructed to be either modified or replaced as data become available on toxicokinetic
22 parameters for the particular agent being assessed. Similarly, the selection of an approach or
23 approaches for dose response assessment is based on a series of decisions that consider the nature and
24 adequacy of available data in choosing among alternative modeling and default approaches.

25 The 1994 NRC report notes (p. 6) that "[a]s scientific knowledge increases, the science policy
26 choices made by the Agency and Congress should have less impact on regulatory decisionmaking.
27 Better data and increased understanding of biological mechanisms should enable risk assessments that
28 are less dependent on conservative default assumptions and more accurate as predictions of human
29 risk." Undoubtedly, this is the trend as scientific understanding increases. However, some gaps in
30 knowledge and data will doubtless continue to be encountered in assessment of even data-rich cases,
31 and it will remain necessary for risk assessments to continue using defaults within the framework set
32 forth here.

1.3.2. Major Defaults

This discussion covers the major default assumptions commonly employed in a cancer risk assessment and adopted in these guidelines. They are predominantly inferences necessary to use data observed under empirical conditions to estimate events and outcomes under environmental conditions. Several inferential issues arise when effects seen in a subpopulation of humans or animals are used to qualitatively infer potential effects in the population of environmentally exposed humans. Several more inferential issues arise in extrapolating the exposure-effect relationship observed empirically to lower-exposure environmental conditions. The following issues cover the major default areas. Typically, an issue has some subissues; they are introduced here, but are discussed in greater detail in subsequent sections.

- C Is the presence or absence of effects observed in a human population predictive of effects in another exposed human population?
- C Is the presence or absence of effects observed in an animal population predictive of effects in exposed humans?
- C How do metabolic pathways relate across species?
- C How do toxicokinetic processes relate across species?
- C What is the correlation of the observed dose response relationship to the relationship at lower doses?

1.3.2.1. Is the Presence or Absence of Effects Observed in a Human Population Predictive of Effects in Another Exposed Human Population?

When cancer effects in exposed humans are attributed to exposure to an exogenous agent, the default assumption is that such data are predictive of cancer in any other exposed human population. Studies either attributing cancer effects in humans to exogenous agents or reporting no effects are often studies of occupationally exposed humans. By sex, age, and general health, workers are not representative of the general population exposed environmentally to the same agents. In such studies there is no opportunity to observe whether infants and children, males, or females who are under represented in the study, or people whose health is not good, would respond differently. Therefore, it is understood that this assumption could still underestimate the response of certain sensitive human subpopulations, i.e. biologically vulnerable parts of the population may be left out of risk assessments (NRC, 1993a, 1994). Consequently, this is a default that does not err on the side of public health conservatism, as the 1994 NRC report also recognizes.

On the one hand, if effects are seen in a worker population, this may be in fact indicative of heightened effects in sensitive subpopulations. There is not enough knowledge yet to form a basis for any generally applicable, qualitative inference to compensate for this knowledge gap. In these

1 guidelines, this problem is left to case-by-case analysis, to be attended to as future research and
2 information on particular agents allow. When information on a sensitive subpopulation exists, it will be
3 used. The topic of variability is addressed further in the discussion of quantitative default assumptions
4 about dose response relationships below. On the other hand, *when cancer effects are not found in*
5 *an exposed human population, this information by itself is not generally sufficient to conclude*
6 *that the agent poses no carcinogenic hazard to this or other populations of potentially exposed*
7 *humans.* This is because epidemiologic studies usually have low power to detect and attribute
8 responses (section 2.2.1.). This may be particularly true when extrapolating null results from a healthy,
9 worker population to other potentially sensitive exposed humans. Again, the problem is left to case-by-
10 case analysis.

11 12 **1.3.2.2. Is the Presence or Absence of Effects Observed in an Animal Population Predictive** 13 **of Effects in Exposed Humans?**

14 *The default assumption is that positive effects in animal cancer studies indicate that the*
15 *agent under study can have carcinogenic potential in humans.* Thus, if no adequate human data
16 are present, positive effects in animal cancer studies are a basis for assessing the carcinogenic hazard to
17 humans. This assumption is a public health conservative policy, and it is both appropriate and
18 necessary given that we do not test for carcinogenicity in humans. The assumption is supported by the
19 fact that nearly all of the agents known to cause cancer in humans are carcinogenic in animals in tests
20 with adequate protocols (IARC, 1994; Tomatis et al., 1989; Huff, 1994). Moreover, almost one-third
21 of human carcinogens were identified subsequent to animal testing (Huff, 1993). Further support is
22 provided by research on the molecular biology of cancer processes, which has shown that the
23 mechanisms of control of cell growth and differentiation are remarkably homologous among species and
24 highly conserved in evolution. Nevertheless, the same research tools that have enabled recognition of
25 the nature and commonality of cancer processes at the molecular level also have the power to reveal
26 differences and instances in which animal responses are not relevant to humans (Linjinsky, 1993; U.S.
27 EPA, 1991b). Under these guidelines, available mode of action information is studied for its
28 implications in both hazard and dose response assessment and its effect on default assumptions.

29 There may be instances in which the use of an animal model would identify a hazard in animals
30 that is not truly a hazard in humans (e.g., the alpha-2u-globulin association with renal neoplasia in male
31 rats (U.S. EPA, 1991b)). The extent to which animal studies may yield false positive indications for
32 humans is a matter of scientific debate. To demonstrate that a response in animals is not relevant to any
33 human situation, adequate data to assess the relevancy issue must be available.

34 Animal studies are conducted at high doses in order to provide statistical power, the highest
35 dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often

1 arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with
2 compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity
3 of the tested agent. There is little doubt that this may happen in some cases, but skepticism exists
4 among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al., 1993a;
5 Melnick et al., 1993b; Barrett, 1993). In light of this question, *the default assumption is that effects*
6 *seen at the highest dose tested are appropriate for assessment, but it is necessary that the*
7 *experimental conditions be scrutinized.* If adequate data demonstrate that the effects are solely the
8 result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be
9 regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the
10 agent. This is a matter of expert judgment, considering all of the data available about the agent including
11 effects in other toxicity studies, structure-activity relationships, and effects on growth control and
12 differentiation.

13 *When cancer effects are not found in well-conducted animal cancer studies in two or*
14 *more appropriate species and other information does not support the carcinogenic potential of*
15 *the agent, these data provide a basis for concluding that the agent is not likely to possess human*
16 *carcinogenic potential, in the absence of human data to the contrary.* This default assumption
17 about lack of cancer effects is not public health conservative. For instance, the tested animal species
18 may not be predictive of effects in humans; arsenic shows only minimal or no effect in animals, while it
19 is clearly positive in humans. (Other information, such as absence of mutagenic activity or absence of
20 carcinogenic activity among structural analogues, can increase the confidence that negative results in
21 animal studies indicate a lack of human hazard.) Also, it is recognized that animal studies (and
22 epidemiologic studies as well) have very low power to detect cancer effects. Detection of a 10%
23 tumor incidence is generally the limit of power with currently conducted animal studies (with the
24 exception of rare tumors that are virtually markers for a particular agent, e.g., angiosarcoma caused by
25 vinyl chloride).

26 Target organs of carcinogenesis for agents that cause cancer in both animals and humans are
27 most often concordant at one or more sites (Tomatis et al., 1989; Huff, 1994). However, concordance
28 by site is not uniform. *The default assumption is that target organ concordance is not a*
29 *prerequisite for evaluating the implications of animal study results for humans.* This is a public
30 health conservative science policy. The mechanisms of control of cell growth and differentiation are
31 concordant among species, but there are marked differences among species in the way control is
32 managed in various tissues. For example, in humans, mutation of the tumor suppressor gene p53 is one
33 of the most frequently observed genetic changes in tumors. This tumor suppressor is also observed to
34 be operating in some rodent tissues, but other growth control mechanisms predominate in rodents.

1 Thus, an animal response may be due to changes in a control that are relevant to humans, but appear in
2 animals in a different way. However, it is appropriate under these guidelines to consider the influences
3 of route of exposure, metabolism, and, particularly, hormonal modes of action that may either support
4 or not support target organ concordance between animals and humans. When data allow, these
5 influences are considered in deciding whether the default remains appropriate in individual instances
6 (NRC, 1994, p. 121). An exception to the basic default of not assuming site concordance exists in the
7 context of toxicokinetic modeling. Site concordance is inherently assumed when these models are used
8 to estimate delivered dose in humans based on animal data.

9 As in the approach of the National Toxicology Program and the International Agency for
10 Research on Cancer, *the default is to include benign tumors observed in animal studies in the*
11 *assessment of animal tumor incidence if they have the capacity to progress to the malignancies*
12 *with which they are associated.* This treats the benign and malignant tumors as representative of
13 related responses to the test agent, which is scientifically appropriate. This is a science policy decision
14 that is somewhat more conservative of public health than not including benign tumors in the assessment.
15 Nonetheless, in assessing findings from animal studies, a greater proportion of malignancy is weighed
16 more heavily than a response with a greater proportion of benign tumors. Greater frequency of
17 malignancy of a particular tumor type in comparison with other tumor responses observed in an animal
18 study is also a factor to be considered in selecting the response to be used in dose response
19 assessment.

20 *Benign tumors that are not observed to progress to malignancy are assessed on a case-*
21 *by-case basis.* There is a range of possibilities for their overall significance. They may deserve
22 attention because they are serious health problems even though they are not malignant; for instance,
23 benign tumors may be a health risk because of their effect on the function of a target tissue such as the
24 brain. They may be significant indicators of the need for further testing of an agent if they are observed
25 in a short term test protocol, or such an observation may add to the overall weight of evidence if the
26 same agent causes malignancies in a long term study. Knowledge of the mode of action associated with
27 a benign tumor response may aid in the interpretation of other tumor responses associated with the
28 same agent. In other cases, observation of a benign tumor response alone may have no significant
29 health hazard implications when other sources of evidence show no suggestion of carcinogenicity.
30

31 **1.3.2.3. How Do Metabolic Pathways Relate Across Species?**

32 *The default assumption is that there is a similarity of the basic pathways of metabolism*
33 *and the occurrence of metabolites in tissues in regard to the species-to-species extrapolation of*
34 *cancer hazard and risk.* If comparative metabolism studies were to show no similarity between the

1 tested species and humans and a metabolite(s) were the active form, there would be less support for an
2 inference that the animal response(s) relates to humans. In other cases, parameters of metabolism may
3 vary quantitatively between species; this becomes part of deciding on an appropriate human equivalent
4 dose based on animal studies, optimally in the context of a toxicokinetic model.

6 **1.3.2.4. How Do Toxicokinetic Processes Relate Across Species?**

7 A major issue is how to estimate human equivalent doses in extrapolating from animal studies.
8 *As a default for oral exposure, a human equivalent dose is estimated from data on another*
9 *species by an adjustment of animal oral dose by a scaling factor of body weight to the 0.75*
10 *power.* This adjustment factor is used because it represents scaling of metabolic rate across animals of
11 different size. Because the factor adjusts for a parameter that can be improved on and brought into
12 more sophisticated toxicokinetic modeling, when such data become available, the default assumption of
13 0.75 power can be refined or replaced.

14 *For inhalation exposure, a human equivalent dose is estimated by default methodologies*
15 *that provide estimates of lung deposition and of internal dose.* The methodologies can be refined to
16 more sophisticated forms with data on toxicokinetic and metabolic parameters of the specific agent.
17 This default assumption, like the one with oral exposure, is selected in part because it lays a foundation
18 for incorporating better data. The use of information to improve dose estimation from applied, to
19 internal, to delivered dose is encouraged, including use of toxicokinetic modeling instead of any default,
20 where data are available. Health conservatism is not an element in choosing the default.

21 For a route-to-route of exposure extrapolation, *the default assumption is that an agent that*
22 *causes internal tumors by one route of exposure will be carcinogenic by another route if it is*
23 *absorbed by the second route to give an internal dose.* This is a qualitative assumption and is
24 considered to be public health conservative. The rationale is that for internal tumors an internal dose is
25 significant no matter what the route of exposure. Additionally, the metabolism of the agent will be
26 qualitatively the same for an internal dose. The issue of quantitative extrapolation of the dose-response
27 relationship from one route to another is addressed case by case. Quantitative extrapolation is
28 complicated by considerations such as first-pass metabolism, but is approachable with empirical data.
29 Adequate data are necessary to demonstrate that an agent will act differently by one route versus
30 another route of exposure.

- 1 **1.3.2.5. *What Is the Correlation of the Observed Dose Response Relationship to the***
- 2 ***Relationship at Lower Doses?***

1 The overriding preferred approach is to use a biologically based or case-specific model for
2 both the observed range and extrapolation below that range when there are sufficient data. While
3 biologically based models are still under development, it is likely that they will be used more frequently
4 in the future. *The default procedure for the observed range of data, when the preferred approach*
5 *cannot be used, is to use a curve-fitting model.*

6 In the absence of data supporting a biologically based or case-specific model for extrapolation
7 outside of the observed range, the choice of approach is based on the view of mode of action of the
8 agent arrived at in the hazard assessment. *A linear default approach is used when the mode of*
9 *action information is supportive of linearity or, alternatively, is insufficient to support a*
10 *nonlinear mode of action.* The linear approach is used when a view of the mode of action indicates a
11 linear response, for example, when a conclusion is made that an agent directly causes alterations in
12 DNA, a kind of interaction that not only theoretically requires one reaction, but also is likely to be
13 additive to ongoing, spontaneous gene mutation. Other kinds of activity may have linear implications,
14 e.g., linear rate-limiting steps, that support a linear procedure also. The linear approach is to draw a
15 straight line between a point of departure from observed data, generally, as a default, the LED₁₀, and
16 the origin (zero dose, zero response). Other points of departure may be more appropriate for certain
17 data sets; these may be used instead of the LED₁₀. This approach is generally considered to be public
18 health conservative. The LED₁₀ is the lower 95% limit on a dose that is estimated to cause a 10%
19 response. This level is chosen to account (conservatively) for experimental variability. Additionally, it is
20 chosen because it rewards experiments with better designs in regard to number of doses and dose
21 spacing, since these generally will have narrower confidence limits. It is also an appropriate
22 representative of the lower end of the observed range because the limit of detection of studies of tumor
23 effect is about 10%.

24 The linear default is thought to generally produce an upper bound on potential risk at low doses,
25 e.g., a 1/100,000 to 1/1,000,000 risk; the straight line approach gives numerical results about the same
26 as a linearized multistage procedure (Krewski et al., 1984). This upper bound is thought to cover the
27 range of human variability although, in some cases, it may not completely do so (Bois et al., 1995). The
28 EPA considers the linear default to be inherently conservative of public health, without addition of
29 another factor for human variability. In any case, the size of such a factor would be hard to determine
30 since a good empirical basis on which to construct an estimate does not currently exist. The question of
31 what may be the actual variability in human sensitivity is one that the 1994 NRC report discussed as did
32 the 1993 NRC report on pesticides in children and infants. The NRC has recommended research on
33 the question, and the EPA and other agencies have begun such research.

1 *When adequate data on mode of action show that linearity is not the most reasonable*
2 *working judgment and provide sufficient evidence to support a nonlinear mode of action, the*
3 *default changes to a different approach-- a margin of exposure analysis--which assumes that*
4 *nonlinearity is more reasonable.* The departure point is again generally the LED₁₀. A margin of
5 exposure analysis compares the LED₁₀ with the dose associated with the environmental exposure(s) of
6 interest by computing the ratio between the two.

7 The purpose of a margin of exposure analysis is to provide the risk manager with all available
8 information on how much reduction in risk may be associated with reduction in exposure from the point
9 of departure. This is to support the risk manager's decision as to what constitutes an acceptable margin
10 of exposure, given requirements of the statute under which the decision is being made. There are
11 several factors to be considered. (For perspective, keep in mind that a sufficient basis to support this
12 nonlinear procedure often will include data on responses that are precursors to tumor effects. This
13 means that the point of departure may well be from these biological response data rather than tumor
14 incidence data, e.g., hormone levels, mitogenic effects.) One factor to consider is the slope of the dose
15 response curve at the point of departure. A steeper slope implies an apparent greater reduction in risk
16 as exposure decreases. This may support a smaller margin of exposure. Conversely, a shallow slope
17 may support use of a greater margin of exposure. A second factor is the nature of the response used in
18 the assessment--A precursor effect or frank toxicity or tumor response. The latter two may support a
19 greater margin of exposure. A third factor is the nature and extent of human variability in sensitivity to
20 the phenomenon. A fourth factor is the agent's persistence in the body. Greater variability or
21 persistence argue for greater margins of exposure. A fifth factor is human sensitivity to the phenomenon
22 as compared with experimental animals. The size of the margin of exposure that is acceptable would
23 increase or decrease as this factor increases or decreases. If human variability cannot be estimated
24 based on data, it should be considered to be at least 10-fold. Similarly, if comparison of species
25 sensitivities cannot be estimated from available data, humans can be considered to be 10-fold more
26 sensitive. If it is found that humans are less sensitive than animals a factor that is a fraction no smaller
27 than 1/10 may be assumed. The 10-fold factors are moderately conservative, traditional ones used for
28 decades in the assessment of toxicological effects. It should not be assumed that the numerical factors
29 are the sole components for determination of an acceptable margin of exposure. Each case calls for
30 individual judgment. It should be noted that for cancer assessment the margin of exposure analysis
31 begins from a point of departure that is adjusted for toxicokinetic differences between species to give a
32 human equivalent dose. Since the traditional factor for interspecies difference is thought to contain a
33 measure for toxicokinetics as well as sensitivity to effect, the result of beginning with a human equivalent
34 dose is to add some conservatism. The ultimate judgment whether a particular margin of exposure is

1 acceptable is a risk management decision under applicable law, rather than being inherent in the risk
2 assessment. Nonetheless, the risk assessor is responsible for providing scientific rationale to support
3 the the decision.

4 *When the mode of action information indicates that the dose response may be adequately*
5 *described by both a linear and a nonlinear approach, then the default is to present both the*
6 *linear and margin of exposure analyses. An assessment may use both linear and nonlinear*
7 *approaches either for responses that are thought to result from different modes of action or for*
8 *presenting considerations for a response that appears to be very different at high and low doses due to*
9 *influence of separate modes of action. Also, separate approaches may be used for different induced*
10 *responses (i.e. tumor types) from the same agent. These would also be carried forward and presented*
11 *in the assessment.*

12 Figure 1-1 presents the decision points in deciding on a dose response approach or approaches.

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Data to Support:					
Biologically Based or Case-Specific Model	yes	no	no	no	no
Linearity		yes	no	yes	no
Nonlinearity		no	yes	yes	no
Extrapolation Used:	model	default--linear	default--nonlinear	default--linear and nonlinear	default--linear

Figure 1-1. Decisions on dose response assessment approaches for the range of extrapolation.

1 *A default assumption is made that cumulative dose received over a lifetime, expressed as*
2 *a lifetime average daily dose, is an appropriate measure of dose.* This assumes that a high dose of
3 such an agent received over a shorter period of time is equivalent to a low dose spread over a lifetime.
4 This is thought to be a relatively public health conservative assumption and has empirical support
5 (Monro, 1992). An example of effects of short-term, high exposure that results in subsequent cancer
6 development is treatment of cancer patients with certain chemotherapeutic agents. An example of
7 cancer from long-term exposure to an agent of relatively low potency is smoking. Whether the
8 cumulative dose measure is exactly the correct measure in both such instances is not certain and should
9 be assessed case by case and altered when data are available to support another approach. Other
10 measures of dose that consider dose rate and duration are appropriate, e.g., when an agent acts by
11 causing cell toxicity or hormone disruption. In these cases both agent concentration and duration are
12 likely to be important, because such effects are generally observed to be reversible at cessation of
13 short-term exposure.

14 15 **1.4. CHARACTERIZATIONS**

16 The risk characterization process first summarizes findings on hazard, dose response, and
17 exposure characterizations, then develops an integrative analysis of the whole risk case. It ends in a
18 nontechnical Risk Characterization Summary. The Risk Characterization Summary is a presentation for
19 risk managers who may or may not be familiar with the scientific details of cancer assessment. It also
20 provides information for other interested readers. The initial steps in the risk characterization process
21 are to make building blocks in the form of characterizations of the assessments of hazard, dose
22 response, and exposure. The individual assessments and characterizations are then integrated to arrive
23 at risk estimates for exposure scenarios of interest. There are two reasons for individually
24 characterizing the hazard, dose response, and exposure assessments. One is that they are often done
25 by different people than those who do the integrative analyses. The second is that there is very often a
26 lapse of time between the conduct of hazard and dose response analyses and the conduct of exposure
27 assessment and integrative analysis. Thus, it is necessary to capture characterizations of assessments as
28 the assessments are done to avoid the need to go back and reconstruct them. Figure 1-2 shows the
29 relationships of analyses. The figure does not necessarily correspond to the number of documents
30 involved; there may be one or several. "Integrative analysis" is a generic term. At EPA, the documents
31 of various programs that contain integrative analyses have other names such as the "Staff Paper" that
32 discusses air quality criteria issues. In the following sections, the elements of this figure are discussed.

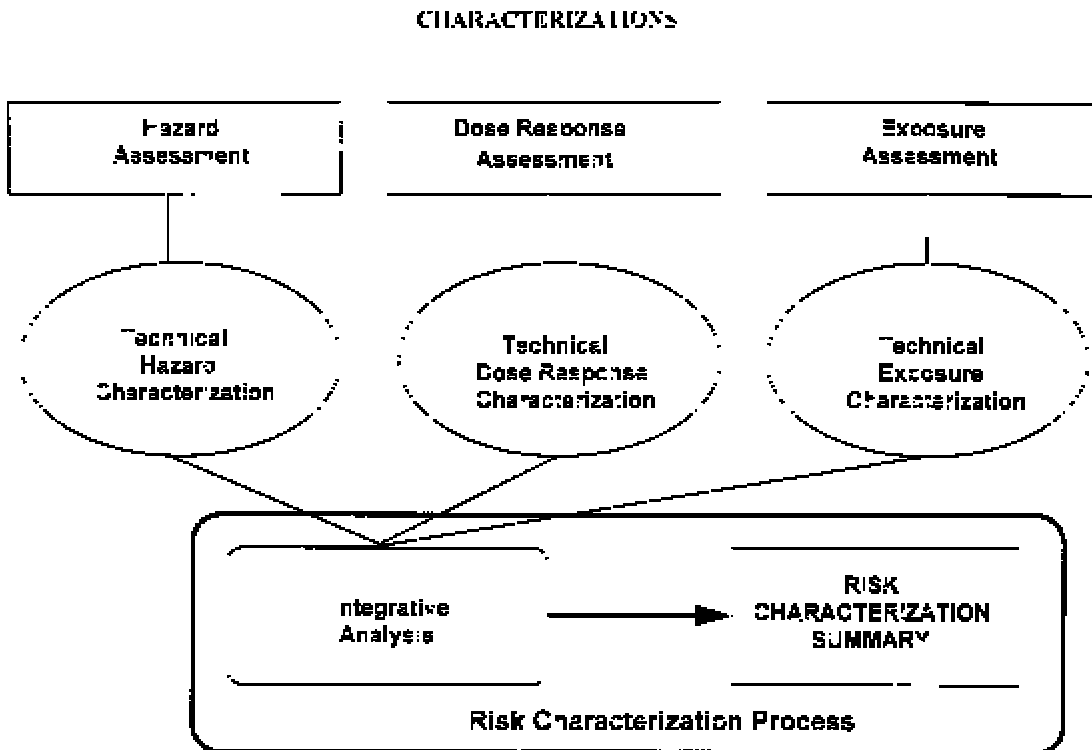


Figure 1-2. Risk Characterization

2. HAZARD ASSESSMENT

2.1. OVERVIEW OF HAZARD ASSESSMENT AND CHARACTERIZATION

2.1.1. Analyses of Data

The purpose of hazard assessment is to review and evaluate data pertinent to two questions: (1) whether an agent may pose a carcinogenic hazard to human beings and (2) under what circumstances an identified hazard may be expressed (NRC, 1994, p. 142). Hazard assessment is composed of analyses of a variety of data that may range from observations of tumor responses to analysis of structure-activity relationships. The purpose of the assessment is not simply to assemble these separate evaluations; its purpose is to construct a total case analysis examining the biological story the data reveal as a whole about carcinogenic effects, mode of action, and implications of these for human hazard and dose response evaluation. Weight of evidence conclusions come from the combined strength and coherence of inferences appropriately drawn from all of the available evidence. To the extent that data permit, hazard assessment addresses the mode of action question as both an initial step in considering appropriate approaches to dose response assessment and as a part of identifying human hazard potential.

The topics in this section include analysis of tumor data, both animal and human, and analysis of other key information about properties and effects that relate to carcinogenic potential. The section addresses how information can be used to evaluate potential modes of action. It also provides guidance on performing a weight of evidence evaluation.

2.1.2. Cross-Cutting Topics for Data Integration

Two topics are included in the analysis of each kind of available data: first, gathering information from available data about the conditions of expression of hazard and second, gathering perspectives on the agent's potential mode of action.

2.1.2.1. *Conditions of Expression*

Information on the significance of the route of exposure may be available from human or animal studies on the agent itself or on structural analogues. This information may be found in studies of the agent or analogue for toxicological endpoints other than cancer under acute or subchronic or chronic exposure regimens. Studies of metabolism or toxicokinetics of the agent similarly may provide pertinent data.

Each kind of data is also examined for information on conditions that affect expression of carcinogenic effect such as presence or absence of metabolic pathways. If carcinogenicity is secondary

1 to another toxic effect, the physiological or tissue changes that mark the other toxicity are examined.
2 Comparison of metabolic processes and toxicity processes in humans and animals also bears on the
3 relevance of animal responses to human hazard. Included in the examination are the questions of the
4 potential range of human variability and whether any special sensitivity may occur because of age, sex,
5 preexisting disease, or other condition.
6

7 **2.1.2.2. *Mode of Action***

8 Information on an agent's potential mode(s) of action is important in considering the relevance
9 of animal effects to assessment of human hazard. It also plays an important role in selecting dose
10 response approach(es), which are generally either biologically based models or case-specific models
11 incorporating mode of action data or default procedures based on more limited data that support
12 inferences about the likely shape of the dose response curve.

13 Each kind of data may provide some insight about mode of action and insights are gathered
14 from each to be considered together as discussed in section 2.4. In Appendix C, is a background
15 discussion of some of the development of views about carcinogenic processes.
16

17 **2.1.3. Presentation of Results**

18 Presentation of the results of hazard assessment follows Agency guidance as discussed in
19 section 2.7. The results are presented in a technical hazard characterization that serves as a support to
20 later risk characterization. It includes:

- 21 ! a summary of the evaluations of hazard data,
- 22 ! the rationales for its conclusions, and
- 23 ! an explanation of the significant strengths or limitations of the conclusions.

24 Another presentation feature is the use of a weight of evidence narrative that includes both a
25 conclusion about the weight of evidence of carcinogenic potential and a summary of the data on which
26 the conclusion rests. This narrative is a brief summary that replaces the alphanumerical classification
27 system used in EPA's previous guidelines.
28

29 **2.2. ANALYSIS OF TUMOR DATA**

30 Evidence of carcinogenicity comes from finding tumor increases in humans or laboratory
31 animals exposed to a given agent, or from finding tumors following exposure to structural analogues to
32 the compound under review. The significance of observed or anticipated tumor effects is evaluated in
33 reference to all of the other key data on the agent. This section contains guidance for analyzing human
34 and animal studies to decide whether there is an association between exposure to an agent or a

1 structural analogue and occurrence of tumors. Note that the use of the term "tumor" here is generic,
2 meaning malignant neoplasms or a combination of malignant and corresponding benign neoplasms.

3 Observation of only benign neoplasias may or may not have significance. Benign tumors that
4 are not observed to progress to malignancy are assessed on a case-by-case basis. There is a range of
5 possibilities for their overall significance. They may deserve attention because they are serious health
6 problems even though they are not malignant; for instance, benign tumors may be a health risk because
7 of their effect on the function of a target tissue such as the brain. They may be significant indicators of
8 the need for further testing of an agent if they are observed in a short term test protocol, or such an
9 observation may add to the overall weight of evidence if the same agent causes malignancies in a long
10 term study. Knowledge of the mode of action associated with a benign tumor response may aid in the
11 interpretation of other tumor responses associated with the same agent. In other cases, observation of
12 a benign tumor response alone may have no significant health hazard implications when other sources of
13 evidence show no suggestion of carcinogenicity.

14 15 **2.2.1. Human Data**

16 Human data may come from epidemiologic studies or case reports. Epidemiology is the study
17 of the distributions and causes of disease within human populations. The goals of cancer epidemiology
18 are to identify differences in cancer risk between different groups in a population or between different
19 populations, and then to determine the extent to which these differences in risk can be attributed
20 causally to specific exposures to exogenous or endogenous factors. Epidemiologic data are extremely
21 useful in risk assessment because they provide direct evidence that a substance produces cancer in
22 humans, thereby avoiding the problem of species to species inference. Thus, when available human
23 data are extensive and of good quality, they are generally preferable over animal data and should be
24 given greater weight in hazard characterization and dose response assessment, although both are
25 utilized.

26 Null results from a single epidemiologic study cannot prove the absence of carcinogenic
27 effects because they can arise either from being truly negative or from inadequate statistical power,
28 inadequate design, imprecise estimates, or confounding factors. However, null results from a well-
29 designed and well-conducted epidemiologic study that contains usable exposure data can help to define
30 upper limits for the estimated dose of concern for human exposure if the overall weight of the evidence
31 indicates that the agent is potentially carcinogenic in humans.

32 Epidemiology can also complement experimental evidence in corroborating or clarifying the
33 carcinogenic potential of the agent in question. For example, observations from epidemiologic studies
34 that elevated cancer incidence occurs at sites corresponding to those at which laboratory animals

1 experience increased tumor incidence can strengthen the weight of evidence of human carcinogenicity.
2 On the other hand, strong nonpositive epidemiologic data alone or in conjunction with compelling
3 mechanistic information can lend support to a conclusion that animal responses may not be predictive of
4 a human response. Furthermore, the advent of biochemical or molecular epidemiology may help
5 improve understanding of the mechanisms of human carcinogenesis.

6 7 **2.2.1.1. *Types of Studies***

8 The major types of cancer epidemiologic studies are analytical epidemiologic studies and
9 descriptive or correlation epidemiologic studies. Each study type has well-known strengths and
10 weaknesses that affect interpretation of study results as summarized below (Kelsey et al., 1986;
11 Lilienfeld and Lilienfeld, 1979; Matusner and Kramer, 1985; Rothman, 1986).

12 Analytical epidemiologic studies are most useful for identifying an association between human
13 exposure and adverse health effects. Analytical study designs include case-control studies and cohort
14 studies. In case-control studies, groups of individuals with (cases) and without (controls) a particular
15 disease are identified and compared to determine differences in exposure. In cohort studies, a group of
16 "exposed" and "nonexposed" individuals are identified and studied over time to determine differences in
17 disease occurrence. Cohort studies can either be performed prospectively or retrospectively from
18 historical records.

19 Descriptive or correlation epidemiologic studies (sometimes called ecological studies) examine
20 differences in disease rates among populations in relation to age, gender, race, and differences in
21 temporal or environmental conditions. In general, these studies can only identify patterns or trends in
22 disease occurrence over time or in different geographical locations but cannot ascertain the causal agent
23 or degree of exposure. These studies, however, are often very useful for generating hypotheses for
24 further research.

25 Biochemical or molecular epidemiologic studies are studies in which laboratory methods are
26 incorporated in analytical investigations. The application of techniques for measuring cellular and
27 molecular alterations due to exposure to specific environmental agents may allow conclusions to be
28 drawn about the mechanisms of carcinogenesis. The use of biological biomarkers in epidemiology may
29 improve assessment of exposure and internal dose.

30 Case reports describe a particular effect in an individual or group of individuals who were
31 exposed to a substance. These reports are often anecdotal or highly selected in nature and are of
32 limited use for hazard assessment. However, reports of cancer cases can identify associations
33 particularly when there are unique features such as an association with an uncommon tumor (e.g., vinyl
34 chloride and angiosarcoma or diethylstilbestrol and clear-cell carcinoma of the vagina).

1 **2.2.1.2. *Criteria for Assessing Adequacy of Epidemiologic Studies***

2 Criteria for assessing the adequacy of epidemiologic studies are well recognized.
3 Characteristics that are desirable in these studies include (1) clear articulation of study objectives or
4 hypothesis, (2) proper selection and characterization of the exposed and control groups, (3) adequate
5 characterization of exposure, (4) sufficient length of follow-up for disease occurrence, (5) valid
6 ascertainment of the causes of cancer morbidity and mortality, (6) proper consideration of bias and
7 confounding factors, (7) adequate sample size to detect an effect, (8) clear, well-documented, and
8 appropriate methodology for data collection and analysis, (9) adequate response rate and methodology
9 for handling missing data, and (10) complete and clear documentation of results. Ideally, these
10 conditions should be satisfied, where appropriate, but rarely can a study meet all of them. No single
11 criterion determines the overall adequacy of a study. The following discussions highlight the major
12 factors included in an analysis of epidemiologic studies.

13
14 ***Population Issues***

15 The ideal comparison would be between two populations that differ only in exposure to the
16 agent in question. Because this is seldom the case, it is important to identify sources of bias inherent in
17 a study's design or data collection methods. Bias can arise from several sources, including
18 noncomparability between populations of factors such as general health (McMichael, 1976), diet,
19 lifestyle, or geographic location; differences in the way case and control individuals recall past events;
20 differences in data collection that result in unequal ascertainment of health effects in the populations; and
21 unequal follow-up of individuals. Both acceptance of studies for assessment and judgment of their
22 strengths or weaknesses depend on identifying their sources of bias and the effects on study results.

23
24 ***Exposure Issues***

25 For epidemiologic data to be useful in determining whether there is an association between
26 health effects and exposure to an agent, there must be adequate characterization of exposure
27 information. In general, greater weight should be given to studies with more precise and specific
28 exposure estimates.

29 Questions to address about exposure are: What can one reliably conclude about the level,
30 duration, route, and frequency of exposure of individuals in one population as compared with another?
31 How sensitive are study results to uncertainties in these parameters?

32 Actual exposure measurements are not available for many retrospective studies. Therefore,
33 surrogates are often used to reconstruct exposure parameters when historical measurements are not
34 available. These may involve attributing exposures to job classifications in a workplace or to broader

1 occupational or geographic groupings. Use of surrogates carries a potential for misclassification in that
2 individuals may be placed in the incorrect exposure group. Misclassification generally leads to reduced
3 ability of a study to detect differences between study and referent populations.

4 When either current or historical monitoring data are available, the exposure evaluation includes
5 consideration of the error bounds of the monitoring and analytic methods and whether the data are from
6 routine or accidental exposures. The potentials for misclassification and measurement errors are
7 amenable to both qualitative and quantitative analysis. These are essential analyses for judging a study's
8 results because exposure estimation is the most critical part of a retrospective study.

9 Biological markers potentially offer excellent measures of exposure (Hulka and Margolin, 1992;
10 Peto and Darby, 1994). Validated markers of exposure such as alkylated hemoglobin from exposure
11 to ethylene oxide (van Sittert et al., 1985) or urinary arsenic (Enterline et al., 1987) can greatly improve
12 estimates of dose. Markers closely identified with effects promise to greatly increase the ability of
13 studies to distinguish real effects from bias at low levels of relative risk between populations (Taylor et
14 al., 1994; Biggs et al., 1993) and to resolve problems of confounding risk factors.

15 16 *Confounding Factors*

17 Because epidemiologic studies are mostly observational, it is not possible to guarantee the
18 control of confounding variables, which may affect the study outcome. A confounding variable is a risk
19 factor, independent of the putative agent, that is distributed unequally among the exposed and
20 unexposed populations (e.g., smoking habits, lifestyle). Adjustment for possible confounding factors
21 can occur either in the design of the study (e.g., matching on critical factors) or in the statistical analysis
22 of the results. The influence of a potential confounding factor is limited by the effect of the exposure of
23 interest. For example, a twofold effect of an exposure requires that the confounder effect be at least as
24 big. The latter may not be possible due to the presentation of the data or because needed information
25 was not collected during the study. In this case, indirect comparisons may be possible. For example,
26 in the absence of data on smoking status among individuals in the study population, an examination of
27 the possible contribution of cigarette smoking to increased lung cancer risk may be based on
28 information from other sources such as the American Cancer Society's longitudinal studies (Hammand,
29 1966; Garfinkel and Silverberg, 1991). The effectiveness of adjustments contributes to the ability to
30 draw inferences from a study.

31 Different studies involving exposure to an agent may have different confounding factors. If
32 consistent increases in cancer risk are observed across a collection of studies with different confounding
33 factors, the inference that the agent under investigation was the etiologic factor is strengthened, even

1 though complete adjustment for confounding factors cannot be made and no single study supports a
2 strong inference.

3 It also may be the case that the agent of interest is a risk factor in conjunction with another
4 agent. This relationship may be revealed in a collection of studies such as in the case of asbestos
5 exposure and smoking.

6 *Sensitivity*

8 Sensitivity, or the ability of a study to detect real effects, is a function of several factors.
9 Greater size of the study population(s) (sample size) increases sensitivity, as does greater exposure
10 (levels and duration) of the population members. Because of the often long latency period in cancer
11 development, sensitivity also depends on whether adequate time has elapsed since exposure began for
12 effects to occur. A unique feature that can be ascribed to the effects of a particular agent (such as a
13 tumor type that is seen only rarely in the absence of the agent) can increase sensitivity by permitting
14 separation of bias and confounding factors from real effects. Similarly, a biomarker particular to the
15 agent can permit these distinctions. Statistical reanalyses of data, particularly an examination of different
16 exposure indices, can give insight on potential exposure-response relationships. These are all factors to
17 explore in statistical analysis of the data.

18 *Statistical Considerations*

20 The analysis applies appropriate statistical methods to ascertain whether or not there is any
21 significant association between exposure and effects. A description of the method or methods should
22 include the reasons for their selection. Statistical analyses of the potential effects of bias or confounding
23 factors are part of addressing the significance of an association, or lack of one, and whether a study is
24 able to detect any effect.

25 The analysis augments examination of the results for the whole population with exploration of
26 the results for groups with comparatively greater exposure or time since first exposure. This may
27 support identifying an association or establishing a dose response trend. When studies show no
28 association, such exploration may apply to determining an upper limit on potential human risk for
29 consideration alongside results of animal tumor effects studies.

30 *Combining Statistical Evidence Across Studies*

32 Meta-analysis is a means of comparing and synthesizing studies dealing with similar health
33 effects and risk factors. It is intended to introduce consistency and comprehensiveness into what
34 otherwise might be a more subjective review of the literature. When utilized appropriately, meta-

1 analysis can enhance understanding of associations between sources and their effects that may not be
2 apparent from examination of epidemiologic studies individually. Whether to conduct a meta-analysis
3 depends on several issues. These include the importance of formally examining sources of
4 heterogeneity, the refinement of the estimate of the magnitude of an effect, and the need for information
5 beyond that provided by individual studies or a narrative review. Meta-analysis may not be useful in
6 some circumstances. These include when the relationship between exposure and disease is obvious
7 without a more formal analysis, when there are only a few studies of the key health outcomes, when
8 there is insufficient information from available studies related to disease, risk estimate, or exposure
9 classification, or when there are substantial confounding or other biases that cannot be adjusted for in
10 the analysis (Blair et al., 1995; Greenland, 1987; Peto, 1992).

11 12 **2.2.1.3. *Criteria for Causality***

13 A causal interpretation is enhanced for studies to the extent that they meet the criteria described
14 below. None of the criteria is conclusive by itself, and the only criterion that is essential is the temporal
15 relationship. These criteria are modeled after those developed by Bradford Hill in the examination of
16 cigarette smoking and lung cancer (Rothman, 1986) and they need to be interpreted in the light of all
17 other information on the agent being assessed.

- 18 ! **Temporal relationship**: The development of cancers require certain latency periods, and
19 while latency periods vary, existence of such periods is generally acknowledged. Thus, the
20 disease has to occur within a biologically reasonable time after initial exposure. This feature
21 must be present if causality is to be considered.
- 22 ! **Consistency**: Associations occur in several independent studies of a similar exposure in
23 different populations, or associations occur consistently for different subgroups in the same
24 study. This feature usually constitutes strong evidence for a causal interpretation when the
25 same bias or confounding is not also duplicated across studies.
- 26 ! **Magnitude of the association**: A causal relationship is more credible when the risk estimate
27 is large and precise (narrow confidence intervals).
- 28 ! **Biological gradient**: The risk ratio (i.e., the ratio of the risk of disease or death among the
29 exposed to the risk of the unexposed) increases with increasing exposure or dose. A strong
30 dose response relationship across several categories of exposure, latency, and duration is
31 supportive for causality given that confounding is unlikely to be correlated with exposure.
32 The absence of a dose response relationship, however, is not by itself evidence against a
33 causal relationship.

- 1 ! Specificity of the association: The likelihood of a causal interpretation is increased if an
2 exposure produces a specific effect (one or more tumor types also found in other studies) or
3 if a given effect has a unique exposure.
- 4 ! Biological plausibility: The association makes sense in terms of biological knowledge.
5 Information is considered from animal toxicology, toxicokinetics, structure-activity
6 relationship analysis, and short-term studies of the agent's influence on events in the
7 carcinogenic process considered.
- 8 ! Coherence: The cause-and-effect interpretation is in logical agreement with what is known
9 about the natural history and biology of the disease, i.e., the entire body of knowledge about
10 the agent.

11

12 **2.2.1.4. *Assessment of Evidence of Carcinogenicity from Human Data***

13 In the evaluation of carcinogenicity based on epidemiologic studies, it is necessary to critically
14 evaluate each study for the confidence in findings and conclusions as discussed under section 2.2.1.2.
15 All studies that are properly conducted, whether yielding positive or null results, or even suggesting
16 protective carcinogenic effects, should be considered in assessing the totality of the human evidence.
17 Although a single study may be indicative of a cause-effect relationship, confidence in inferring a causal
18 relationship is increased when several independent studies are concordant in showing the association,
19 when the association is strong, and when other criteria for causality are also met. Conclusions about
20 the overall evidence for carcinogenicity from available studies in humans should be summarized along
21 with a discussion of strengths or limitations of the conclusions.

22

23 **2.2.2. *Animal Data***

24 Various kinds of whole animal test systems are currently used or are under development for
25 evaluating potential carcinogenicity. Cancer studies involving chronic exposure for most of the life span
26 of an animal are generally accepted for evaluation of tumor effects (Tomatis et al., 1989; Rall, 1991;
27 Allen et al., 1988; but see Ames and Gold, 1990). Other studies of special design are useful for
28 observing formation of preneoplastic lesions or tumors or investigating specific modes of action.

29

30 **2.2.2.1. *Long-Term Carcinogenicity Studies***

31 The objective of long-term carcinogenesis bioassays is to determine the carcinogenic potential
32 and dose response relationships of the test agent. Long-term rodent studies are designed to examine
33 the production of tumors as well as preneoplastic lesions and other indications of chronic toxicity that
34 may provide evidence of treatment-related effects and insights into the way the test agent produces

1 tumors. Current standardized long-term studies in rodents test at least 50 animals per sex per dose
2 group in each of three treatment groups and in a concurrent control group, usually for 18 to 24 months,
3 depending on the rodent species tested (OECD, 1981; U.S. EPA, 1983a; U.S. EPA, 1983b; U.S.
4 EPA, 1983c). The high dose in long-term studies is generally selected to provide the maximum ability
5 to detect treatment-related carcinogenic effects while not compromising the outcome of the study due
6 to excessive toxicity or inducing inappropriate toxicokinetics (e.g., overwhelming detoxification or
7 absorption mechanisms). The purpose of two or more lower doses is to provide some information on
8 the shape of the dose response curve. Similar protocols have been and continue to be used by many
9 laboratories worldwide.

10 All available studies of tumor effects in whole animals are considered, at least preliminarily. The
11 analysis discards studies judged to be wholly inadequate in protocol, conduct, or results. Criteria for
12 the technical adequacy of animal carcinogenicity studies have been published and should be used as
13 guidance to judge the acceptability of individual studies (NTP, 1984; OSTP, 1985). Care is taken to
14 include studies that provide some evidence bearing on carcinogenicity or help interpret effects noted in
15 other studies even if they have some limitations of protocol or conduct. Such limited, but not wholly
16 inadequate, studies can contribute as their deficiencies permit. The findings of long-term rodent
17 bioassays are always interpreted in conjunction with results of prechronic studies along with
18 toxicokinetic and metabolism studies and other pertinent information, if available. Evaluation of tumor
19 effects requires consideration of both biological and statistical significance of the findings (Haseman,
20 1984, 1985, 1990, 1995). The following sections highlight the major issues in the evaluation of long-
21 term carcinogenicity studies.

22 23 *Dosing Issues*

24 In order to obtain the most relevant information from a long-term carcinogenicity study, it is
25 important to require maximization of exposure to the test material. At the same time, there is a need for
26 caution in using excessive high dose levels that would confound the interpretation of study results to
27 humans. The high dose is conventionally defined as a dose that produces some toxic effects without
28 either unduly affecting mortality from effects other than cancer or producing significant adverse effects
29 on the nutrition and health of the test animals (OECD, 1981; NRC, 1993b). It should be noted that
30 practical upper limits have been established to avoid the use of excessive high doses in long-term
31 carcinogenicity studies (e.g., 5% of the test substance in the feed for dietary studies [OECD, 1981]).

32 Evaluating the appropriateness of the high dose in carcinogenicity studies is based on scientific
33 judgment using all available relevant information. In general, if the test agent does not appear to cause
34 any specific target organ toxicity or perturbation of physiological function, an adequate high dose would

1 be a dose that causes no more than 10% reduction of body weight gain over the life span of the
2 animals. On the other hand, significant increases in mortality from effects other than cancer is accepted
3 as clear evidence of frank toxicity, which indicates that an adequate high dose may have been
4 exceeded. Other signs of treatment-related toxicity that may indicate that an adequate high dose has
5 been exceeded include the following: (a) reduction of body weight gain of 10% or greater, (b)
6 significant increases in abnormal behavioral and clinical signs, (c) significant changes in hematology or
7 clinical chemistry, (d) saturation of absorption and detoxification mechanisms, or (e) marked changes in
8 organ weight, morphology, and histopathology.

9 For dietary studies, weight gain reductions should be evaluated as to whether there is a
10 palatability problem or an issue with food efficiency; certainly, the latter is a toxic manifestation. In the
11 case of inhalation studies with respirable particles, evidence of impairment of normal clearance of
12 particles from the lung should be considered along with other signs of toxicity to the respiratory airways
13 to determine whether the high exposure concentration has been appropriately selected. For dermal
14 studies, evidence of skin irritation may indicate that an adequate high dose has been reached.

15 Interpretation of carcinogenicity study results is profoundly affected by exposure conditions,
16 especially by inappropriate dose selection. This is particularly important in studies that are nonpositive
17 for carcinogenicity, since failure to reach a sufficient dose reduces the sensitivity of a study. A lack of
18 tumorigenic responses at exposure levels that cause significant impairment of animal survival may also
19 not be acceptable as negative findings because of the reduced sensitivity of the study. On the other
20 hand, overt toxicity or inappropriate toxicokinetics due to excessive high doses may result in tumor
21 effects that are secondary to the toxicity rather than directly attributable to the agent.

22 There are several possible outcomes regarding the study interpretation of the significance and
23 relevance of tumorigenic effects associated with exposure or dose levels below, at, or above an
24 adequate high dose. General guidance is given here that should not be taken as prescriptive; for each
25 case, the information at hand is evaluated and a rationale should be given for the position taken.

26 ! Adequate high dose: If an adequate high dose has been utilized, tumor effects are judged
27 positive or negative depending on the presence or absence of significant tumor incidence
28 increases, respectively.

29 ! Excessive high dose: If toxicity or mortality is excessive at the high dose, interpretation
30 depends on the finding of tumors or not.

31 (a) Studies that show tumor effects only at excessive doses may be compromised and
32 may or may not carry weight, depending on the interpretation in the context of other
33 study results and other lines of evidence. Results of such studies, however, are
34 generally not considered suitable for risk extrapolation.

1 (b) Studies that show tumors at lower doses, even though the high dose is excessive and
2 may be discounted, should be evaluated on their own merits.

3 (c) If a study does not show an increase in tumor incidence at a toxic high dose and
4 appropriately spaced lower doses are used without such toxicity or tumors, the study
5 is generally judged as negative for carcinogenicity.

6 ! Inadequate high dose: Studies of inadequate sensitivity where an adequate high dose has
7 not been reached may be used to bound the dose range where carcinogenic effects might be
8 expected.

10 *Statistical Considerations*

11 The main aim of statistical evaluation is to determine whether exposure to the test agent is
12 associated with an increase of tumor development. Statistical analysis of a long-term study should be
13 performed for each tumor type separately. The incidence of benign and malignant lesions of the same
14 cell type, usually within a single tissue or organ, are considered separately and are combined when
15 scientifically defensible (McConnell et al., 1986).

16 Trend tests and pairwise comparison tests are the recommended tests for determining whether
17 chance, rather than a treatment-related effect, is a plausible explanation for an apparent increase in
18 tumor incidence. A trend test such as the Cochran-Armitage test (Snedecor and Cochran, 1967) asks
19 whether the results in all dose groups together increase as dose increases. A pairwise comparison test
20 such as the Fisher exact test (Fisher, 1932) asks whether an incidence in one dose group is increased
21 over the control group. By convention, for both tests a statistically significant comparison is one for
22 which $p < 0.05$ that the increased incidence is due to chance. Significance in either kind of test is
23 sufficient to reject the hypothesis that chance accounts for the result. A statistically significant response
24 may or may not be biologically significant and vice versa. The selection of a significance level is a policy
25 choice based on a trade-off between the risks of false positives and false negatives. A significance level
26 of greater or less than 5% is examined to see if it confirms other scientific information. When the
27 assessment departs from a simple 5% level, this should be highlighted in the risk characterization. A
28 two-tailed test or a one-tailed test can be used. In either case a rationale is provided.

29 Considerations of multiple comparisons should also be taken into account. Haseman (1983)
30 analyzes typical animal bioassays testing both sexes of two species and concludes that, because of
31 multiple comparisons, a single tumor increase for a species-sex-site combination that is statistically
32 significant at the 1% level for common tumors or 5% for rare tumors corresponds to a 7-8%
33 significance level for the study as a whole. Therefore, animal bioassays presenting only one significant
34 result that falls short of the 1% level for a common tumor may be treated with caution.

Concurrent and Historical Controls

1
2 The standard for determining statistical significance of tumor incidence comes from a
3 comparison of tumors in dosed animals as compared with concurrent control animals. Additional
4 insights about both statistical and biological significance can come from an examination of historical
5 control data (Tarone, 1982; Haseman, 1995). Historical control data can add to the analysis
6 particularly by enabling identification of uncommon tumor types or high spontaneous incidence of a
7 tumor in a given animal strain. Identification of common or uncommon situations prompts further
8 thought about the meaning of the response in the current study in context with other observations in
9 animal studies and with other evidence about the carcinogenic potential of the agent. These other
10 sources of information may reinforce or weaken the significance given to the response in the hazard
11 assessment. Caution should be exercised in simply looking at the ranges of historical responses
12 because the range ignores differences in survival of animals among studies and is related to the number
13 of studies in the database.

14 In analyzing results for uncommon tumors in a treated group that are not statistically significant
15 in comparison to concurrent controls, the analyst can use the experience of historical controls to
16 conclude that the result is in fact unlikely to be due to chance. In analyzing results for common tumors,
17 a different set of considerations comes into play. Generally speaking, statistically significant increases in
18 tumors should not be discounted simply because incidence rates in the treated groups are within the
19 range of historical controls or because incidence rates in the concurrent controls are somewhat lower
20 than average. Random assignment of animals to groups and proper statistical procedures provide
21 assurance that statistically significant results are unlikely to be due to chance alone. However, caution
22 should be used in interpreting results that are barely statistically significant or in which incidence rates in
23 concurrent controls are unusually low in comparison with historical controls.

24 In cases where there may be reason to discount the biological relevance to humans of increases
25 in common animal tumors, such considerations should be weighed on their own merits and clearly
26 distinguished from statistical concerns.

27 When historical control data are used, the discussion needs to address several issues that affect
28 comparability of historical and concurrent control data. Among these issues are the following: genetic
29 drift in the laboratory strains; differences in pathology examination at different times and in different
30 laboratories (e.g., in criteria for evaluating lesions; variations in the techniques for preparation or reading
31 of tissue samples among laboratories); comparability of animals from different suppliers. The most
32 relevant historical data come from the same laboratory and same supplier, gathered within 2 or 3 years
33 one way or the other of the study under review; other data should be used only with extreme caution.
34

Assessment of Evidence of Carcinogenicity from Long-Term Animal Studies

In general, observation of tumor effects under different circumstances lends support to the significance of the findings for animal carcinogenicity. Significance is a function of the number of factors present, and for a factor such as malignancy, the severity of the observed pathology. The following observations add significance to the tumor findings:

- ! uncommon tumor types
- ! tumors at multiple sites
- ! tumors by more than one route of administration
- ! tumors in multiple species, strains, or both sexes
- ! progression of lesions from preneoplastic to benign to malignant
- ! reduced latency of neoplastic lesions
- ! metastases
- ! unusual magnitude of tumor response
- ! proportion of malignant tumors
- ! dose-related increases

These guidelines adopt the science policy position that tumor findings in animals indicate that an agent may produce such effects in humans. Moreover, the absence of tumor findings in well-conducted, long-term animal studies in at least two species provides reasonable assurance that an agent may not be a carcinogenic concern for humans. Each of these is a default assumption that may be adopted, when appropriate, after evaluation of tumor data and other key evidence.

Site concordance of tumor effects between animals and humans is an issue to be considered in each case. Thus far, there is evidence that growth control mechanisms at the level of the cell are homologous among mammals, but there is no evidence that these mechanisms are site concordant. Moreover, agents observed to produce tumors in both humans and animals have produced tumors either at the same (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not assumed a priori. On the other hand, certain processes with consequences for particular tissue sites (e.g., disruption of thyroid function) may lead to an anticipation of site concordance.

2.2.2.2. Other Studies

Various intermediate-term studies often use protocols that screen for carcinogenic or preneoplastic effects, sometimes in a single tissue. Some involve the development of various proliferative lesions, like foci of alteration in the liver (Goldsworthy et al., 1986). Others use tumor endpoints, like the induction of lung adenomas in the sensitive strain A mouse (Maronpot et al., 1986)

1 or tumor induction in initiation-promotion studies using various organs such as the bladder, intestine,
2 liver, lung, mammary gland, and thyroid (Ito et al., 1992). In these tests, the selected tissue is, in a
3 sense, the test system rather than the whole animal. Important information concerning the steps in the
4 carcinogenic process and mode of action can be obtained from "start/stop" experiments. In these
5 protocols, an agent is given for a period of time to induce particular lesions or effects, then stopped to
6 evaluate the progression or reversibility of processes (Todd, 1986; Marsman and Popp, 1994).

7 Assays in genetically engineered rodents may provide insight into the chemical and gene
8 interactions involved in carcinogenesis (Tennant et al., 1995a). These mechanistically based
9 approaches involve activated oncogenes that are introduced (transgenic) or tumor suppressor genes
10 that are deleted (knocked-out). If appropriate genes are selected, not only may these systems provide
11 information on mechanisms, but the rodents typically show tumor development earlier than the standard
12 bioassay. Transgenic mutagenesis assays also represent a mechanistic approach for assessing the
13 mutagenic properties of agents as well as developing quantitative linkages between exposure, internal
14 dose, and mutation related to tumor induction (Morrison and Ashby, 1994; Sisk et al., 1994; Hayward
15 et al., 1995). These systems use a stable genomic integration of a lambda shuttle vector that carries a
16 *lacI* target gene and a *lacZ* reporter gene.

17 The support that these studies give to a determination of carcinogenicity rests on their
18 contribution to the consistency of other evidence about an agent. For instance, benzoyl peroxide has
19 promoter activity on the skin, but the overall evidence may be less supportive (Kraus et al., 1995).
20 These studies also may contribute information about mode of action. One needs to recognize the
21 limitations of these experimental protocols such as short duration, limited histology, lack of complete
22 development of tumors, or experimental manipulation of the carcinogenic process that may limit their
23 contribution to the overall assessment. Generally, their results are appropriate as aids in the assessment
24 for interpreting other toxicological evidence (e.g., rodent chronic bioassays), especially regarding
25 potential modes of action. With sufficient validation, these studies may partially or wholly replace
26 chronic bioassays in the future (Tennant et al., 1995).

27 28 **2.2.3. Structural Analogue Data**

29 For some chemical classes, there is significant information available on the carcinogenicity of
30 analogues, largely in rodent bioassays. Analogue effects are instructive in investigating carcinogenic
31 potential of an agent as well as identifying potential target organs, exposures associated with effects,
32 and potential functional class effects or modes of action. All appropriate studies are included and
33 analyzed, whether indicative of a positive effect or not. Evaluation includes tests in various animal
34 species, strains, and sexes; with different routes of administration; and at various doses, as data are

1 available. Confidence in conclusions is a function of how similar the analogues are to the agent under
2 review in structure, metabolism, and biological activity. This confidence needs to be considered to
3 ensure a balanced position.
4

5 **2.3. ANALYSIS OF OTHER KEY DATA**

6 The physical, chemical, and structural properties of an agent, as well as data on endpoints that
7 are thought to be critical elements of the carcinogenic process, provide valuable insights into the
8 likelihood of human cancer risk. The following sections provide guidance for analyses of these data.
9

10 **2.3.1. Physicochemical Properties**

11 Physicochemical properties affect an agent's absorption, tissue distribution (bioavailability),
12 biotransformation, and degradation in the body and are important determinants of hazard potential (and
13 dose response analysis). Properties to analyze include, but are not limited to, the following: molecular
14 weight, size, and shape; valence state; physical state (gas, liquid, solid); water or lipid solubility, which
15 can influence retention and tissue distribution; and potential for chemical degradation or stabilization in
16 the body.

17 An agent's potential for chemical reaction with cellular components, particularly with DNA and
18 proteins, is also important. The agent's molecular size and shape, electrophilicity, and charge
19 distribution are considered in order to decide whether they would facilitate such reactions.
20

21 **2.3.2. Structure-Activity Relationships**

22 Structure-activity relationship (SAR) analyses and models can be used to predict molecular
23 properties, surrogate biological endpoints, and carcinogenicity. Overall, these analyses provide
24 valuable initial information on agents, which may strengthen or weaken the concern for an agent's
25 carcinogenic potential.

26 Currently, SAR analysis is useful for chemicals and metabolites that are believed to initiate
27 carcinogenesis through covalent interaction with DNA (i.e., DNA-reactive, mutagenic, electrophilic, or
28 proelectrophilic chemicals) (Ashby and Tennant, 1991). For organic chemicals, the predictive
29 capability of SAR analysis combined with other toxicity information has been demonstrated (Ashby and
30 Tennant, 1994). The following parameters are useful in comparing an agent to its structural analogues
31 and congeners that produce tumors and affect related biological processes such as receptor binding and
32 activation, mutagenicity, and general toxicity (Woo and Arcos, 1989):

33 ! nature and reactivity of the electrophilic moiety or moieties present,

- 1 ! potential to form electrophilic reactive intermediate(s) through chemical, photochemical, or
- 2 metabolic activation,
- 3 ! contribution of the carrier molecule to which the electrophilic moiety(ies) is attached,
- 4 ! physicochemical properties (e.g., physical state, solubility, octanol-water partition
- 5 coefficient, half-life in aqueous solution),
- 6 ! structural and substructural features (e.g., electronic, steric, molecular geometric),
- 7 ! metabolic pattern (e.g., metabolic pathways and activation and detoxification ratio), and
- 8 ! possible exposure route(s) of the agent.

9 Suitable SAR analysis of non-DNA-reactive chemicals and of DNA-reactive chemicals that do
10 not appear to bind covalently to DNA requires knowledge or postulation of the probable mode(s) of
11 action of closely related carcinogenic structural analogues (e.g., receptor-mediated, cytotoxicity-
12 related). Examination of the physicochemical and biochemical properties of the agent may then provide
13 the rest of the information needed in order to make an assessment of the likelihood of the agent's
14 activity by that mode of action.

16 **2.3.3. Comparative Metabolism and Toxicokinetics**

17 Studies of the absorption, distribution, biotransformation, and excretion of agents permit
18 comparisons among species to assist in determining the implications of animal responses for human
19 hazard assessment, supporting identification of active metabolites, identifying changes in distribution and
20 metabolic pathway or pathways over a dose range, and making comparisons among different routes of
21 exposure.

22 If extensive data are available (e.g., blood/tissue partition coefficients and pertinent
23 physiological parameters of the species of interest), physiologically based pharmacokinetic models can
24 be constructed to assist in a determination of tissue dosimetry, species-to-species extrapolation of dose,
25 and route-to-route extrapolation (Connolly and Andersen, 1991; see section 3.2.2). If it is not contrary
26 to available data, it is assumed as a default that toxicokinetic and metabolic processes are qualitatively
27 comparable between species. Discussion of the defaults regarding quantitative comparison and their
28 modifications appears in section 3.

29 The qualitative question of whether an agent is absorbed by a particular route of exposure is
30 important for weight of evidence classification discussed in section 2.7.1. Decisions whether route of
31 exposure is a limiting factor on expression of any hazard, in that absorption does not occur by a route,
32 are based on studies in which effects of the agent, or its structural analogues, have been observed by
33 different routes, on physical-chemical properties, or on toxicokinetics studies.

1 Adequate metabolism and pharmacokinetic data can be applied toward the following as data
2 permit. Confidence in conclusions is enhanced when in vivo data are available.

- 3 ! Identifying metabolites and reactive intermediates of metabolism and determining whether
4 one or more of these intermediates are likely to be responsible for the observed effects.
5 This information on the reactive intermediates will appropriately focus SAR analysis,
6 analysis of potential modes of action, and estimation of internal dose in dose response
7 assessment (D'Souza et al., 1987; Krewski et al., 1987).
- 8 ! Identifying and comparing the relative activities of metabolic pathways in animals with those
9 in humans. This analysis can provide insights for extrapolating results of animal studies to
10 humans.
- 11 ! Describing anticipated distribution within the body and possibly identifying target organs.
12 Use of water solubility, molecular weight, and structure analysis can support qualitative
13 inferences about anticipated distribution and excretion. In addition, describing whether the
14 agent or metabolite of concern will be excreted rapidly or slowly or will be stored in a
15 particular tissue or tissues to be mobilized later can identify issues in comparing species and
16 formulating dose response assessment approaches.
- 17 ! Identifying changes in toxicokinetics and metabolic pathways with increases in dose. These
18 changes may result in important differences in disposition of the agent or its generation of
19 active forms of the agent between high and low dose levels. These studies play an
20 important role in providing a rationale for dose selection in carcinogenicity studies.
- 21 ! Determining bioavailability via different routes of exposure by analyzing uptake processes
22 under various exposure conditions. This analysis supports identification of hazards for
23 untested routes. In addition, use of physicochemical data (e.g., octanol-water partition
24 coefficient information) can support an inference about the likelihood of dermal absorption
25 (Flynn, 1990).

26 In all of these areas, attempts are made to clarify and describe as much as possible the
27 variability to be expected because of differences in species, sex, age, and route of exposure. The
28 analysis takes into account the presence of subpopulations of individuals who are particularly vulnerable
29 to the effects of an agent because of toxicokinetic or metabolic differences (genetically or
30 environmentally determined) (Bois et al., 1995).

31 32 **2.3.4. Toxicological and Clinical Findings**

33 Toxicological findings in experimental animals and clinical observations in humans are an
34 important resource to the cancer hazard assessment. Such findings provide information on

1 physiological effects, effects on enzymes, hormones, and other important macromolecules as well as on
2 target organs for toxicity. Given that the cancer process represents defects in terminal differentiation,
3 growth control, and cell death, developmental studies of agents may provide an understanding of the
4 activity of an agent that carries over to cancer assessment. Toxicity studies in animals by different routes
5 of administration support comparison of absorption and metabolism by those routes. Data on human
6 variability in standard clinical tests may provide insight into the range of human sensitivity and common
7 mechanisms to agents that affect the tested parameters.

8 9 **2.3.5. Mode of Action-Related Endpoints and Short-Term Tests**

10 A myriad of biochemical and biological endpoints relevant to the carcinogenic process provide
11 important information in determining whether a cancer hazard exists and include, but are not limited to,
12 mutagenesis, inhibition of gap junctional intercellular communication, increased cell proliferation,
13 inhibition of programmed cell death, receptor activation, and immunosuppression. These precursor
14 effects are discussed below.

15 16 **2.3.5.1. *Direct DNA Effects***

17 Because cancer is the result of multiple genetic defects in genes controlling proliferation and
18 tissue homeostasis (Vogelstein et al., 1988), the ability of an agent to affect DNA is of obvious
19 importance. It is well known that many carcinogens are electrophiles that interact directly with DNA,
20 resulting in DNA damage and adducts, and subsequent mutations (referred to in these guidelines as
21 direct DNA effects) that are thought to contribute to the carcinogenic process (Shelby and Zeiger,
22 1990; Tinwell and Ashby, 1991). Thus, studies of these phenomena continue to be important in the
23 assessment of cancer hazard. The EPA has published testing guidelines for detecting the ability of
24 agents to affect DNA or chromosomes (EPA, 1991a). Information on agents that induce mutations in
25 animal germ cells also deserves attention; several human carcinogens have been shown to be positive in
26 rodent tests for the induction of genetic damage in both somatic and germ cells (Shelby, 1995).

27 28 **2.3.5.2. *Secondary DNA Effects***

29 Similarly of interest are secondary mechanisms that either increase mutation rates or the number
30 of dividing cells. An increase in mutations might be due to cytotoxic exposures causing regenerative
31 proliferation or mitogenic influences, either of which could result in clonal expansion of initiated cells
32 (Cohen and Ellwein, 1990). An agent might interfere with the enzymes involved in DNA repair and
33 recombination (Barrett and Lee, 1992). Also, programmed cell death (apoptosis) can potentially be
34 blocked by an agent, thereby permitting replication of damaged cells. For example, peroxisome

1 proliferators may act by suppressing apoptosis pathways (Shulte-Hermann et al., 1993; Bayly et al.,
2 1994). An agent may also generate reactive oxygen species that produce oxidative damage to DNA
3 and other important macromolecules that become important elements of the carcinogenic process
4 (Kehrer, 1993; Clayson et al., 1994; Chang et al., 1988). Damage to certain critical DNA repair
5 genes or other genes (e.g., the p53 gene) may result in genomic instability, which predisposes cells to
6 further genetic alterations and increases the probability of neoplastic progression independent of any
7 exogenous agent (Harris and Hollstein, 1993; Levine, 1994).

8 The loss or gain of chromosomes (i.e., aneuploidy) is an effect that can result in genomic
9 instability (Fearon and Vogelstein, 1990; Cavenee et al., 1986). Although the relationship between
10 induced aneuploidy and carcinogenesis is not completely established, several carcinogens have been
11 shown to induce aneuploidy (Gibson et al., 1995; Barrett, 1992). Agents that cause aneuploidy
12 interfere with the normal process of chromosome segregation and lead to chromosomal losses, gains, or
13 aberrations by interacting with the proteins (e.g., microtubules) needed for chromosome movement.

14 15 **2.3.5.3. *Nonmutagenic and Other Effects***

16 A failure to detect DNA damage and mutation induction in several test systems suggests that a
17 carcinogenic agent may act by another mode of action.

18 It is possible for an agent to alter gene expression (transcriptional, translational, or post-
19 translational modifications) by means not involving mutations (Barrett, 1995). For example,
20 perturbation of DNA methylation patterns may cause effects that contribute to carcinogenesis (Jones,
21 1986; Goodman and Counts, 1993; Holliday, 1987). Overexpression of genes by amplification has
22 been observed in certain tumors (Vainio et al., 1992). Other mechanisms may involve cellular
23 reprogramming through hormonal mechanisms or receptor-mediated mechanisms (Ashby et al., 1994;
24 Barrett, 1992).

25 Gap-junctional intercellular communication is widely believed to play a role in tissue and organ
26 development and in the maintenance of a normal cellular phenotype within tissues. A growing body of
27 evidence suggests that chemical interference with gap-junctional intercellular communication is a
28 contributing factor in tumor development; many carcinogens have been shown to inhibit this
29 communication. Thus, such information may provide useful mechanistic data in evaluating cancer
30 hazard (Swierenga and Yamasaki, 1992; Yamasaki, 1995).

31 Both cell death and cell proliferation are mandatory for the maintenance of homeostasis in
32 normal tissue. The balance between the two directly affects the survival and growth of initiated cells, as
33 well as preneoplastic and tumor cell populations (i.e., increase in cell proliferation or decrease in cell
34 death) (Bellamy et al., 1995; Cohen and Ellwein, 1990, 1991; Cohen et al., 1991). In studies of

1 proliferative effects, distinctions should be made between mitogenesis and regenerative proliferation
2 (Cohen and Ellwein, 1990, 1991; Cohen et al., 1991). In applying information from studies on cell
3 proliferation and apoptosis to risk assessment, it is important to identify the tissues and target cells
4 involved, to measure effects in both normal and neoplastic tissue, to distinguish between apoptosis and
5 necrosis, and to determine the dose that affects these processes.

7 **2.3.5.4. *Criteria for Judging Mode of Action***

8 Criteria that are applicable for judging the adequacy of mechanistically based data include the
9 following:

- 10 ! mechanistic relevance of the data to carcinogenicity,
- 11 ! number of studies of each endpoint,
- 12 ! consistency of results in different test systems and different species,
- 13 ! similar dose response relationships for tumor and mode of action-related effects,
- 14 ! tests conducted in accordance with generally accepted protocols, and
- 15 ! degree of consensus and general acceptance among scientists regarding interpretation of the
16 significance and specificity of the tests.

17 Although important information can be gained from in vitro test systems, a higher level of confidence is
18 generally given to data that are derived from in vivo systems, particularly those results that show a site
19 concordance with the tumor data.

21 **2.4. BIOMARKER INFORMATION**

22 Various endpoints can serve as biological markers of events in biological systems or samples.
23 In some cases, these molecular or cellular effects (e.g., DNA or protein adducts, mutation,
24 chromosomal aberrations, levels of thyroid stimulating hormone) can be measured in blood, body fluids,
25 cells and tissues to serve as biomarkers of exposure in both animals and humans (Callemen et al., 1978;
26 Birner et al., 1990). As such, they can do the following:

- 27 ! act as an internal surrogate measure of chemical dose, representing as appropriate, either
28 recent (e.g., serum concentration) or accumulated (e.g., hemoglobin adducts) exposure,
- 29 ! help identify doses at which elements of the carcinogenic process are operating,
- 30 ! aid in interspecies extrapolations when data are available from both experimental animal and
31 human cells, and
- 32 ! under certain circumstances, provide insights into the possible shape of the dose response
33 curve below levels where tumor incidences are observed (e.g., Choy, 1993).

1 Genetic and other findings (like changes in proto-oncogenes and tumor suppressor genes in
2 preneoplastic and neoplastic tissue or possibly measures of endocrine disruption) can indicate the
3 potential for disease and as such serve as biomarkers of effect. They, too, can be used in different
4 ways:

5 ! The spectrum of genetic changes in proliferative lesions and tumors following chemical
6 administration to experimental animals can be determined and compared with those in
7 spontaneous tumors in control animals, in animals exposed to other agents of varying
8 structural and functional activities, and in persons exposed to the agent under study.

9 ! They may provide a linkage to tumor response.

10 ! They may help to identify subpopulations of individuals who may be at an elevated risk for
11 cancer, e.g., cytochrome P450 2D6/debrisoquine sensitivity for lung cancer (Caporaso et
12 al., 1989) or inherited colon cancer syndromes (Kinzler et al., 1991; Peltomäki et al.,
13 1993).

14 ! As with biomarkers of exposure, it may be justified in some cases to use these endpoints for
15 dose response assessment or to provide insight into the potential shape of the dose
16 response curve at doses below those at which tumors are induced experimentally.

17 In applying biomarker data to cancer assessment (particularly assessments based on
18 epidemiologic data), one should consider the following:

19 ! routes of exposure

20 ! exposure to mixtures

21 ! time after exposure

22 ! sensitivity and specificity of biomarkers

23 ! dose response relationships.

24 25 **2.5. MODE OF ACTION--IMPLICATIONS FOR HAZARD CHARACTERIZATION AND** 26 **DOSE RESPONSE**

27 The interaction of the biology of the organism and the chemical properties of the agent
28 determine whether there is an adverse effect. Thus, mode of action analysis is based on physical,
29 chemical, and biological information that helps to explain critical events in an agent's influence on
30 development of tumors. The entire range of information developed in the assessment is reviewed to
31 arrive at a reasoned judgment. An agent may work by more than one mode of action both at different
32 sites and at the same tumor site. It is felt that at least some information bearing on mode of action (e.g.,
33 SAR, screening tests for mutagenicity) is present for most agents undergoing assessment of
34 carcinogenicity, even though certainty about exact molecular mechanisms may be rare.

1 Inputs to mode of action analysis include tumor data in humans, animals, and among structural
2 analogues as well as the other key data. The more complete the data package and generic knowledge
3 about a given mode of action, the more confidence one has and the more one can replace or refine
4 default science policy positions with relevant information. Making reasoned judgments is generally
5 based on a data-rich source of chemical, chemical class, and tumor type-specific information. Many
6 times there will be conflicting data and gaps in the information base; one must carefully evaluate these
7 uncertainties before reaching any conclusion.

8 Some of the questions that need to be addressed include the following:

- 9 ! Has a body of data been developed on the agent that fits with a generally accepted mode of
10 action?
- 11 ! Has the mode of action been published and gained general scientific acceptance through
12 peer-reviewed research or is it still speculative?
- 13 ! Is the mode of action consistent with generally agreed-upon principles and understanding of
14 carcinogenesis?
- 15 ! Is the mode of action reasonably anticipated or assumed, in the absence of specific data, to
16 operate in humans? How is this question influenced by information on comparative uptake,
17 metabolism, and excretion patterns across animals and humans?
- 18 ! Do humans appear to be more or less sensitive to the mode of action than are animals?
- 19 ! Does the agent affect DNA, directly or indirectly?
- 20 ! Are there important determinants in carcinogenicity other than effects on DNA, such as
21 changes in cell proliferation, apoptosis, gene expression, immune surveillance, or other
22 influences?

23 In making decisions about potential modes of action and the relevance of animal tumor findings
24 to humans (Ashby et al., 1990), very often the results of chronic animal studies may give important
25 clues. Some of the important factors to review include the following:

- 26 ! tumor types, e.g., those responsive to endocrine influence, those produced by reactive
27 carcinogens (Ashby and Tennant, 1991),
- 28 ! number of tumor sites, sexes, studies, and species affected or unaffected (Tennant, 1993),
- 29 ! influence of route of exposure; spectrum of tumors; local or systemic sites,
- 30 ! target organ or system toxicity, e.g., urinary chemical changes associated with stone
31 formation, effects on immune surveillance,
- 32 ! presence of proliferative lesions, e.g., hepatic foci, hyperplasias,
- 33 ! progression of lesions from preneoplastic to benign to malignant with dose and time,
- 34 ! ratio of malignant to benign tumors as a function of dose and time,

- 1 ! time of appearance of tumors after commencing exposure,
- 2 ! tumors invading locally, metastasizing, producing death,
- 3 ! tumors at sites in laboratory animals with high or low spontaneous historical incidence,
- 4 ! biomarkers in tumor cells, both induced and spontaneous, e.g., DNA or protein adducts,
- 5 mutation spectra, chromosome changes, oncogene activation, and
- 6 ! shape of the dose response in the range of tumor observation, e.g., linear vs. profound
- 7 change in slope.

8 Some of the myriad of ways that information from chronic animal studies influences mode of
9 action judgments include the following. Multisite and multispecies tumor effects are often associated
10 with mutagenic agents. Tumors restricted to one sex/species may suggest an influence restricted to
11 gender, strain, or species. Late onset of tumors that are primarily benign or are at sites with a high
12 historical background incidence or show reversal of lesions on cessation of exposure may point to a
13 growth-promoting mode of action. The possibility that an agent may act differently in different tissues
14 or have more than one mode of action in a single tissue must also be kept in mind.

15 Simple knowledge of sites of tumor increase in rodent studies can give preliminary clues as to
16 mode of action. Experience at the National Toxicology Program (NTP) indicates that substances that
17 are DNA reactive and produce gene mutations may be unique in producing tumors in certain anatomical
18 sites, while tumors at other sites may arise from both mutagenic or nonmutagenic influences (Ashby and
19 Tennant, 1991; Huff et al., 1991).

20 Effects on tumor sites in rodents and other mode of action information has been explored for
21 certain agents (Alison et al., 1994; Clayson, 1989; ECETOC, 1991; MacDonald et al., 1994;
22 McClain, 1994; Tischer et al., 1991; ILSI, 1995; Cohen and Ellwein, 1991; FASEB, 1994; Havu et
23 al., 1990; U.S. EPA, 1991; Li et al., 1987; Grasso and Hinton, 1991; Larson et al., 1994; IARC,
24 1990; Jack et al., 1983; Stitzel et al., 1989; Ingram and Grasso, 1991; Bus and Popp, 1987;
25 Prahalada et al., 1994; Yamada et al., 1994; Hill et al., 1989; Burek et al., 1988).

26 The selection of a dose response extrapolation procedure for cancer risk estimation considers
27 mode of action information. When information is extensive and there is considerable certainty in a given
28 mode of action, a biologically based or case-specific model that incorporates data on processes
29 involved is preferred. Obviously, use of such a model requires the existence of substantial data on
30 component parameters of the mode of action, and judgments on its applicability must be made on a
31 case-by-case basis.

32 In the absence of information to develop a biologically based or case-specific model,
33 understanding of mode of action should be employed to the extent possible in deciding upon one of
34 three science policy defaults: low-dose linear extrapolation, nonlinear, and both procedures. The

1 overall choice of the default(s) depends upon weighing the various inputs and deciding which best
2 reflect the mode of action understanding. A rationale accompanies whichever default or defaults are
3 chosen.

4 A default assumption of linearity is appropriate when the evidence supports a mode of action of
5 gene mutation due to DNA reactivity or supports another mode of action that is anticipated to be linear.
6 Other elements of empirical data may also support an inference of linearity, e.g., the background of
7 human exposure to an agent might be such that added human exposure is on the linear part of a dose
8 response curve that is sublinear overall. The default assumption of linearity is also appropriate as the
9 ultimate default when evidence shows no DNA reactivity or other support for linearity, but neither is it
10 sufficient evidence of a nonlinear mode of action to support a nonlinear procedure.

11 A default assumption of nonlinearity is appropriate when there is no evidence for linearity and
12 sufficient evidence to support an assumption of nonlinearity and a nonlinear procedure. The mode of
13 action may lead to a dose response relationship that is nonlinear, with response falling much more
14 quickly than linearly with dose, or being most influenced by individual differences in sensitivity.
15 Alternatively, the mode of action may theoretically have a threshold, e.g., the carcinogenicity may be a
16 secondary effect of toxicity that is itself a threshold phenomenon.

17 Both linear and nonlinear procedures may be used in particular cases. If a mode of action
18 analysis finds substantial support for differing modes of action for different tumor sites, an appropriate
19 procedure is used for each. Both procedures may also be appropriate to discuss implications of
20 complex dose response relationships. For example, if it is apparent that an agent is both DNA reactive
21 and is highly active as a promotor at high doses, and there are insufficient data for modeling, both linear
22 and nonlinear default procedures may be needed to decouple and consider the contribution of both
23 phenomena.

24 25 **2.6. WEIGHT OF EVIDENCE EVALUATION FOR POTENTIAL HUMAN** 26 **CARCINOGENICITY**

27 A weight of evidence evaluation is a collective evaluation of all pertinent information so that the
28 full impact of biological plausibility and coherence are adequately considered. Identification and
29 characterization of human carcinogenicity is based on human and experimental data, the nature,
30 advantages and limitations of which have been discussed in the preceding sections.

31 The subsequent sections outline: (1) the basics of weighing individual lines of evidence and
32 combining the entire body of evidence to make an informed judgment, (2) classification descriptors of
33 cancer hazard, and (3) some case study examples to illustrate how the principles of guidance can be
34 applied to arrive at a classification.

1 **2.6.1. Weight of Evidence Analysis**

2 Judgment about the weight of evidence involves considerations of the quality and adequacy of
3 data and consistency of responses induced by the agent in question. The weight of evidence judgment
4 requires combined input of relevant disciplines. Initial views of one kind of evidence may change
5 significantly when other information is brought to the interpretation. For example, a positive animal
6 carcinogenicity finding may be diminished by other key data; a weak association in epidemiologic
7 studies may be bolstered by consideration of other key data and animal findings. Factors typically
8 considered are illustrated in figures below. Generally, no single weighing factor on either side
9 determines the overall weight. The factors are not scored mechanically by adding pluses and minuses;
10 they are judged in combination.

11
12 *Human Evidence*

13 Analyzing the contribution of evidence from a body of human data requires examining available
14 studies and weighing them in the context of well-accepted criteria for causation (see section 2.2.1). A
15 judgment is made about how closely they satisfy these criteria, individually and jointly, and how far they
16 deviate from them. Existence of temporal relationships, consistent results in independent studies, strong
17 association, reliable exposure data, presence of dose-related responses, freedom from biases and
18 confounding factors, and high level of statistical significance are among the factors leading to increased
19 confidence in a conclusion of causality.

20 Generally, the weight of human evidence increases with the number of adequate studies that
21 show comparable results on populations exposed to the same agent under different conditions. The
22 analysis takes into account all studies of high quality, whether showing positive associations or null
23 results, or even protective effects. In weighing positive studies against null studies, possible reasons for
24 inconsistent results should be sought, and results of studies that are judged to be of high quality are
25 given more weight than those from studies judged to be methodologically less sound. See figure 2-1.

Human Evidence Factors

Increase Weight	Decrease Weight
Number of independent studies with consistent results	Few studies Equally well designed and conducted studies with null results
Most causal criteria satisfied:	
Temporal relationship	
Strong association	
Reliable exposure data	Few causal criteria satisfied
Dose response relationship	
Freedom from bias and confounding	
Biological plausibility	
High statistical significance	

Figure 2-1. Factors for Weighing Human Evidence

Animal Evidence Factors

Increase Weight	Decrease Weight
Number of independent studies with consistent results	Single study Inconsistent results
Same site across species, structural analogues	
Multiple observations Species Sites Sexes	Single site/species/sex
Severity and progression of lesions Early in life tumors/malignancy Dose response relationships Lesion progression Uncommon tumor	Benign tumors only High background of incidence tumors
Route of administration like human exposure	Route of administration unlike human exposure

Figure 2-2. Factors for Weighing Animal Evidence

1 ***Other Key Evidence***

2 Additional information bearing on the qualitative assessment of carcinogenic potential may be
3 gained from comparative pharmacokinetic and metabolism studies, genetic toxicity studies, SAR
4 analysis, and other studies of an agent's properties. See figure 2-3. Information from these studies
5 helps to elucidate potential modes of action and biological fate and disposition. The knowledge gained
6 supports interpretation of cancer studies in humans and animals and provides a separate source of
7 information about carcinogenic potential.

Other Key Evidence Factors

Increase Weight	Decrease Weight
A rich set of other key data are available	Few or poor data
Physicochemical data	or
Data indicate reactivity with macromolecules	Inadequate data necessitate use of default assumptions
Structure activity relationships support hazard potential	or
Comparable metabolism and toxicokinetics between species	Data show that animal findings are not relevant to humans
Toxicological and human clinical data support tumor findings	
Biomarker data support attribution of effects to agent	
Mode of action data support causal interpretation of human evidence or relevance of animal evidence	

Figure 2-3. Factors for Weighing Other Key Evidence

1
2
3
4
5

Totality of Evidence

In reaching a view of the entire weight of evidence, all data and inferences are merged. Figure 2-4 indicates the generalities. In fact, possible weights of evidence span a broad continuum that cannot be capsulized. Most of the time the data in various lines of evidence fall in the middle of the weights represented in the four figures in this section.

Totality of Evidence Factors

Increase Weight	Decrease Weight
Evidence of human causality	Data not available or do not show causality
Evidence of animal effects relevant to humans	Data not available or not relevant
Coherent inferences	Conflicting data
Comparable metabolism and toxicokinetics between species	Metabolism and toxicokinetics not comparable
Mode of action comparable across species	Mode of action not comparable across species

Figure 2-4. Factors for Weighing Totality of Evidence

1 The following section and the weight of evidence narrative discussed in 2.7.2. provide a way to
2 state a conclusion and capture this complexity in a consistent way.

3 4 **2.6.2. Descriptors for Classifying Weight of Evidence**

5 Hazard classification uses three categories of descriptors for human carcinogenic potential:
6 "known/likely," "cannot be determined," and "not likely." Each category has associated subdescriptors
7 to further define the conclusion. The descriptors are not meant to replace an explanation of the nuances
8 of the biological evidence, but rather to summarize it. Each category spans a wide variety of potential
9 data sets and weights of evidence. There will always be gray areas, gradations, and borderline cases.
10 That is why the descriptors are presented only in the context of a weight of evidence narrative whose
11 format is given in section 2.7.2. Using them within a narrative preserves and presents the complexity
12 that is an essential part of the hazard classification. Applying a descriptor is a matter of judgment and
13 cannot be reduced to a formula. Risk managers should consider the entire range of information
14 included in the narrative rather than focusing simply on the descriptor.

15 A single agent may be categorized in more than one way if, for instance, the agent is likely to be
16 carcinogenic by one route of exposure but not by another (section 2.3.3).

17 The descriptors and subdescriptors are standardized and are to be used consistently from case
18 to case. The discussions below explain descriptors and subdescriptors which appear in italics, and
19 along with Appendix A and section 2.6.3, illustrate their use.

20 21 ***"Known/Likely"***

22 This category of descriptors is appropriate when the available tumor effects and other key data
23 are adequate to convincingly demonstrate carcinogenic potential for humans; it includes:

- 24 C agents *known* to be carcinogenic in humans based on either epidemiologic evidence or a
25 combination of epidemiologic and experimental evidence, demonstrating causality between
26 human exposure and cancer,
- 27 C agents that should be treated *as if* they were *known* human carcinogens, based on a
28 combination of epidemiologic data showing a plausible causal association (not
29 demonstrating it definitively) and strong experimental evidence.
- 30 C agents that are *likely* to produce cancer in humans due to the production or anticipated
31 production of tumors by modes of action that are relevant or assumed to be relevant to
32 human carcinogenicity.

33 Modifying descriptors for particularly high or low ranking in the "known/likely" group can be applied
34 based on scientific judgment and experience and are as follows:

1 C agents that are *likely* to produce cancer in humans based on data that are at the *high end* of
2 the weights of evidence typical of this group,

3 C agents that are *likely* to produce cancer in humans based on data that are at the *low end* of
4 the weights of evidence typical of this group.

6 ***"Cannot Be Determined"***

7 This category of descriptors is appropriate when available tumor effects or other key data are
8 suggestive or conflicting or limited in quantity and, thus, are not adequate to convincingly demonstrate
9 carcinogenic potential for humans. In general, further agent specific and generic research and testing are
10 needed to be able to describe human carcinogenic potential. The descriptor *cannot be determined* is
11 used with a subdescriptor that captures the rationale:

12 C agents whose carcinogenic potential *cannot be determined*, but for which there is
13 *suggestive* evidence that raises concern for carcinogenic effects,

14 C agents whose carcinogenic potential *cannot be determined* because the existing evidence is
15 composed of *conflicting data* (e.g., some evidence is suggestive of carcinogenic effects,
16 but other equally pertinent evidence does not confirm any concern),

17 C agents whose carcinogenic potential *cannot be determined* because there are *inadequate*
18 *data* to perform an assessment,

19 C agents whose carcinogenic potential *cannot be determined* because *no data* are available
20 to perform an assessment.

22 ***"Not Likely"***

23 This is the appropriate descriptor when experimental evidence is satisfactory for deciding that
24 there is no basis for human hazard concern, as follows (in the absence of human data suggesting a
25 potential for cancer effects):

26 C agents *not likely* to be carcinogenic to humans because they have been evaluated in at least
27 two well conducted studies in two appropriate animal species without demonstrating
28 carcinogenic effects,

29 C agents *not likely* to be carcinogenic to humans because they have been appropriately
30 evaluated in animals and show only carcinogenic effects that have been shown not to be
31 relevant to humans (e.g., showing only effects in the male rat kidney due to accumulation of
32 alpha_{2u}-globulin),

33 C agents *not likely* to be carcinogenic to humans when carcinogenicity is dose or route
34 dependent. For instance, *not likely* below a certain dose range (categorized as *likely*

1 above that range) or *not likely* by a certain route of exposure (may be categorized as *likely*
2 by another route of exposure). To qualify, agents will have been appropriately evaluated in
3 animal studies and the only effects show a dose range or route limitation or a route limitation
4 is otherwise shown by empirical data.

5 C agents not likely to be carcinogenic to humans based on extensive human experience that
6 demonstrates lack of effect (e.g., phenobarbital).
7

8 **2.6.3. Case Study Examples**

9 This section provides examples of substances that fit the three broad categories described
10 above. These examples are based on available information about real substances and are selected to
11 illustrate the principles for weight-of-evidence evaluation and the application of the classification
12 scheme.

13 These case studies show the interplay of differing lines of evidence in making a conclusion.
14 Some particularly illustrate the role that “other key data” can play in conclusions.
15

16 ***Example 1: "Known Human Carcinogen"--Route-Dependent/Linear Extrapolation***

17 Human Data

18 Substance 1 is an aluminosilicate mineral that exists in nature with a fibrous habit. Several
19 descriptive epidemiologic studies have demonstrated very high mortality from malignant mesothelioma,
20 mainly of the pleura, in three villages in Turkey, where there was a contamination of this mineral and
21 where exposure had occurred from birth. Both sexes were equally affected and at an unusually young
22 age.
23

24 Animal Data

25 Substance 1 has been studied in a single long-term inhalation study in rats at one exposure
26 concentration that showed an extremely high incidence of pleural mesothelioma (98% in treated animals
27 versus 0% in concurrent controls). This is a rare malignant tumor in the rat and the onset of tumors
28 occurred at a very early age (as early as 1 year of age). Several studies involving injection into the
29 body cavities of rats or mice (i.e., pleural or peritoneal cavities) also produced high incidences of
30 pleural or peritoneal mesotheliomas. No information is available on the carcinogenic potential of
31 substance 1 in laboratory animals via oral and dermal exposures.
32

33 Other Key Data

1 Information on the physical and chemical properties of substance 1 indicates that it is highly
2 respirable to humans and laboratory rodents. It is highly insoluble and is not likely to be readily
3 degraded in biological fluid.

4 No information is available on the deposition, translocation, retention, lung clearance, and
5 excretion of the substance after inhalation exposure or ingestion. Lung burden studies have shown the
6 presence of elevated levels of the substance in lung tissue samples of human cases of pleural
7 mesotheliomas from contaminated villages compared with control villages.

8 No data are available on genetic or related effects in humans. The substance has been shown
9 to induce unscheduled DNA synthesis in human cells in vitro and transformation and unscheduled DNA
10 synthesis in mouse cells.

11 The mechanisms by which this substance causes cancer in humans and animals are not
12 understood, but appear to be related to its unique physical, chemical, and surface properties. Its fiber
13 morphology is similar to a known group of naturally occurring silicate minerals that have been known to
14 cause respiratory cancers (including pleural mesothelioma) from inhalation exposure and genetic
15 changes in humans.

16 17 Evaluation

18 Human evidence is judged to establish a causal link between exposure to substance 1 and
19 human cancer. Even though the human evidence does not satisfy all criteria for causality, this judgment
20 is based on a number of unusual observations: large magnitude of the association, specificity of the
21 association, demonstration of environmental exposure, biological plausibility, and coherence based on
22 the entire body of knowledge of the etiology of mesothelioma.

23 Animal evidence demonstrates a causal relationship between exposure and cancer in laboratory
24 animals. Although available data are not optimal in terms of design (e.g., the use of single dose, one sex
25 only), the judgment is based on the unusual findings from the only inhalation experiment in rats (i.e.,
26 induction of an uncommon tumor, an extremely high incidence of malignant neoplasms, and onset of
27 tumors at an early age). Additional evidence is provided by consistent results from several injection
28 studies showing an induction of the same tumors by different modes of administration in more than one
29 species.

30 Other key data, while limited, support the human and animal evidence of carcinogenicity. It can
31 be inferred from human and animal data that this substance is readily deposited in the respiratory
32 airways and deep lung and is retained for extended periods of time since first exposure. Information on
33 related fibrous substances indicates that the modes of action are likely mediated by the physical and

1 chemical characteristics of the substance (e.g., fiber shape, high aspect ratio, a high degree of
2 insolubility in lung tissues).

3 Insufficient data are available to evaluate the human carcinogenic potential of substance 1 by
4 oral exposure. Even though there is no information on its carcinogenic potential via dermal uptake, it is
5 not expected to pose a carcinogenic hazard to humans by that route because it is very insoluble and is
6 not likely to penetrate the skin.

7 8 Conclusion

9 It is concluded that substance 1 is a *known human carcinogen by inhalation exposure*. The
10 weight of evidence of human carcinogenicity is based on (a) exceptionally increased incidence of
11 malignant mesothelioma in epidemiologic studies of environmentally exposed human populations; (b)
12 significantly increased incidence of malignant mesothelioma in a single inhalation study in rats and in
13 several injection studies in rats and mice; and (c) supporting information on related fibrous substances
14 that are known to cause cancer via inhalation and genetic damage in exposed mammalian and human
15 mesothelial cells. The human carcinogenic potential of substance 1 via oral exposure cannot be
16 determined on the basis of insufficient data. It is not likely to pose a carcinogenic hazard to humans via
17 dermal uptake because it is not anticipated to penetrate the skin.

18 The mode of action of this substance is not understood. In addition to this uncertainty, dose
19 response information is lacking for both human and animal data. Epidemiologic studies contain
20 observations of significant excess cancer risks at relatively low levels of environmental exposure. The
21 use of *linear* extrapolation in a dose response relationship assessment is appropriate as a default since
22 mode of action data are not available.

23 24 ***Example 2: "As If Known Human Carcinogen"--Any Exposure*** 25 ***Conditions/Linear Extrapolation***

26 Human Data

27 Substance 2 is an alkene oxide. Several cohort studies of workers using substance 2 as a
28 sterilant have been conducted. In the largest and most informative study, mortality from lymphatic and
29 hematopoietic cancer was marginally elevated, but a significant trend was found, especially for
30 lymphatic leukemia and non-Hodgkin's lymphoma, in relation to estimated cumulative exposure to the
31 substance. Nonsignificant excesses of lymphatic and hematopoietic cancer were found in three other
32 smaller studies of sterilization personnel.

33 In one cohort study of chemical workers exposed to substance 2 and other agents, mortality
34 rate from lymphatic and hematopoietic cancer was elevated, but the excess was confined to a small

1 subgroup with only occasional low-level exposure to substance 2. Six other studies of chemical
2 workers are considered more limited due to a smaller number of deaths. Four studies found an excess
3 of lymphatic and hematopoietic cancer (which were significant in two); no increase in mortality rate was
4 observed in the other two studies.

5 6 Animal Data

7 Substance 2 was studied in an oral gavage study in rats. Treatment of substance 2 resulted in a
8 dose-dependent increased incidence in forestomach tumors that were mainly squamous-cell
9 carcinomas.

10 Substance 2 was also studied in two inhalation studies in mice and two inhalation studies in rats.
11 In the first mouse study, dose-dependent increases in combined benign and malignant tumors at several
12 tissue sites were induced in mice of both sexes (lung tumors and tumors of the Harderian gland in each
13 sex, and uterine adenocarcinomas, mammary carcinomas, and malignant lymphomas in females). In a
14 second study--a screening study for pulmonary tumors in mice--inhalation exposure to substance 2
15 resulted in a dose-dependent increase in lung tumors. In the two inhalation studies in rats, increased
16 incidences of mononuclear-cell leukemia and brain tumors were induced in exposed animals of each
17 sex; increased incidences of peritoneal tumors in the region of the testis and subcutaneous fibromas
18 were induced in exposed male rats.

19 Substance 2 induced local sarcomas in mice following subcutaneous injection. No tumors were
20 found in a limited skin painting study in mice.

21 22 Other Key Data

23 Substance 2 is a flammable gas at room temperature. The gaseous form is readily taken up in
24 humans and rats, and in aqueous solution it can penetrate human skin. Studies in rats indicate that, once
25 absorbed, substance 2 is uniformly distributed throughout the body. It is eliminated metabolically by
26 hydrolysis and by conjugation with glutathione. The ability to form glutathione conjugate varies across
27 animal species, with the rat being most active, followed by mice and rabbits.

28 Substance 2 is a directly acting alkylating agent. It has been shown to form adducts with
29 hemoglobin in both humans and animals and with DNA in animals. The increased frequency of
30 hemoglobin adducts, which have been used as markers of internal dose, has been found to correlate
31 with the level and cumulative exposure to substance 2. Significant increases in chromosomal
32 aberrations and sister chromatid exchanges in peripheral lymphocytes and induction of micronuclei in
33 the bone marrow cells have been observed in exposed workers.

1 Substance 2 also induced chromosomal aberrations and sister chromatid exchanges in
2 peripheral lymphocytes of monkeys exposed in vivo. It also induced gene mutation, specific locus
3 mutation, sister chromatid exchanges, chromosomal aberrations, micronuclei, dominant lethal mutations,
4 and heritable translocation in rodents exposed in vivo. In human cells in vitro, it induced sister
5 chromatid exchanges, chromosomal aberrations, and unscheduled DNA synthesis. Similar genetic and
6 related effects were observed in rodent cells in vitro and in nonmammalian systems.

7 8 Evaluation

9 Available epidemiologic studies, taken together, suggest that a causal association between
10 exposure to substance 2 and elevated risk of cancer is plausible. This judgment is based on small but
11 consistent excesses of lymphatic and hematopoietic cancer in the studies of sterilization workers.
12 Interpretation of studies of chemical workers is difficult because of possible confounding exposures.
13 Nevertheless, findings of elevated risks of cancer at similar sites in chemical workers support the
14 findings in studies of sterilization workers. Additional support is provided by observations of DNA
15 damage in the same tissue in which elevated cancer was seen in exposed workers.

16 Extensive evidence indicates that substance 2 is carcinogenic to laboratory animals. Positive
17 results were consistently observed in all well-designed and well-conducted studies. Substance 2 causes
18 dose-related increased incidences of tumors at multiple tissue sites in rats and mice of both sexes by
19 two routes of exposure (oral and inhalation). The only dermal study that yielded a nonpositive finding is
20 considered of limited quality.

21 Other key data significantly add support to the potential carcinogenicity of substance 2. There
22 is strong evidence of heritable mutations of exposed rodents and mutagenicity and clastogenicity both in
23 vivo and in vitro. These findings are reinforced by observations of similar genetic damage in exposed
24 workers. Additional support is based on SAR analysis that indicates that substance 2 is a highly DNA-
25 reactive agent. Structurally related chemicals, i.e., low-molecular-weight epoxides, also exhibit
26 carcinogenic effects in laboratory animals.

27 28 Conclusion

29 Substance 2 should be considered *as if it were a known human carcinogen by all routes of*
30 *exposure*. The weight of evidence of human carcinogenicity is based on (a) consistent evidence of
31 carcinogenicity in rats and mice by oral and inhalation exposure; (b) epidemiologic evidence suggestive
32 of a causal association between exposure and elevated risk of lymphatic and hematopoietic cancer; (c)
33 evidence of genetic damage in blood lymphocytes and bone marrow cells of exposed workers; (d)
34 mutagenic effects in numerous in vivo and in vitro test systems; (e) membership in a class of DNA-

1 reactive compounds that have been shown to cause carcinogenic and mutagenic effects in animals; and
2 (f) ability to be absorbed by all routes of exposure, followed by rapid distribution throughout the body.

3 Although the exact mechanisms of carcinogenic action of substance 2 are not completely
4 understood, available data strongly indicate a mutagenic mode of action. Linear extrapolation should be
5 assumed in dose response assessment.

6
7 ***Example 3: "Likely Human Carcinogen"--Any Exposure Conditions/Linear Extrapolation***

8 Human Data

9 Substance 3 is a brominated alkane. Three studies have investigated the cancer mortality of
10 workers exposed to this substance. No statistically significant increase in cancer at any site was found
11 in a study of production workers exposed to substance 3 and several other chemicals. Elevated cancer
12 mortality was reported in a much smaller study of production workers. An excess of lymphoma was
13 reported in grain workers who may have had exposure to substance 3 and other chemical compounds.
14 These studies are considered inadequate due to their small cohort size; lack of, or poorly characterized,
15 exposure concentrations; or concurrent exposure of the cohort to other potential or known
16 carcinogens.

17
18 Animal Data

19 The potential carcinogenicity of substance 3 has been extensively studied in an oral gavage
20 study in rats and mice of both sexes, two inhalation studies of rats of different strains of both sexes, an
21 inhalation study in mice of both sexes, and a skin painting study in female mice.

22 In the oral study, increased incidences of squamous-cell carcinoma of the forestomach were
23 found in rats and mice of both sexes. Additionally, there were increased incidences of liver carcinomas
24 in female rats, hemangiosarcomas in male rats, and alveolar/bronchiolar adenoma of the lung of male
25 and female mice. Excessive toxicity and mortality were observed in the rat study, especially in the high-
26 dose groups, which resulted in early termination of study, and similar time-weighted average doses for
27 the high- and low-treatment groups.

28 In the first inhalation study in rats and mice, increased incidences of carcinomas and
29 adenocarcinomas of the nasal cavity and hemangiosarcoma of the spleen were found in exposed
30 animals of each species of both sexes. Treated female rats also showed increased incidences of
31 alveolar/bronchiolar carcinoma of the lung and mammary gland fibroadenomas. Treated male rats
32 showed an increased incidence of peritoneal mesothelioma. In the second inhalation study in rats
33 (single exposure only), significantly increased incidences of hemangiosarcoma of the spleen and adrenal
34 gland tumors were seen in exposed animals of both sexes. Additionally, increased incidences of

1 subcutaneous mesenchymal tumors and mammary gland tumors were induced in exposed male and
2 female rats, respectively.

3 Lifetime dermal application of substance 3 to female mice resulted in significantly increased
4 incidences of skin papillomas and lung tumors.

5 Several chemicals structurally related to substance 3 are also carcinogenic in rodents. The
6 spectrum of tumor responses induced by related substances was similar to those seen with substance 3
7 (e.g., forestomach, mammary gland, lung tumors).

8 9 Other Key Data

10 Substance 3 exists as a liquid at room temperature and is readily absorbed by ingestion,
11 inhalation, and dermal contact. It is widely distributed in the body and is eliminated in the urine mainly
12 as metabolites (e.g., glutathione conjugate).

13 Substance 3 is not itself DNA-reactive, but is biotransformed to reactive metabolites as inferred
14 by findings of its covalent binding to DNA and induction of DNA strand breaks, both in vivo and in
15 vitro. Substance 3 has been shown to induce sister chromatid exchanges, mutations, and unscheduled
16 DNA synthesis in human and rodent cells in vitro. Reverse and forward mutations have been
17 consistently produced in bacterial assays and in vitro assays using eukaryotic cells. Substance 3,
18 however, did not induce dominant lethal mutations in mice or rats, or chromosomal aberrations or
19 micronuclei in bone marrow cells of mice treated in vivo.

20 21 Evaluation

22 Available epidemiologic data are considered inadequate for an evaluation of a causal
23 association of exposure to the substance and excess of cancer mortality due to major study limitations.

24 There is extensive evidence that substance 3 is carcinogenic in laboratory animals. Increased
25 incidences of tumors at multiple sites have been observed in multiple studies in two species of both
26 sexes with different routes of exposure. It induces tumors both at the site of entry (e.g., nasal tumors
27 via inhalation, forestomach tumors by ingestion, skin tumor with dermal exposure) and at distal sites
28 (e.g., mammary gland tumors). Additionally, it induced tumors at the same sites in both species and
29 sexes via different routes of exposure (e.g., lung tumors). With the exception of the oral study in which
30 the employed doses caused excessive toxicity and mortality, the other studies are considered
31 adequately designed and well conducted. Overall, given the magnitude and extent of animal
32 carcinogenic responses to substance 3, coupled with similar responses to structurally related
33 substances, these animal findings are judged to be highly relevant and predictive of human responses.

1 Other key data, while not very extensive, are judged to be supportive of carcinogenic potential.
2 Substance 3 has consistently been shown to be mutagenic in mammalian cells, including human cells,
3 and nonmammalian cells; thus, mutation is likely a mode of action for its carcinogenic activity.
4 However, the possible involvement of other modes of action has not been fully investigated.
5 Furthermore, induction of genetic changes from in vivo exposure to substance 3 has not been
6 demonstrated.

7 8 Conclusion

9 Substance 3 is *likely to be a human carcinogen by any route of exposure*. In comparison
10 with other agents designated as likely human carcinogens, the overall weight of evidence for substance
11 3 puts it at the *high end* of the grouping.

12 The weight of evidence of human carcinogenicity is based on animal evidence and other key
13 evidence. Human data are inadequate for an evaluation of human carcinogenicity. The overall weight
14 of evidence is based on (a) extensive animal evidence showing induction of increases of tumors at
15 multiple sites in both sexes of two rodent species via three routes of administration relevant to human
16 exposure; (b) tumor data of structural analogues exhibiting similar patterns of tumors in treated rodents;
17 (c) in vitro evidence for mutagenic effects in mammalian cells and nonmammalian systems; and (d) its
18 ability to be absorbed by all routes of exposure followed by rapid distribution throughout the body.

19 Some uncertainties are associated with the mechanisms of carcinogenicity of substance 3.
20 Although there is considerable evidence indicating that mutagenic events could account for carcinogenic
21 effects, there is still a lack of adequate information on the mutagenicity of substance 3 in vivo in animals
22 or humans. Moreover, alternative modes of action have not been explored. Nonetheless, available
23 data indicate a likely mutagenic mode of action. Linear extrapolation should be assumed in dose
24 response assessment.

25 26 ***Example 4: "Likely Human Carcinogen"--All Routes/Linear and Nonlinear Extrapolation***

27 Human Data

28 Substance 4 is a chlorinated alkene solvent. Several cohort studies of dry cleaning and laundry
29 workers exposed to substance 4 and other solvents reported significant excesses of mortality due to
30 cancers of the lung, cervix, esophagus, kidney, bladder, lymphatic and hematopoietic system, colon, or
31 skin. No significant cancer risks were observed in a subcohort of one these investigations of dry
32 cleaning workers exposed mainly to substance 4. Possible confounding factors such as smoking,
33 alcohol consumption, or low socioeconomic status were not considered in the analyses of these studies.

1 A large case-control study of bladder cancer did not show any clear association with dry
2 cleaning. Several case-control studies of liver cancer identified an increased risk of liver cancer with
3 occupational exposure to organic solvents. The specific solvents to which workers were exposed and
4 exposure levels were not identified.

5 6 Animal Data

7 The potential carcinogenicity of substance 4 has been investigated in two long-term studies in
8 rats and mice of both sexes by oral administration and inhalation.

9 Significant increases in hepatocellular carcinomas were induced in mice of both sexes treated
10 with substance 4 by oral gavage. No increases in tumor incidence were observed in treated rats.
11 Limitations in both experiments included control groups smaller than treated groups, numerous dose
12 adjustments during the study, and early mortality due to treatment-related nephropathy.

13 In the inhalation study, there were significantly increased incidences of hepatocellular adenoma
14 and carcinoma in exposed mice of both sexes. In rats of both sexes, there were marginally significant
15 increased incidences of mononuclear cell leukemia (MCL) when compared with concurrent controls.
16 The incidences of MCL in control animals, however, were higher than historical controls from the
17 conducting laboratory. The tumor finding was also judged to be biologically significant because the time
18 to onset of tumor was decreased and the disease was more severe in treated than in control animals.
19 Low incidences of renal tubular cell adenomas or adenocarcinomas were also observed in exposed
20 male rats. The tumor incidences were not statistically significant but there was a significant trend.

21 22 Other Key Data

23 Substance 4 has been shown to be readily and rapidly absorbed by inhalation and ingestion in
24 humans and laboratory animals. Absorption by dermal exposure is slow and limited. Once absorbed,
25 substance 4 is primarily distributed to and accumulated in adipose tissue and the brain, kidney, and
26 liver. A large percentage of substance 4 is eliminated unchanged in exhaled air, with urinary excretion
27 of metabolites comprising a much smaller percentage. The absorption and distribution profiles of
28 substance 4 are similar across species including humans.

29 Two major metabolites (trichloroacetic acid (TCA), and trichloroethanol), which are formed by
30 a P-450-dependent mixed-function oxidase enzyme system, have been identified in all studied species,
31 including humans. There is suggestive evidence for the formation of an epoxide intermediate based on
32 the detection of two other metabolites (oxalic acid and trichloroacetyl amide). In addition to oxidative
33 metabolism, substance 4 also undergoes conjugation with glutathione. Further metabolism by renal
34 beta-lyases could lead to two minor active metabolites (trichlorovinyl thiol and dichlorothiokente).

1 Toxicokinetic studies have shown that the enzymes responsible for the metabolism of substance
2 4 can be saturated at high exposures. The glutathione pathway was found to be a minor pathway at
3 low doses, but more prevalent following saturation of the cytochrome P-450 pathway. Comparative in
4 vitro studies indicate that mice have the greater capacity to metabolize to TCA than rats and humans.
5 Inhalation studies also indicate saturation of oxidative metabolism of substance 4, which occurs at
6 higher dose levels in mice than in rats and humans. Based on these findings, it has been postulated that
7 the species differences in the carcinogenicity of substance 4 between rats and mice may be related to
8 the differences in the metabolism to TCA and glutathione conjugates.

9 Substance 4 is a member of the class of chlorinated organics that often cause liver and kidney
10 toxicity and carcinogenesis in rodents. Like many chlorinated organics, substance 4 itself does not
11 appear to be mutagenic. Substance 4 was generally negative in in vitro bacterial systems and in vivo
12 mammalian systems. However, a minor metabolite formed in the kidney by the glutathione conjugation
13 pathway has been found to be a strong mutagen.

14 The mechanisms of induced carcinogenic effects of substance 4 in rats and mice are not
15 completely understood. It has been postulated that mouse liver carcinogenesis is related to liver
16 peroxisomal proliferation and toxicity of the metabolite TCA. Information on whether or not TCA
17 induces peroxisomal proliferation in humans is not definitive. The induced renal tumors in male rats may
18 be related either to kidney toxicity or the activity of a mutagenic metabolite. The mechanisms of
19 increases in MCL in rats are not known.

20 21 Evaluation

22 Available epidemiologic studies, taken together, provide suggestive evidence of a possible
23 causal association between exposure to substance 4 and cancer incidence in the laundry and dry
24 cleaning industries. This is based on consistent findings of elevated cancer risks in several studies of
25 different populations of dry cleaning and laundry workers. However, each individual study is
26 compromised by a number of study deficiencies including small numbers of cancers, confounding
27 exposure to other solvents, and poor exposure characterization. Others may interpret these findings
28 collectively as inconclusive.

29 There is considerable evidence that substance 4 is carcinogenic to laboratory animals. It
30 induces tumors in mice of both sexes by oral and inhalation exposure and in rats of both sexes via
31 inhalation. However, due to incomplete understanding of the mode of mechanism of action, the
32 predictivity of animal responses to humans is uncertain.

33 Animal data of structurally related compounds showing common target organs of toxicity and
34 carcinogenic effects (but lack of mutagenic effects) provide additional support for the carcinogenicity of

1 substance 4. Comparative toxicokinetic and metabolism information indicates that the mouse may be
2 more susceptible to liver carcinogenesis than rats and humans. This may indicate differences of the
3 degree and extent of carcinogenic responses, but does not detract from the qualitative weight of
4 evidence of human carcinogenicity. The toxicokinetic information also indicates that oral and inhalation
5 are the major routes of human exposure.

6 7 Conclusion

8 Substance 4 is *likely to be carcinogenic to humans by all routes of exposure*. The weight
9 of evidence of human carcinogenicity is based on: (a) demonstrated evidence of carcinogenicity in two
10 rodent species of both sexes via two relevant routes of human exposure; (b) the substance's similarity in
11 structure to other chlorinated organics that are known to cause liver and kidney toxicity and
12 carcinogenesis in rodents; (c) suggestive evidence of a possible association between exposure to the
13 substance in the laundry and dry cleaning industries and increased cancer incidence; and (d) human and
14 animal data indicating that the substance is absorbed by all routes of exposure.

15 In comparison with other agents designated as likely carcinogens, the overall weight of evidence
16 places it the lower end of the grouping. This is because there is a lack of good evidence that observed
17 excess cancer risk in exposed workers is due solely to substance 4. Moreover, there is considerable
18 scientific uncertainty about the human significance of certain rodent tumors associated with substance 4
19 and related compounds. In this case, the human relevance of the animal evidence of carcinogenicity
20 relies on the default assumption.

21 Overall, there is not enough evidence to give high confidence in a conclusion about any single
22 mode of action; it appears that more than one is plausible in different rodent tissues. Nevertheless, the
23 lack of mutagenicity of substance 4 and its general growth-promoting effect on high background tumors
24 as well as its toxicity toward mouse liver and rat kidney tissue support the view that the predominant
25 mode is growth-promoting rather than mutagenic. A mutagenic contribution to carcinogenicity due to a
26 metabolite cannot be ruled out. The dose response assessment should, therefore, adopt both default
27 approaches, nonlinear and linear extrapolations. The latter approach is very conservative since it likely
28 overestimates risk at low doses in this case, and is primarily useful for screening analyses.

29 30 ***Example 5: "Likely/Not Likely Human Carcinogen"--Range of Dose*** 31 ***Limited, Margin-of-Exposure Extrapolation***

32 Human Data

33 Substance 5 is a metal-conjugated phosphonate. No human tumor or toxicity data exist on this
34 chemical.

1 Animal Data

2 Substance 5 caused a statistically significant increase in the incidence of urinary bladder tumors
3 in male, but not female, rats at 30,000 ppm (3%) in the diet in a long-term study. Some of these
4 animals had accompanying urinary tract stones and toxicity. No bladder tumors or adverse urinary
5 tract effects were seen in two lower dose groups (2,000 and 8,000 ppm) in the same study. A chronic
6 dietary study in mice at doses comparable to those in the rat study showed no tumor response or
7 urinary tract effects. A 2-year study in dogs at doses up to 40,000 ppm showed no adverse urinary
8 tract effects.

9
10 Other Key Data

11 Subchronic dosing of rats confirmed that there was profound development of stones in the male
12 bladder at doses comparable to those causing cancer in the chronic study, but not at lower doses.
13 Sloughing of the epithelium of the urinary tract accompanied the stones.

14 There was a lack of mutagenicity relevant to carcinogenicity. In addition, there is nothing about
15 the chemical structure of substance 5 to indicate DNA-reactivity or carcinogenicity.

16 Substance 5 is composed of a metal, ethanol, and a simple phosphorus-oxygen-containing
17 component. The metal is not absorbed from the gut, whereas the other two components are absorbed.
18 At high doses, ethanol is metabolized to carbon dioxide, which makes the urine more acidic; the
19 phosphorus level in the blood is increased and calcium in the urine is increased. Chronic testing of the
20 phosphorus-oxygen-containing component alone in rats did not show any tumors or adverse effects on
21 the urinary tract.

22 Because substance 5 is a metal complex, it is not likely to be readily absorbed from the skin.

23
24 Evaluation

25 Substance 5 produced cancer of the bladder and urinary tract toxicity in male, but not female
26 rats and mice, and dogs failed to show the toxicity noted in male rats. The mode of action developed
27 from the other key data to account for the toxicity and tumors in the male rats is the production of
28 bladder stones. At high but not lower subchronic doses in the male rat, substance 5 leads to elevated
29 blood phosphorus levels; the body responds by releasing excess calcium into the urine. The calcium
30 and phosphorus combine in the urine and precipitate into multiple stones in the bladder. The stones are
31 very irritating to the bladder; the bladder lining is eroded, and cell proliferation occurs to compensate
32 for the loss of the lining. Cell layers pile up, and finally, tumors develop. Stone formation does not
33 involve the chemical per se but is secondary to the effects of its constituents on the blood and,

1 ultimately, the urine. Bladder stones, regardless of their cause, commonly produce bladder tumors in
2 rodents, especially the male rat.

3 4 Conclusion

5 Substance 5, a metal aliphatic phosphonate, is *likely* to be carcinogenic to humans only under
6 high-exposure conditions following *oral and inhalation exposure* that lead to bladder stone formation,
7 but is *not likely* to be carcinogenic under low-exposure conditions. It is *not likely to be a human*
8 *carcinogen* via the *dermal* route, given that the compound is a metal conjugate that is readily ionized
9 and its dermal absorption is not anticipated. The weight of evidence is based on (a) bladder tumors
10 only in male rats; (b) the absence of tumors at any other site in rats or mice; (c) the formation of
11 calcium-phosphorus-containing bladder stones in male rats at high, but not low, exposures that erode
12 bladder epithelium and result in profound increases in cell proliferation and cancer; and (d) the absence
13 of structural alerts or mutagenic activity.

14 There is a strong mode of action basis for the requirements of (a) high doses of substance 5, (b)
15 which lead to excess calcium and increased acidity in the urine, (c) which result in the precipitation of
16 stones and (d) the necessity of stones for toxic effects and tumor hazard potential. Lower doses fail to
17 perturb urinary constituents, lead to stones, produce toxicity, or give rise to tumors. Therefore, dose
18 response assessment should assume nonlinearity.

19 A major uncertainty is whether the profound effects of substance 5 may be unique to the rat.
20 Even if substance 5 produced stones in humans, there is only limited evidence that humans with bladder
21 stones develop cancer. Most often human bladder stones are either passed in the urine or lead to
22 symptoms resulting in their removal. However, since one cannot totally dismiss the male rat findings,
23 some hazard potential may exist in humans following intense exposures. Only fundamental research
24 could illuminate this uncertainty.

25 26 ***Example 6: "Cannot Be Determined"--Suggestive Evidence***

27 Human Data

28 Substance 6 is an unsaturated aldehyde. In a cohort study of workers in a chemical plant
29 exposed to a mixture of chemicals with substance 6 as a minor component, an elevated risk of cancer
30 than was expected was reported. This study is considered inadequate because of multiple exposures,
31 small cohort, and poor exposure characterization.

1 Substance 6 was tested for potential carcinogenicity in a drinking water study in rats, an
2 inhalation study in hamsters, and a skin painting study in mice. No significant increases in tumors were
3 observed in male rats treated with substance 6 at three dose levels in drinking water. However, a
4 significant increase of adrenal cortical adenomas was found in the only treated female dose group
5 administered a dose equivalent to the high dose of males. This study used a small number of animals
6 (20 per dose group).

7 No significant finding was detected in the inhalation study in hamsters. This study is inadequate
8 due to the use of too few animals, short duration of exposure, and inappropriate dose selection (use of
9 a single exposure that was excessively toxic as reflected by high mortality).

10 No increase in tumors was induced in the skin painting study in mice. This study is of
11 inadequate design for carcinogenicity evaluation because of several deficiencies: small number of
12 animals, short duration of exposure, lack of reporting about the sex and age of animals, and purity of
13 test material.

14 Substance 6 is structurally related to lowmolecularweight aldehydes that generally exhibit
15 carcinogenic effects in the respiratory tracts of laboratory animals via inhalation exposure. Three skin
16 painting studies in mice and two subcutaneous injection studies of rats and mice were conducted to
17 evaluate the carcinogenic potential of a possible metabolite of substance 6 (identified in vitro).
18 Increased incidences of either benign or combined benign and malignant skin tumors were found in the
19 dermal studies. In the injection studies of rats and mice, increased incidences of local sarcomas or
20 squamous cell carcinoma were found at the sites of injection. All of these studies are limited by the
21 small number of test animals, the lack of characterization of test material, and the use of single doses.
22

23 Other Key Data

24 Substance 6 is a flammable liquid at room temperature. Limited information on its
25 toxicokinetics indicates that it can be absorbed by all routes of exposure. It is eliminated in the urine
26 mainly as glutathione conjugates. Substance 6 is metabolized in vitro by rat liver and lung microsomal
27 preparations to a dihydroxylated aldehyde.

28 No data were available on the genetic and related effects of substance 6 in humans. It did not
29 induce dominant lethal mutations in mice. It induced sister chromatid exchanges in rodent cells in vitro.
30 The mutagenicity of substance 6 is equivocal in bacteria. It did not induce DNA damage or mutations
31 in fungi.
32

1 Available human data are judged inadequate for an evaluation of any causal relationship
2 between exposure to substance 6 and human cancer.

3 The carcinogenic potential of substance 6 has not been adequately studied in laboratory animals
4 due to serious deficiencies in study design, especially the inhalation and dermal studies. There is some
5 evidence of carcinogenicity in the drinking water study in female rats. However, the significance and
6 predictivity of that study to human response are uncertain since the finding is limited to occurrence of
7 benign tumors, one sex, and at the high dose only. Additional suggestion for animal carcinogenicity
8 comes from observation that a possible metabolite is carcinogenic at the site of administration. This
9 metabolite, however, has not been studied in vivo. Overall, the animal evidence is judged to be
10 suggestive for human carcinogenicity.

11 Other key data, taken together, do not add significantly to the overall weight of evidence of
12 carcinogenicity. SAR analysis indicates that substance 6 would be DNA-reactive. However,
13 mutagenicity data are inconclusive. Limited in vivo data do not support a mutagenic effect. While there
14 is some evidence of DNA damage in rodent cells in vitro, there is either equivocal or no evidence of
15 mutagenicity in nonmammalian systems.

16 17 Conclusion

18 The human carcinogenicity potential of substance 6 cannot be determined on the basis of
19 available information. Both human and animal data are judged inadequate for an evaluation. There is
20 evidence suggestive of potential carcinogenicity on the basis of limited animal findings and SAR
21 considerations. Data are not sufficient to judge whether there is a mode of carcinogenic action.
22 Additional studies are needed for a full evaluation of the potential carcinogenicity of substance 6.
23 Hence, dose response assessment is not appropriate.

24 25 ***Example 7: "Not Likely Human Carcinogen"--Appropriately Studied Chemical in*** 26 ***Animals Without Tumor Effects***

27 Human Data

28 Substance 7, a plant extract, has not been studied for its toxic or carcinogenic potential in
29 humans.

30 31 Animal Data

32 Substance 7 has been studied in four chronic studies in three rodent species. In a feeding study
33 in rats, males showed a nonsignificant increase in benign tumors of the parathyroid gland in the high-
34 dose group, where the incidence in concurrent controls greatly exceeded the historical control range.

1 Females demonstrated a significant increase in various subcutaneous tumors in the low-dose group, but
2 findings were not confirmed in the high-dose group, and there was no dose response relationship.
3 These effects were considered as not adding to the evidence of carcinogenicity. No tumor increases
4 were noted in a second adequate feeding study in male and female rats. In a mouse feeding study, no
5 tumor increases were noted in dosed animals. There was some question as to the adequacy of the
6 dosing; however it was noted that in the mouse 90-d subchronic study, a dose of twice the high dose in
7 the chronic study led to significant decrements in body weight. In a hamster study there were no
8 significant increases in tumors at any site. No structural analogues of substance 7 have been tested for
9 cancer.

10 11 Other Key Data

12 There are no structural alerts that would suggest that substance 7 is a DNA-reactive
13 compound. It is negative for gene mutations in bacteria and yeast, but positive in cultured mouse cells.
14 Tests for structural chromosome aberrations in cultured mammalian cells and in rats are negative;
15 however, the animals were not tested at sufficiently high doses. Substance 7 binds to proteins of the
16 cell division spindle; therefore, there is some likelihood for producing numerical chromosome
17 aberrations, an endpoint that is sometimes noted in cancers. In sum, there is limited and conflicting
18 information concerning the mutagenic potential of the agent.

19 The compound is absorbed via oral and inhalation exposure but only poorly via the skin.

20 21 Evaluation

22 The only indication of a carcinogenic effect comes from the finding of benign tumors in male rats
23 in a single study. There is no confirmation of a carcinogenic potential from dosed females in that study,
24 in males and females in a second rat study, or from mouse and hamster studies.

25 There is no structural indication that substance 7 is DNA-reactive, there is inconsistent
26 evidence of gene mutations, and chromosome aberration testing is negative. The agent binds to cell
27 division spindle proteins and may have the capacity to induce numerical chromosome anomalies.
28 Further information on gene mutations and in vivo structural and numerical chromosome aberrations
29 may be warranted.

30 31 Conclusion

32 Substance 7 is *not likely* to be carcinogenic to humans via all relevant routes of exposure. This
33 weight of evidence judgment is largely based on the absence of significant tumor increases in chronic

1 rodent studies. Adequate cancer studies in rats, mice, and hamsters fail to show any carcinogenic
2 effect; a second rat study showed an increase in benign tumors at a site in dosed males, but not females.
3

4 **2.7. PRESENTATION OF RESULTS**

5 The results of the hazard assessment are presented in the form of an overall technical hazard
6 characterization. Additionally, a weight of evidence narrative is used when the conclusion as to
7 carcinogenic potential needs to be presented separately from the overall characterization.
8

9 **2.7.1. Technical Hazard Characterization**

10 The hazard characterization has two functions. First, it presents results of the hazard
11 assessment and an explanation of how the weight of evidence conclusion was reached. It explains the
12 potential for human hazard, anticipated attributes of its expression, and mode of action considerations
13 for dose response. Second, it contains the information needed for eventual incorporation into a risk
14 characterization consistent with EPA guidance on risk characterization (U.S. EPA, 1995).

15 The characterization qualitatively describes the conditions under which the agent's effects may
16 be expressed in human beings. These qualitative hazard conditions are ones that are observable in the
17 toxicity data without having done either quantitative dose response or exposure assessment. The
18 description includes how expression is affected by route of exposure and dose levels and durations of
19 exposure.

20 The discussion of limitations of dose as a qualitative aspect of hazard addresses the question of
21 whether reaching a certain dose range appears to be a precondition for a hazard to be expressed; for
22 example, when carcinogenic effects are secondary to another toxic effect that appears only when a
23 certain dose level is reached. The assumption is made that an agent that causes internal tumors by one
24 route of exposure will be carcinogenic by another route, if it is absorbed by the second route to give an
25 internal dose. Conversely, if there is a route of exposure by which the agent is not absorbed (does not
26 cross an absorption barrier; e.g., the exchange boundaries of skin, lung, and digestive tract through
27 uptake processes) to any significant degree, hazard is not anticipated by that route. An exception to the
28 latter statement would be when the site of contact is also the target tissue of carcinogenicity. Duration
29 of exposure may be a precondition for hazard if, for example, the mode of action requires cytotoxicity
30 or a physiologic change, or is mitogenicity, for which exposure must be sustained for a period of time
31 before effects occur. The characterization could note that one would not anticipate a hazard from
32 isolated, acute exposures. The above conditions are qualitative ones regarding preconditions for
33 effects, not issues of relative absorption or potency at different dose levels. The latter are dealt with

1 under dose response assessment (section 3), and their implications can only be assessed after human
2 exposure data are applied in the characterization of risk.

3 The characterization describes conclusions about mode of action information and its support for
4 recommending dose response approaches.

5 The hazard characterization routinely includes the following in support of risk characterization:

- 6 ! a summary of results of the assessment,
- 7 ! identification of the kinds of data available to support conclusions and explanation of how
8 the data fit together, highlighting the quality of the data in each line of evidence, e.g., tumor
9 effects, short-term studies, structure-activity relationships), and highlighting the coherence of
10 inferences from the different kinds of data,
- 11 ! strengths and limitations (uncertainties) of the data and assessment, including identification of
12 default assumptions invoked in the face of missing or inadequate data,
- 13 ! identification of alternative interpretations of data that are considered equally plausible,
- 14 ! identification of any subpopulations believed to be more susceptible to the hazard than the
15 general population,
- 16 ! conclusions about the agent's mode of action and recommended dose response
17 approaches,
- 18 ! significant issues regarding interpretation of data that arose in the assessment. Typical ones
19 may include:
 - 20 -- determining causality in human studies,
 - 21 -- dosing (MTD), background tumor rates, relevance of animal tumors to humans,
 - 22 -- weighing studies with positive and null results, considering the influence of other
23 available kinds of evidence,
 - 24 -- drawing conclusions based on mode of action data versus using a default assumption
25 about the mode of action.

27 **2.7.2. Weight of Evidence Narrative**

28 The weight of evidence narrative summarizes the results of hazard assessment employing the
29 descriptors defined in section 2.6.1. The narrative (about two pages in length) explains an agent's
30 human carcinogenic potential and the conditions of its expression. If data do not allow a conclusion as
31 to carcinogenicity, the narrative explains the basis of this determination. An example narrative appears
32 below. More examples appear in Appendix A.

33 The items regularly included in a narrative are:

- 34 ! name of agent and Chemical Abstracts Services number, if available,

- 1 ! conclusions (by route of exposure) about human carcinogenicity, using a standard descriptor
- 2 from section 2.6.1,
- 3 ! summary of human and animal tumor data on the agent or its structural analogues, their
- 4 relevance, and biological plausibility,
- 5 ! other key data (e.g., structure-activity data, toxicokinetics and metabolism, short-term
- 6 studies, other relevant toxicity or clinical data),
- 7 ! discussion of possible mode(s) of action and appropriate dose response approach(es),
- 8 ! conditions of expression of carcinogenicity, including route, duration, and magnitude of
- 9 exposure.

Example Narrative

Aromatic Compound

CAS# XXX

CANCER HAZARD SUMMARY

Aromatic compound (AR) is *known* to be carcinogenic to humans by all routes of exposure.

The weight of evidence of human carcinogenicity is based on (a) consistent evidence of elevated leukemia incidence in studies of exposed workers and significant increases of genetic damage in bone marrow cells and blood lymphocytes of exposed workers; (b) significantly increased incidence of cancer in both sexes of several strains of rats and mice; (c) genetic damage in bone marrow cells of exposed rodents and effects on intracellular signals that control cell growth.

AR is readily absorbed by all routes of exposure and rapidly distributed throughout the body. The mode of action of AR is not understood. A dose response assessment that assumes linearity of the relationship is recommended as a default.

SUPPORTING INFORMATION

Data include numerous human epidemiologic and biomonitoring studies, long-term bioassays, and other data on effects of AR on genetic material and cell growth processes. The key epidemiologic studies and animal studies are well conducted and reliable. The other data are generally of good quality also.

Human Effects

Numerous epidemiologic and case studies have reported an increased incidence or a causal relationship associating exposure to AR and leukemia. Among the studies are five for which the design and performance as well as follow-up are considered adequate to demonstrate the causal relationship.

1 Biomonitoring studies of exposed workers have found dose-related increases in chromosomal
2 aberrations in bone marrow cells and blood lymphocytes.

3
4 **Animal Effects**

5 AR caused increased incidence of tumors in various tissues in both sexes of several rat and
6 mouse strains. AR also caused chromosomal aberrations in rabbits, mice, and rats--as it does in
7 humans.

8
9 **Other Key Data**

10 AR itself is not DNA-reactive and is not mutagenic in an array of test systems both in vitro and
11 in vivo. Metabolism of AR yields several metabolites that have been separately studied for effects on
12 carcinogenic processes. Some have mutagenic activity in test systems and some have other effects on
13 cell growth controls inside cells.

14
15 **MODE OF ACTION**

16 No rodent tumor precisely matches human leukemia in pathology. The closest parallel is a
17 mouse cancer of blood-forming tissue. Studies of the effects of AR at the cell level in this model system
18 are ongoing. As yet, the mode of action of AR is unclear, but most likely the carcinogenic activity is
19 associated with one or a combination of its metabolites. It is appropriate to apply a linear approach to
20 the dose response assessment pending a better understanding because: (a) genetic damage is a typical
21 effect of AR exposure in mammals and (b) metabolites of AR produce mutagenic effects in addition to
22 their other effects on cell growth controls; AR is a multitissue carcinogen in mammals suggesting that it is
23 affecting a common controlling mechanism of cell growth.

3. DOSE RESPONSE ASSESSMENT

Dose response assessment first addresses the relationship of dose² to the degree of response observed in an experiment or human study. When environmental exposures are outside of the range of observation, extrapolations are necessary in order to estimate or characterize the dose relationship (ILSI, 1995). In general, three extrapolations may be made: from high to low doses, from animal to human responses, and from one route of exposure to another.

The dose response assessment proceeds in two parts. The first is assessment of the data in the range of empirical observation. This is followed by extrapolations either by modeling, if there are sufficient data to support a model, or by a default procedure based as much as possible on information about the agent's mode of action. The following discussion covers the assessment of observed data and extrapolation procedures, followed by sections on analysis of response data and analysis of dose data. The final section discusses dose response characterization.

3.1. DOSE RESPONSE RELATIONSHIP

In the discussion that follows, reference to “response” data includes measures of tumorigenicity as well as other responses related to carcinogenicity. The other responses may include effects such as changes in DNA, chromosomes, or other key macromolecules, effects on growth signal transduction, induction of physiological or hormonal changes, effects on cell proliferation, or other effects that play a role in the process. Responses other than tumorigenicity may be considered part of the observed range in order either to extend the tumor dose response analysis or to inform it. The nontumor response or responses also may be used in lieu of tumor data if they are considered to be a more informative representation of the carcinogenic process for an agent (see section 3.2).

3.1.1. Analysis in the Range of Observation

Biologically Based and Case-Specific Models

A biologically based model is one whose parameters are calculated independently of curve-fitting of tumor data. If data are sufficient to support a biologically based model specific to the agent and the purpose of the assessment is such as to justify investing resources supporting use, this is the first choice for both the observed tumor and related response data and for extrapolation below the range of

For this discussion, “exposure” means contact of an agent with the outer boundary of an organism. “Applied dose” means the amount of an agent presented to an absorption barrier and available for absorption. “Internal dose” means the amount crossing an absorption barrier (e.g., the exchange boundaries of skin, lung, and digestive tract) through uptake processes. “Delivered dose” for an organ or cell means the amount available for interaction with that organ or cell (U.S. EPA, 1992a).

1 observed data in either animal or human studies. Examples are the two-stage models of initiation plus
2 clonal expansion and progression developed by Moolgavkar and Knudson (1981) and Chen and
3 Farland (1991). Such models require extensive data to build the form of the model as well as to
4 estimate how well it conforms with the observed carcinogenicity data. Theoretical estimates of process
5 parameters, such as cell proliferation rates, are not used to enable application of such a model (Portier,
6 1987).

7 Similarly preferred as a first choice are dose response models based on general concepts of
8 mode of action and data on the agent. For a case-specific model, model parameters and data are
9 obtained from studies on the agent.

10 In most cases, a biologically based or case-specific model will not be practicable, either
11 because the necessary data do not exist or the decisions that the assessment are to support do not
12 justify or permit, the time and resources required. In these cases, the analysis proceeds using curve-
13 fitting models followed by default procedures for extrapolation, based, to the extent possible, on mode
14 of action and other biological information about the agent. These methods and assumptions are
15 described below.

16 *Curve-Fitting and Point of Departure for Extrapolation*

17 Curve-fitting models are used that are appropriate to the kind of response data in the observed
18 range. Any of several models can be used; e.g., the models developed for benchmark dose estimation
19 for noncancer endpoints may be applied (Barnes et al., 1995). For some data sets, particularly those
20 with extreme curvature, the impact of model selection can be significant. In these cases, the choice is
21 rationalized on biological grounds as possible. In other cases, the nature of the data or the way it is
22 reported will suggest other types of models; for instance, when longitudinal data on tumor development
23 are available, time to tumor or survival models may be necessary and appropriate to fit the data.

24 A point of departure for extrapolation is estimated. This is a point that is either a data point or
25 an estimated point that can be considered to be in the range of observation, without any significant
26 extrapolation. The LED₁₀--the lower 95% confidence limit on a dose associated with 10% extra risk--
27 is such a point and is the standard point of departure, adopted as a matter of science policy to remain
28 as consistent and comparable from case to case as possible.³ It is also a comparison point for
29 noncancer endpoints (U.S. EPA, 1991f). The central estimate of the ED₁₀ also may be appropriate for
30 use in relative hazard and potency ranking.
31

It is appropriate to report the central estimate of the ED₁₀, the upper and lower 95% confidence limits, and a graphical representation of model fit.

1 For some data sets, a choice of point of departure other than the LED₁₀ may be appropriate.
2 For example, if the observed response is below the LED₁₀, then a lower point may be a better choice.
3 Moreover, some forms of data may not be amenable to curve-fitting estimation, but to estimation of a
4 "low-" or "no-observable-adverse-effect level" (LOAEL, NOAEL) instead, e.g., certain continuous
5 data.

6 The rationale supporting the use of the LED₁₀ is that it a 10% response is at or just below the
7 limit of sensitivity of discerning a significant difference in most long-term rodent studies. The lower
8 confidence limit on dose is used to appropriately account for experimental uncertainty (Barnes et al.,
9 1995) and for consistency with the "benchmark dose" approach for noncancer assessment; it does not
10 provide information about human variability. In laboratory studies of cancer or noncancer endpoints,
11 the level of dose at which increased incidence of effects can be detected, as compared to controls, is a
12 function of the size of the sample (e.g., number of animals), dose spacing, and other design aspects. In
13 noncancer assessment, the dose at which significant effects are not observed is traditionally termed the
14 NOAEL. This is not, in fact, a level of zero effect. The NOAEL in most study protocols is about the
15 same as an LED₅ or LED₁₀--the lower 95% confidence limit on a dose associated with a 5% or 10%
16 increased effect (Faustman et al., 1994; Haseman, 1983). Adopting parallel points of departure for
17 cancer and noncancer assessment is intended to make discussion and comparison of the two kinds of
18 assessment more comparable because of their similar science and science policy bases and similar
19 analytic approaches.

20 Analysis of human studies in the observed range is designed case by case, depending on the
21 type of study and how dose and response are measured in the study. In some cases the agent may
22 have discernible interactive effects with another agent (e.g., asbestos and smoking), making possible
23 estimation of contribution of the agent and others as risk factors. Also, in some cases, estimation of
24 population risk in addition, or in lieu of, individual risk may be appropriate.

26 **3.1.2. Analysis in the Range of Extrapolation**

27 Extrapolation to lower doses is usually necessary, and in the absence of a biologically based or
28 case-specific model, is based on one of the three default procedures described below. The Agency
29 has adopted these three procedures as a matter of science policy based on current hypotheses of the
30 likely shapes of dose response curves for differing modes of action. The choice of the procedure to be
31 used in an individual case is a judgment based on the agent's modes of action.

1 A default assumption of linearity is appropriate when the evidence supports a mode of action of
2 gene mutation due to DNA reactivity or supports another mode of action that is anticipated to be linear.
3 Other elements of empirical support may also support an inference of linearity, e.g., the background of
4 human exposure to an agent might be such that added human exposure is on the linear part of a dose
5 response curve that is sublinear overall. The default assumption of linearity is also appropriate as the
6 ultimate science policy default when evidence shows no DNA reactivity or other support for linearity,
7 but neither does it show sufficient evidence of a nonlinear mode of action to support a nonlinear
8 procedure.

9 For linear extrapolation, a straight line is drawn from the point of departure to the origin--zero
10 dose, zero response (Flamm and Winbush, 1984; Gaylor and Kodell, 1980; Krewski et al., 1984).
11 This approach is generally conservative of public health, in the absence of information about the extent
12 of human variability in sensitivity to effects. When a linear extrapolation procedure is used, the risk
13 characterization summary displays the degree of extrapolation that is being made from empirical data
14 and discusses its implications for the interpretation of the resulting quantitative risk estimates.

15 16 *Nonlinear*

17 A default assumption of nonlinearity is appropriate when there is no evidence for linearity and
18 sufficient evidence to support an assumption of nonlinearity. The mode of action may lead to a dose
19 response relationship that is nonlinear, with response falling much more quickly than linearly with dose,
20 or being most influenced by individual differences in sensitivity. Alternatively, the mode of action may
21 theoretically have a threshold, e.g., the carcinogenicity may be a secondary effect of toxicity or of an
22 induced physiological change (see example 5, section 2.6.3) that is itself a threshold phenomenon.

23 As a matter of science policy under this analysis, nonlinear probability functions are not fitted to
24 the response data to extrapolate quantitative low-dose risk estimates because different models can lead
25 to a very wide range of results, and there is currently no basis, generally, to choose among them.
26 Sufficient information to choose leads to a biologically based or case-specific model. In cases of
27 nonlinearity, the risk is not extrapolated as a probability of an effect at low doses. A margin of
28 exposure analysis is used, as described below, to evaluate concern for levels of exposure. The margin
29 of exposure is the LED₁₀ or other point of departure divided by the environmental exposure of interest.
30 The EPA does not generally try to distinguish between modes of action that might imply a "true
31 threshold" from others with a nonlinear dose response relationship. Except in unusual cases where
32 extensive information is available, it is not possible to distinguish between these empirically.

33 The environmental exposures of interest, for which margins of exposure are estimated, may be
34 actual or projected future levels. The risk manager decides whether a given margin of exposure is

1 acceptable under applicable management policy criteria. The risk assessment provides supporting
2 information to assist the decisionmaker.

3 The EPA often conducts margin of exposure analyses to accompany estimates of reference
4 doses or concentrations (RfD, RfC) for noncancer endpoints.⁴ The procedure for a margin of exposure
5 analysis for a response related to carcinogenicity is operationally analogous, the difference being that a
6 threshold of cancer response is not necessarily presumed. If, in a particular case, the evidence indicates
7 a threshold, as in the case of carcinogenicity being secondary to another toxicity that has a threshold,
8 the margin of exposure analysis for the toxicity is the same as is done for a noncancer endpoint, and an
9 RfD or RfC for that toxicity also may be estimated and considered in cancer assessment.

10 The analogy between margin of exposure analysis for noncancer and cancer responses begins
11 with the analogy of points of departure; for both it is an effect level, either LED₁₀ or other point
12 (presented as a human equivalent dose or concentration), as data support. For cancer responses,
13 when animal data are used, the point of departure is a human equivalent dose or concentration arrived
14 at by interspecies dose adjustment or toxicokinetic analysis. It is likely that many of the margin of
15 exposure analyses for cancer will be for responses other than tumor incidence. This is because the
16 impetus for considering a carcinogenic agent to have a nonlinear dose response will be a conclusion that
17 there is sufficient evidence to support that view, and this evidence will often be information about a
18 response that is a precursor to tumors.

19 To support a risk manager's consideration of the margin of exposure, information is provided in
20 a risk assessment about current understanding of the phenomena that may be occurring as dose
21 (exposure) decreases substantially below the observed data. The goal is to provide as much
22 information as possible about the risk reduction that accompanies lowering of exposure. To this end,
23 some important points to address include:

- 24 ! the slope of the observed dose response relationship at the point of departure and its
25 uncertainties and implications for risk reduction associated with exposure reduction (a
26 shallow slope suggests less reduction than a steep slope),
- 27 ! the nature of the response used for the dose response assessment,
- 28 ! the nature and extent of human variability in sensitivity to the phenomena involved,
- 29 ! persistence of the agent in the body,
- 30 ! human sensitivity to the phenomena as compared with experimental animals.

An RfD or RfC is an estimate with uncertainty spanning perhaps an order of magnitude of daily exposure to the human population (including sensitive subgroups) that is anticipated to be without appreciable deleterious effects during a lifetime. It is arrived at by dividing empirical data on effects by uncertainty factors that consider inter- and intraspecies variability, extent of data on all important chronic exposure toxicity endpoints, and availability of chronic as opposed to subchronic data.

1 As a default assumption for two of these points, a factor of no less than 10-fold each may be
2 employed to account for human variability and for interspecies differences in sensitivity when humans
3 may be more sensitive than animals. When humans are found to be less sensitive than animals, a default
4 factor of no smaller than a 1/10 fraction may be employed to account for this. If any information about
5 human variability or interspecies differences is available, it is used instead of the default or to modify it
6 as appropriate. In the case of analysis based on human studies, obviously, interspecies differences are
7 not a factor. It should be noted that the dose response relationship and inter- or intraspecies variability
8 in sensitivity are independent. That is, reduction of dose reduces risk; it does not change variability. To
9 support consideration of acceptability of a margin of exposure by the risk manager, the assessment
10 considers all of the hazard and dose response factors together; hence, the factors for inter- and
11 intraspecies differences alone are not to be considered a default number for an acceptable margin of
12 exposure. (See Section 1.3.2.5.)

13 It is appropriate to provide a graphical representation of the data and dose response modeling
14 in the observed range, also showing exposure levels of interest to the decisionmaker. (See figure 3-1.)
15 In order to provide a frame of reference, by way of comparison, a straight line extrapolation may be
16 displayed to show what risk levels would be associated with decreasing dose, if the dose response
17 were linear. If this is done, the

1
2
3

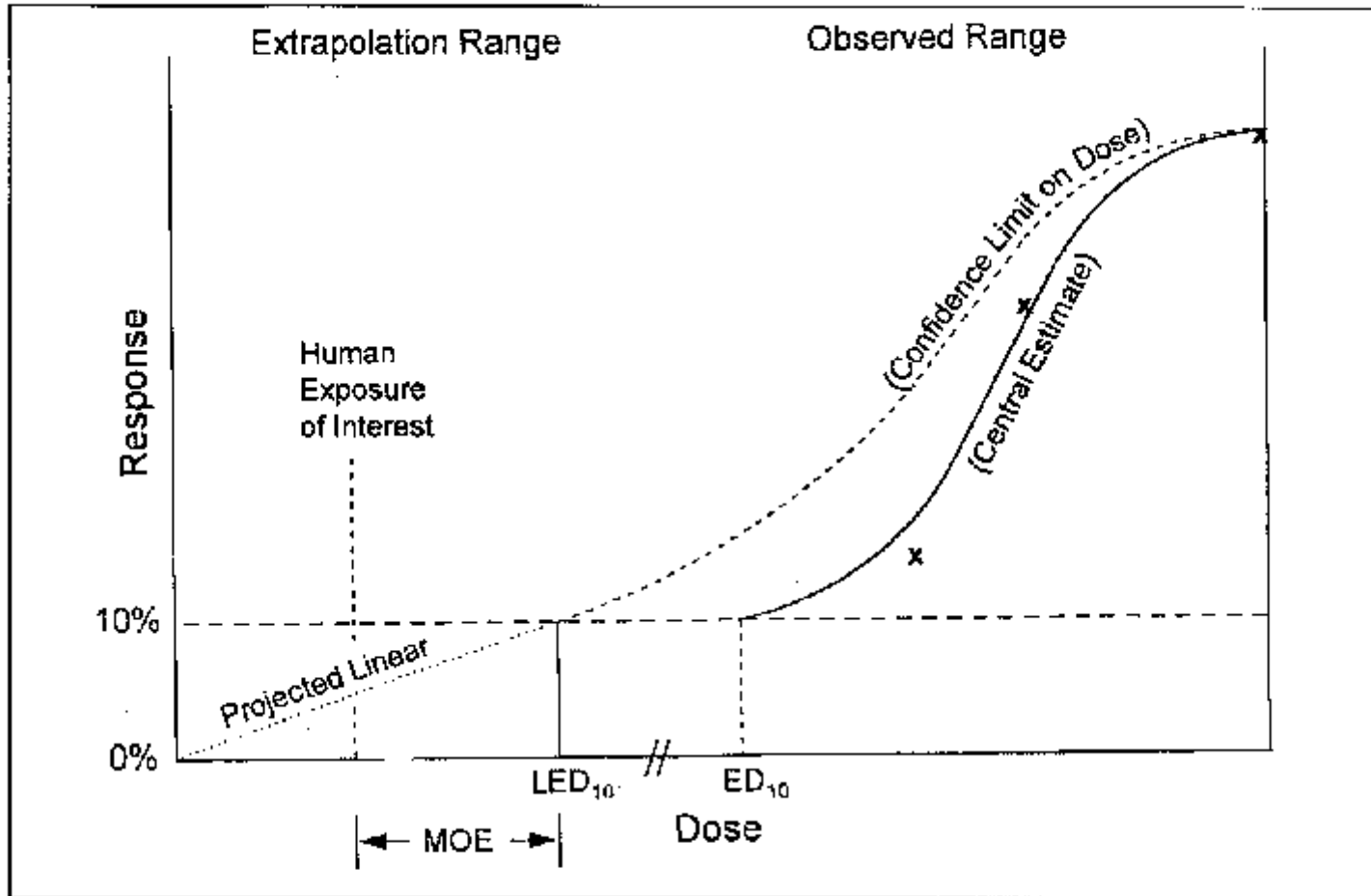


Figure 3-1. Graphical presentation of data and extrapolation.

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1 clear accompanying message is that, in this case of nonlinearity, the response falls disproportionately
2 with decreasing dose.

3 4 *Linear and Nonlinear*

5 Both linear and nonlinear procedures may be used in particular cases. If a mode of action
6 analysis finds substantial support for differing modes of action for different tumor sites, an appropriate
7 procedure is used for each. Both procedures may also be appropriate to discuss implications of
8 complex dose response relationships. For example, if it is apparent that an agent is both DNA reactive
9 and is highly active as a promotor at high doses, and there are insufficient data for modeling, both linear
10 and nonlinear default procedures may be needed to decouple and consider the contribution of both
11 phenomena.

12 13 **3.1.3. Use of Toxicity Equivalence Factors and Relative Potency Estimates**

14 A toxicity equivalence factor (TEF) procedure is one used to derive quantitative dose response
15 estimates for agents that are members of a category or class of agents. TEFs are based on shared
16 characteristics that can be used to order the class members by carcinogenic potency when cancer
17 bioassay data are inadequate for this purpose (U.S. EPA, 1991c). The ordering is by reference to the
18 characteristics and potency of a well-studied member or members of the class. Other class members
19 are indexed to the reference agent(s) by one or more shared characteristics to generate their TEFs.
20 The TEFs are usually indexed at increments of a factor of 10. Very good data may permit a smaller
21 increment to be used. Shared characteristics that may be used are, for example, receptor-binding
22 characteristics, results of assays of biological activity related to carcinogenicity, or structure-activity
23 relationships.

24 TEFs are generated and used for the limited purpose of assessment of agents or mixtures of
25 agents in environmental media when better data are not available. When better data become available
26 for an agent, its TEF should be replaced or revised. Criteria for constructing TEFs are given in U.S.
27 EPA (1991b). The criteria call for data that are adequate to support summing doses of the agents in
28 mixtures. To date, adequate data to support use of TEF's has been found in only one class of
29 compounds (dioxins) (U.S. EPA, 1989a).

30 Relative potencies can be similarly derived and used for agents with carcinogenicity or other
31 supporting data. These are conceptually similar to TEFs, but they are less firmly based in science and
32 do not have the same level of data to support them. They are used only when there is no better
33 alternative.

1 The uncertainties associated with both TEFs and relative potencies are explained whenever
2 they are used.

3 4 **3.2. RESPONSE DATA**

5 Response data for analysis include tumor incidence data from human or animal studies as well
6 as data on other responses as they relate to an agent's carcinogenicity, such as effects on growth
7 control processes or cell macromolecules or other toxic effects. Tumor incidence data are ordinarily
8 the basis of dose response assessment, but other response data can augment such assessment or
9 provide separate assessments of carcinogenicity or other important effects.

10 Data on carcinogenic processes underlying tumor effects may be used to support biologically
11 based or case-specific models. Other options for such data exist. If confidence is high in the linkage of
12 a precursor effect and the tumor effect, the assessment of tumor incidence may be extended to lower
13 dose levels by linking it to the assessment of the precursor effect (Swenberg et al., 1987). Even if a
14 quantitative link is not appropriate, the assessment for a precursor effect may provide a view of the
15 likely shape of the dose response curve for tumor incidence below the range of tumor observation
16 (Cohen and Ellwein, 1990; Choy, 1993). If responses other than tumor incidence are regarded as
17 better representations of the carcinogenicity of the agent, they may be used in lieu of tumor responses.
18 For example, if it is concluded that the carcinogenic effect is secondary to another toxic effect, the dose
19 response for the other effect will likely be more pertinent for risk assessment. As another example, if
20 disruption of hormone activity is the key mode of action of an agent, data on hormone activity may be
21 used in lieu of tumor incidence data.

22 If adequate positive human epidemiologic response data are available, they provide an
23 advantageous basis for analysis since concerns about interspecies extrapolation do not arise.
24 Adequacy of human exposure data for quantification is an important consideration in deciding whether
25 epidemiologic data are the best basis for analysis in a particular case. If adequate exposure data exist
26 in a well-designed and well-conducted epidemiologic study that detects no effects, it may be possible to
27 obtain an upper-bound estimate of the potential human risk to provide a check on plausibility of
28 available estimates based on animal tumor or other responses, e.g., do confidence limits on one overlap
29 the point estimate of the other?

30 When animal studies are used, response data from a species that responds most like humans
31 should be used if information to this effect exists. If this is unknown and an agent has been tested in
32 several experiments involving different animal species, strains, and sexes at several doses and different
33 routes of exposure, all of the data sets are considered and compared, and a judgment is made as to the

1 data to be used to best represent the observed data and important biological features such as mode of
2 action. Appropriate options for presenting results include:

- 3 ! use of a single data set,
- 4 ! combining data from different experiments (Stiteler et al., 1993; Vater et al., 1993),
- 5 ! showing a range of results from more than one data set,
- 6 ! showing results from analysis of more than one statistically significant tumor response based
7 on differing modes of action,
- 8 ! representing total response in a single experiment by combining animals with statistically
9 significant tumors at more than one site, or
- 10 ! a combination of these options.

11 The approach judged to best represent the data is presented with the rationale for the judgment,
12 including the biological and statistical considerations involved. The following are some points to
13 consider:

- 14 ! quality of study protocol and execution,
- 15 ! proportion of malignant neoplasms,
- 16 ! latency of onset of neoplasia,
- 17 ! number of data points to define the relationship of dose and response,
- 18 ! background incidence in test animal,
- 19 ! differences in range of response among species, sexes, strains,
- 20 ! most sensitive responding species, and
- 21 ! availability of data on related precursor events to tumor development.

22 Analyses of carcinogenic effects other than tumor incidence are similarly presented and evaluated for
23 their contribution to a best judgment on how to represent the biological data for dose response
24 assessment.

26 **3.3. DOSE DATA**

27 Whether animal experiments or epidemiologic studies are the sources of data, questions need to
28 be addressed in arriving at an appropriate measure of dose for the anticipated environmental exposure.
29 Among these are:

- 30 ! whether the dose is expressed as an environmental concentration, applied dose, or
31 delivered dose to the target organ,
- 32 ! whether the dose is expressed in terms of a parent compound, one or more metabolites, or
33 both,
- 34 ! the impact of dose patterns and timing where significant,

- 1 ! conversion from animal to human doses, where animal data are used, and
- 2 ! the conversion metric between routes of exposure where necessary and appropriate.

3 In practice, there may be little or no information on the concentration or identity of the active form at a
4 target; being able to compare the applied and delivered doses between routes and species is the ideal,
5 but is rarely attained. Even so, the objective is to use available data to obtain as close to a measure of
6 internal or delivered dose as possible.

7 The following discussion assumes that the analyst will have data of varying detail in different
8 cases about toxicokinetics and metabolism. Discussed below are approaches to basic data that are
9 most frequently available, as well as approaches and judgments for improving the analysis based on
10 additional data. The estimation of dose in human studies is tailored to the form of dose data available.

11 12 **3.3.1. Interspecies Adjustment of Dose**

13 When adequate data are available, the doses used in animal studies can be adjusted to
14 equivalent human doses using toxicokinetic information on the particular agent. The methods used
15 should be tailored to the nature of the data on a case-by-case basis. In rare cases, it may also be
16 possible to make adjustments based on toxicodynamic considerations. In most cases, however, there
17 are insufficient data available to compare dose between species. In these cases, the estimate of human
18 equivalent dose is based on science policy default assumptions. The defaults described below are
19 modified or replaced whenever better comparative data on toxicokinetic or metabolic relationships are
20 available. The availability and discussion of the latter also may permit reduction or discussion of
21 uncertainty in the analysis.

22 For oral exposure, the default assumption is that delivered doses are related to applied dose by
23 a power of body weight. This assumption rests on the similarities of mammalian anatomy, physiology,
24 and biochemistry generally observed across species. This assumption is more appropriate at low
25 applied dose concentrations where sources of nonlinearity, such as saturation or induction of enzyme
26 activity, are less likely to occur. To derive an equivalent human oral dose from animal data, the default
27 procedure is to scale daily applied doses experienced for a lifetime in proportion to body weight raised
28 to the 0.75 power ($W^{0.75}$). Equating exposure concentrations in parts per million units for food or
29 water is an alternative version of the same default procedure because daily intakes of these are in
30 proportion to $W^{0.75}$. The rationale for this factor rests on the empirical observation that rates of
31 physiological processes consistently tend to maintain proportionality with $W^{0.75}$. A more extensive
32 discussion of the rationale and data supporting the Agency's adoption of this scaling factor is in U.S.
33 EPA, 1992b. Information such as blood levels or exposure biomarkers or other data that are available
34 for interspecies comparison are used to improve the analysis when possible.

1 The default procedure to derive an human equivalent concentration of inhaled particles and
2 gases is described in U.S. EPA (1994) and Jarabek (1995a,b). The methodology estimates respiratory
3 deposition of inhaled particles and gases and provides methods for estimating internal doses of gases
4 with different absorption characteristics. The method is able to incorporate additional toxicokinetics
5 and metabolism to improve the analysis if such data are available.
6

7 **3.3.2. Toxicokinetic Analyses**

8 Physiologically based mathematical models are potentially the most comprehensive way to
9 account for toxicokinetic processes affecting dose. Models build on physiological compartmental
10 modeling and attempt to incorporate the dynamics of tissue perfusion and the kinetics of enzymes
11 involved in metabolism of an administered compound.

12 A comprehensive model requires the availability of empirical data on the carcinogenic activity
13 contributed by parent compound and metabolite or metabolites and data by which to compare kinetics
14 of metabolism and elimination between species. A discussion of issues of confidence accompanies
15 presentation of model results (Monro, 1992). This includes considerations of model validation and
16 sensitivity analysis that stress the predictive performance of the model. When a delivered dose measure
17 is used in animal to human extrapolation of dose response data, the assessment should discuss the
18 confidence in the assumption that the toxicodynamics of the target tissue(s) will be the same in both
19 species. Toxicokinetic data can improve dose response assessment by accounting for sources of
20 change in proportionality of applied to internal or delivered dose at various levels of applied dose.
21 Many of the sources of potential nonlinearity involve saturation or induction of enzymatic processes at
22 high doses. An analysis that accounts for nonlinearity (for instance, due to enzyme saturation kinetics)
23 can assist in avoiding overestimation or underestimation of low dose response otherwise resulting from
24 extrapolation from a sublinear or supralinear part of the experimental dose response curve (Gillette,
25 1983). Toxicokinetic processes tend to become linear at low doses, an expectation that is more robust
26 than low-dose linearity of response (Hattis, 1990). Accounting for toxicokinetic nonlinearities allows
27 better description of the shape of the curve at relatively high levels of dose in the range of observation,
28 but cannot determine linearity or nonlinearity of response at low dose levels (Lutz, 1990a; Swenberg et
29 al., 1987).

30 Toxicokinetic modeling results may be presented as the preferred method of estimating human
31 equivalent dose or in parallel discussion with default assumptions depending on relative confidence in
32 the modeling.
33

3.3.3. Route-to-Route Extrapolation

Judgments frequently need to be made about the carcinogenicity of an agent through a route of exposure different than the one in the underlying studies. For example, exposures of interest may be through inhalation of an agent tested primarily through animal feeding studies or through ingestion of an agent that showed positive results in human occupational studies from inhalation exposure.

Route-to-route extrapolation has both qualitative and quantitative aspects. For the qualitative aspect, the assessor weighs the degree to which positive results through one route of exposure in human or animal studies support a judgment that similar results would have been observed in appropriate studies using the route of exposure of interest. In general, confidence in making such a judgment is strengthened when the tumor effects are observed at a site distant from the portal of entry and when absorption through the route of exposure of interest is similar to absorption via the tested routes. In the absence of contrary data, the qualitative default assumption is that, if the agent is absorbed by a route to give an internal dose, it may be carcinogenic by that route. (See section 2.7.1.)

When a qualitative extrapolation can be supported, quantitative extrapolation may still be problematic in the absence of adequate data. The differences in biological processes among routes of exposure (oral, inhalation, dermal) can be great because of, for example, first-pass effects and differing results from different exposure patterns. There is no generally applicable method for accounting for these differences in uptake processes in quantitative route-to-route extrapolation of dose response data in the absence of good data on the agent of interest. Therefore, route-to-route extrapolation of dose data relies on a case-by-case analysis of available data. When good data on the agent itself are limited, an extrapolation analysis can be based on expectations from physical and chemical properties of the agent, properties and route-specific data on structurally analogous compounds, or in vitro or in vivo uptake data on the agent. Route-to-route uptake models may be applied if model parameters are suitable for the compound of interest. Such models are currently considered interim methods; further model development and validation is awaiting the development of more extensive data (see generally, Gerrity and Henry, 1990). For screening or hazard ranking, route-to-route extrapolation may be based on assumed quantitative comparability as a default, as long as it is reasonable to assume absorption by compared routes. When route-to-route extrapolation is used, the assessor's degree of confidence in both the qualitative and quantitative extrapolation should be discussed in the assessment and highlighted in the dose response characterization.

3.3.4. Dose Averaging

The cumulative dose received over a lifetime, expressed as lifetime average daily dose, is generally considered an appropriate default measure of exposure to a carcinogen (Monro, 1992). The

1 assumption is made that a high dose of a carcinogen received over a short period of time is equivalent
2 to a corresponding low dose spread over a lifetime. While this is a reasonable default assumption
3 based on theoretical considerations, departures from it are expected. Another approach is needed in
4 some cases, such as when dose-rate effects are noted (e.g., formaldehyde). Cumulative dose may be
5 replaced, as appropriate and justified by the data, with other dose measures. In such cases,
6 modifications to the default assumption are made to take account of these effects; the rationale for the
7 selected approach is explained.

8 In cases where a mode of action or other feature of the biology has been identified that has
9 special dose implications for sensitive subpopulations (e.g., differential effects by sex or
10 disproportionate impacts of early-life exposure), these are explained and are recorded to guide
11 exposure assessment and risk characterization. Special problems arise when the human exposure
12 situation of concern suggests exposure regimens (e.g., route and dosing schedule) that are substantially
13 different from those used in the relevant animal studies. These issues are explored and pointed out for
14 attention in the exposure assessment and risk characterization.

16 **3.4. DISCUSSION OF UNCERTAINTIES**

17 The exploration of significant uncertainties in data for dose and response and in extrapolation
18 procedures is part of the assessment. The presentation distinguishes between model uncertainty and
19 parameter uncertainty. Model uncertainty is an uncertainty about a basic biological question. For
20 example, a default, linear dose response extrapolation may have been made based on tumor and other
21 key evidence supporting the view that the model for an agent's mode of action is a DNA-reactive
22 process. Discussion of the confidence in the extrapolation is appropriately done qualitatively or by
23 showing results for alternatives that are equally plausible. It is not useful, for example, to conduct
24 quantitative uncertainty analysis running multiple forms of linear models. This would obviate the function
25 of the policy default.

26 Parameter uncertainties deal with numbers representing statistical or analytical measures of
27 variance or error in data or estimates. Uncertainties in parameters are described quantitatively, if
28 practicable, through sensitivity analysis and statistical uncertainty analysis. With the recent expansion of
29 readily available computing capacity, computer methods are being adapted to create simulated
30 biological data that are comparable with observed information. These simulations can be used for
31 sensitivity analysis, for example, to analyze how small, plausible variations in the observed data could
32 affect dose response estimates. These simulations can also provide information about experimental
33 uncertainty in dose response estimates, including a distribution of estimates that are compatible with the
34 observed data. Because these simulations are based on the observed data, they cannot assist in

1 evaluating the extent to which the observed data as a whole are idiosyncratic rather than typical of the
2 true situation. If quantitative analysis is not possible, significant parameter uncertainties are described
3 qualitatively. In either case, the discussion highlights uncertainties that are specific to the agent being
4 assessed, as distinct from those that are generic to most assessments.

5 Estimation of the applied dose in a human study has numerous uncertainties such as the
6 exposure fluctuations that humans experience compared with the controlled exposures received by
7 animals on test. In a prospective cohort study, there is opportunity to monitor exposure and human
8 activity patterns for a period of time that supports estimation of applied dose (U.S. EPA, 1992a). In a
9 retrospective study, exposure may be based on monitoring data but is often based on human activity
10 patterns and levels reconstructed from historical data, contemporary data, or a combination of the two.
11 Such reconstruction is accompanied by analysis of uncertainties considered with sensitivity analysis in
12 the estimation of dose (Wyzga, 1988; U.S. EPA, 1986a). These uncertainties can also be assessed for
13 any confounding factor for which a quantitative adjustment of dose response data is made (U.S. EPA,
14 1984).

16 **3.5. TECHNICAL DOSE RESPONSE CHARACTERIZATION**

17 As with hazard characterization, the dose response characterization serves the dual purposes of
18 presenting a technical characterization of the assessment results and supporting the risk characterization.

19 The characterization presents the results of analyses of dose data, of response data, and of
20 dose response. When alternative approaches are plausible and persuasive in selecting dose data,
21 response data, or extrapolation procedures, the characterization follows the alternative paths of analysis
22 and presents the results. The discussion covers the question of whether any should be preferred over
23 others because it (or they) better represents the available data or corresponds to the view of the
24 mechanism of action developed in the hazard assessment. The results for different tumor types by sex
25 and species are provided along with the one(s) preferred. Similarly, results for responses other than
26 tumor incidence are shown if appropriate.

27 Numerical dose response estimates are presented to one significant figure. Numbers are
28 qualified as to whether they represent central tendency or upper bounds and whether the method used
29 is inherently more likely to overestimate or underestimate (Krewski et al., 1984).

30 In cases where a mode of action or other feature of the biology has been identified that has
31 special implications for early-life exposure, differential effects by sex, or other concerns for sensitive
32 subpopulations, these are explained. Similarly, any expectations that high dose-rate exposures may
33 alter the risk picture for some portion of the population are described. These and other perspectives
34 are recorded to guide exposure assessment and risk characterization. Whether the lifetime average

1 daily dose or another measure of dose should be considered for differing exposure scenarios is
2 discussed.

3 Uncertainty analyses, qualitative or quantitative if possible, are highlighted in the
4 characterization.

5 The dose response characterization routinely includes the following, as appropriate for the data
6 available:

- 7 ! identification of the kinds of data available for analysis of dose and response and for dose
8 response assessment,
- 9 ! results of assessment as above,
- 10 ! explanation of analyses in terms of quality of data available,
- 11 ! selection of study/response and dose metric for assessment,
- 12 ! discussion of implications of variability in human susceptibility, including for susceptible
13 subpopulation,
- 14 ! applicability of results to varying exposure scenarios--issues of route of exposure, dose rate,
15 frequency, and duration,
- 16 ! discussion of strengths and limitations (uncertainties) of the data and analyses that are
17 quantitative as well as qualitative, and
- 18 ! special issues of interpretation of data, such as:
 - 19 -- selecting dose data, response data, and dose response approach(es),
 - 20 -- use of meta-analysis,
 - 21 -- uncertainty and quantitative uncertainty analysis.

5. RISK CHARACTERIZATION

5.1. PURPOSE

The risk characterization process includes an integrative analysis followed by a presentation in a Risk Characterization Summary, of the major results of the risk assessment. The Risk Characterization Summary is a nontechnical discussion that minimizes the use of technical terms. It is an appraisal of the science that supports the risk manager in making public health decisions, as do other decisionmaking analyses of economic, social, or technology issues. It also serves the needs of other interested readers. The summary is an information resource for preparation of risk communication information, but being somewhat technical, is not itself the usual vehicle for communication with every audience.

The integrative analysis brings together the assessments and characterizations of hazard, dose response, and exposure to make risk estimates for the exposure scenarios of interest. This analysis is generally much more extensive than the Risk Characterization Summary. It may be peer-reviewed or subject to public comment along with the summary in preparation for an Agency decision. The integrative analysis may be titled differently by different EPA programs (e.g., "Staff Paper" for criteria air pollutants), but it typically will identify exposure scenarios of interest in a decisionmaking and present risk analyses associated with them. Some of the analyses may concern scenarios in several media, others may examine, for example, only drinking water risks. It also may be the document that contains quantitative analyses of uncertainty.

The values supported by a risk characterization throughout the process are *transparency* in environmental decisionmaking, *clarity* in communication, *consistency* in core assumptions and science policies from case to case, and *reasonableness*. While it is appropriate to err on the side of protection of health and the environment in the face of scientific uncertainty, common sense and reasonable application of assumptions and policies are essential to avoid unrealistic estimates of risk (U.S. EPA, 1995). Both integrative analyses and the Risk Characterization Summary present an integrated and balanced picture of the analysis of the hazard, dose response, and exposure. The risk analyst should provide summaries of the evidence and results and describe the quality of available data and the degree of confidence to be placed in the risk estimates. Important features include the constraints of available data and the state of knowledge, significant scientific issues, and significant science and science policy choices that were made when alternative interpretations of data existed (U.S. EPA, 1995). Choices made about using default assumptions or data in the assessment are explicitly discussed in the course of analysis, and if a choice is a significant issue, it is highlighted in the summary.

1 **5.2. APPLICATION**

2 Risk characterization is a necessary part of generating any Agency report on risk, whether the
3 report is preliminary to support allocation of resources toward further study or comprehensive to
4 support regulatory decisions. In the former case, the detail and sophistication of the characterization
5 are appropriately small in scale; in the latter case, appropriately extensive. Even if a document covers
6 only parts of a risk assessment (hazard and dose response analyses for instance), the results of these
7 are characterized.

8 Risk assessment is an iterative process that grows in depth and scope in stages from screening
9 for priority-making, to preliminary estimation, to fuller examination in support of complex regulatory
10 decisionmaking. Default assumptions are used at every stage because no database is ever complete,
11 but they are predominant at screening stages and are used less as more data are gathered and
12 incorporated at later stages. Various provisions in EPA-administered statutes require decisions based
13 on findings that represent all stages of iteration. There are close to 30 provisions within the major
14 statutes that require decisions based on risk, hazard, or exposure assessment. For example, Agency
15 review of premanufacture notices under section 5 of the Toxic Substances Control Act relies on
16 screening analyses, while requirements for industry testing under section 4 of that Act rely on
17 preliminary analyses of risk or simply of exposure. At the other extreme, air quality criteria under the
18 Clean Air Act rest on a rich data collection required by statute to undergo reassessment every few
19 years. There are provisions that require ranking of hazards of numerous pollutants--by its nature a
20 screening level of analysis--and other provisions that require a full assessment of risk. Given this range
21 in the scope and depth of analyses, not all risk characterizations can or should be equal in coverage or
22 depth. The risk assessor must carefully decide which issues in a particular assessment are important to
23 present, choosing those that are noteworthy in their impact on results. For example, health effect
24 assessments typically rely on animal data since human data are rarely available. The objective of
25 characterization of the use of animal data is not to recount generic issues about interpreting and using
26 animal data. Agency guidance documents cover these. Instead, the objective is to call out any
27 significant issues that arose within the particular assessment being characterized and inform the reader
28 about significant uncertainties that affect conclusions.

29
30 **5.3. PRESENTATION OF RISK CHARACTERIZATION SUMMARY**

31 The presentation is a nontechnical discussion of important conclusions, issues, and uncertainties
32 that uses the hazard, dose-response, exposure, and integrative analyses for technical support. The
33 primary technical supports within the risk assessment are the hazard characterization, dose response

1 characterization, and exposure characterization described in this guideline. The risk characterization is
2 derived from these. The presentation should fulfill the aims outlined in the purpose section above.

4 **5.4. CONTENT OF RISK CHARACTERIZATION SUMMARY**

5 Specific guidance on hazard, dose response, and exposure characterization appears in previous
6 sections. Overall, the risk characterization routinely includes the following, capturing the important
7 items covered in hazard, dose response, and exposure characterization.

- 8 ! primary conclusions about hazard, dose response, and exposure, including equally plausible
9 alternatives,
- 10 ! nature of key supporting information and analytic methods,
- 11 ! risk estimates and their attendant uncertainties, including key uses of default assumptions
12 when data are missing or uncertain,
- 13 ! statement of the extent of extrapolation of risk estimates from observed data to exposure
14 levels of interest (i.e., margin of exposure) and its implications for certainty or uncertainty in
15 quantifying risk,
- 16 ! significant strengths and limitations of the data and analyses, including any major peer
17 reviewers' issues,
- 18 ! appropriate comparison with similar EPA risk analyses or common risks with which people
19 may be familiar, and
- 20 ! comparison with assessment of the same problem by another organization.

1 **APPENDIX A**

2
3 This appendix contains several general illustrations of weight of evidence narratives. In
4 addition, after narrative #5 is an example of a briefing summary format.

5
6 **NARRATIVE #1**
7 **Chlorinated Alkene**
8 **CAS# XXX**

9 **CANCER HAZARD SUMMARY**

10 Chlorinated alkene (cl-alkene) is *likely to be carcinogenic to humans by all routes of*
11 *exposure*. The weight of evidence of human carcinogenicity of cl-alkene is based on (a) findings of
12 carcinogenicity in rats and mice of both sexes by oral and inhalation exposures; (b) its similarity in
13 structure to other chlorinated organics that are known to cause liver and kidney damage, and liver and
14 kidney tumors in rats and mice; (c) suggestive evidence of a possible association between cl-alkene
15 exposure of workers in the laundry and dry cleaning industries and increased cancer risk in a number of
16 organ systems; and (d) human and animal data indicating that cl-alkene is absorbed by all routes of
17 exposure.

18 In comparison with other agents designated as likely carcinogens, the overall weight of evidence
19 for cl-alkene places it at the *low end* of the grouping. This is because one cannot attribute observed
20 excess cancer risk in exposed workers solely to cl-alkene. Moreover, there is considerable scientific
21 uncertainty about the human significance and relevance of certain rodent tumors associated with
22 exposure to cl-alkene and other chlorinated organics, but insufficient evidence about mode of action for
23 the animal tumors. Hence, the human relevance of the animal evidence of carcinogenicity relies on a
24 default assumption of relevance.

25 There is no clear evidence about the mode of action for each tumor type induced in rats and
26 mice. Available evidence suggests that cl-alkene induces cancer mainly by promoting cell growth rather
27 than via direct mutagenic action, although a mutagenic mode of action for rat kidney tumors cannot be
28 ruled out. The dose response assessment should, therefore, adopt *both default approaches,*
29 *nonlinear and linear*. It is recognized that the latter approach likely overestimates risk at low doses if
30 the mode of action is primarily growth-promoting. This approach, however, may be useful for
31 screening analyses.

32
33 **SUPPORTING INFORMATION**

34 **Human Data**

1 A number of epidemiologic studies of dry cleaning and laundry workers that have reported
2 elevated incidences of lung, cervix, esophagus, kidney, blood and lymphoid cancers. Many of these
3 studies are confounded by co-exposure to other petroleum solvents, making them limited for
4 determining whether the observed increased cancer risks are causally related to cl-alkene. The only
5 investigation of dry cleaning workers with no known exposure to other chemicals did not evaluate other
6 confounding factors such as smoking, alcohol consumption, and low socioeconomic status to exclude
7 the possible contribution of these factors to cancer risks.

8 9 **Animal Data**

10 The carcinogenic potential of cl-alkene has been adequately investigated in two chronic studies
11 in two rodent species, the first study by gavage and the second study by inhalation. Cl-alkene is
12 carcinogenic in the liver in both sexes of mice when tested by either route of exposure. It causes
13 marginally increased incidences of mononuclear cell leukemia (MCL) in both sexes of rats and low
14 incidences of a rare kidney tumor in male rats by inhalation. No increases in tumor incidence were
15 found in rats treated with cl-alkene by gavage. This rat study was considered limited because of high
16 mortality of the animals.

17 Although cl-alkene causes increased incidences of tumors at multiple sites in two rodent
18 species, controversy surrounds each of the tumor endpoints concerning their relevance and/or
19 significance to humans (see discussion under Mode of Action).

20 21 **Other Key Data**

22 Cl-alkene is a member of a class of chlorinated organics that often cause liver and kidney
23 toxicity and carcinogenesis in rodents. Like many chlorinated hydrocarbons, cl-alkene itself is tested
24 negative in a battery of standard genotoxicity tests using bacterial and mammalian cells systems
25 including human lymphocytes and fibroblast cells. There is evidence, however, that a minor metabolite
26 generated by an enzyme found in rat kidney tissue is mutagenic. This kidney metabolite has been
27 hypothesized to be related to the development of kidney tumors in the male rat. This metabolic
28 pathway appears to be operative in the human kidney.

29 Human data indicate that cl-alkene is readily absorbed via inhalation but to a much lesser extent
30 by skin contact. Animal data show that cl-alkene is absorbed well by the oral route.

31 32 **MODE OF ACTION**

33 The mechanisms of cl-alkene-induced mouse liver tumors are not completely understood. One
34 mechanism has been hypothesized to be mediated by a genotoxic epoxide metabolite generated by

1 enzymes found in the mouse liver, but there is a lack of direct evidence in support of this mechanism. A
2 more plausible mechanism that still needs to be further defined is related to liver peroxisomal
3 proliferation and toxicity by TCA (trichloroacetic acid), a major metabolite of cl-alkene. However,
4 there are no definitive data indicating that TCA induces peroxisomal proliferation in humans.

5 The mechanisms by which cl-alkene induces kidney tumors in male rats are even less well
6 understood. The rat kidney response may be related to either kidney toxicity or the activity of a
7 mutagenic metabolite of the parent compound.

8 The human relevance of cl-alkene-induced MCL in rats is unclear. The biological significance
9 of marginally increased incidences of MCL has been questioned by some, since this tumor occurs
10 spontaneously in the tested rat strain at very high background rates. On the other hand, it has been
11 considered by others to be a true finding because there was a decreased time to onset of the disease
12 and the disease was more severe in treated as compared with untreated control animals. The exact
13 mechanism by which cl-alkene increases incidences of MCL in rats is not known.

14 Overall, there is not enough evidence to give high confidence in a conclusion about any single
15 mode of action; it would appear that more than a single mode operates in different rodent tissues. The
16 apparent lack of mutagenicity of cl-alkene itself and its general growth-promoting effect on high
17 background tumors as well as its toxicity toward mouse liver and rat kidney tissue support the view that
18 its predominant mode of action is cell growth promoting rather than mutagenic. A mutagenic
19 contribution to the renal carcinogenicity due to a metabolite cannot be entirely ruled out.

21 **NARRATIVE #2**

22 **Unsaturated Aldehyde**

23 **CAS# XXX**

24 **CANCER HAZARD SUMMARY**

25 The potential human hazard of unsaturated aldehyde (UA) *cannot be determined*, but there
26 are *suggestive* data for carcinogenicity.

27 The evidence on carcinogenicity consists of (a) data from an oral animal study showing a
28 response only at the highest dose in female rats, with no response in males and (b) the fact that other
29 low-molecular-weight aldehydes have shown tumorigenicity in the respiratory tract after inhalation. The
30 one study of UA effects by the inhalation route was not adequately performed. The available evidence
31 is too limited to describe human carcinogenicity potential or support dose response assessment.

32 **SUPPORTING INFORMATION**

33 **Human Data**

1 An elevated incidence of cancer was reported in a cohort of workers in a chemical plant who
2 were exposed to a mixture of chemicals including UA as a minor component. The study is considered
3 inadequate because of the small size of the cohort studied and the lack of adequate exposure data.
4

5 **Animal Data**

6 In a long-term drinking water study in rats, an increased incidence of adrenal cortical adenomas
7 was found in the highest-dosed females. No other significant finding was made. The oral rat study was
8 well conducted by a standard protocol. In a 1-year study in hamsters at one inhalation dose, no tumors
9 were seen. This study was inadequate due to high mortality and consequent short duration. The
10 chemical is very irritating and is a respiratory toxicant in mammals. The animal data are too limited for
11 conclusions to be drawn.
12

13 **Structural Analogue Data**

14 UA's structural analogues, formaldehyde and acetaldehyde, both have carcinogenic effects on
15 the rat respiratory tract.
16

17 **Other Key Data**

18 The weight of results of mutagenicity tests in bacteria, fungi, fruit flies, and mice result in an
19 overall conclusion of not mutagenic; UA is lethal to bacteria to a degree that makes testing difficult and
20 test results difficult to interpret. The chemical is readily absorbed by all routes.
21

22 **MODE OF ACTION**

23 Data are not sufficient to judge whether there is a carcinogenic mode of action.
24

25 **NARRATIVE #3**

26 **Alkene Oxide**

27 **CAS# XXX**

28 **CANCER HAZARD SUMMARY**

29 Alkene oxide (AO) should be dealt with *as if* it were a *known* human carcinogen by *all routes*
30 *of exposure*. Several studies in workers, when considered together, suggest an elevated risk of
31 leukemia and lymphoma after long-term exposure to AO, even though no single study conclusively
32 demonstrates that AO caused the cancer. In addition, animal cancer and mutagenicity studies as well
33 as short-term tests of mutagenicity have strongly consistent results that support a level of concern equal
34 to having conclusive human studies.

1 The weight of evidence of human carcinogenicity is based on (a) consistent evidence of
2 carcinogenicity of AO in rats and mice by both oral and inhalation exposure; (b) studies in workers that
3 taken together suggest elevated risk of leukemia and lymphoma to workers exposed to AO and show
4 genetic damage in blood lymphocytes in exposed workers; (c) mutagenic effects in numerous test
5 systems and heritable gene mutations in animals; and (d) membership in a class of DNA-reactive
6 compounds that are regularly observed to cause cancer in animals.

7 Due to its ready absorption by all routes of exposure and rapid distribution throughout the
8 body, AO is expected to pose a risk by any route of exposure. The strong evidence of a mutagenic
9 mode of action supports dose response assessment that assumes *linearity* of the relationship.

10 11 **SUPPORTING INFORMATION**

12 **Human Data**

13 Elevated risks of lymphatic cancer and cancer of blood-forming tissue have been reported in
14 exposed workers in several studies. The interpretation of the studies separately is complicated by
15 exposures to other agents in each so there is no single study that demonstrates that AO caused the
16 effects; nevertheless, several of the studies together are considered suggestive of AO carcinogenicity
17 because they consistently show cancer elevation in the same tissues. Biomonitoring studies of exposed
18 workers find DNA damage in blood lymphocytes and the degree of DNA damage correlates with the
19 level and duration of AO exposure. Finding this damage in the same tissue in which elevated cancer
20 was seen in workers adds further weight to the positive suggestion from the worker cancer studies.
21 The human data are from well-conducted studies.

22 23 **Animal Data**

24 AO causes cancer in multiple tissue sites in rats and mice of both sexes by oral and inhalation
25 exposure. The database is more extensive than usual and the studies are good. The observation of
26 multisite, multispecies carcinogenic activity by an agent is considered to be very strong evidence and is
27 often the case with highly mutagenic agents. There are also good studies showing that AO causes
28 heritable germ cell mutations in mice after inhalation exposure--a property that is very highly correlated
29 with carcinogenicity.

30 31 **Structural Analogue Data**

32 Organic epoxides are commonly found to have carcinogenic effects in animals, particularly the
33 low-molecular-weight ones.

1 **Other Key Data**

2 The structure and DNA reactivity of AO support potential carcinogenicity. Both properties are
3 highly correlated with carcinogenicity. Positive mutagenicity tests *in vitro* and *in vivo* add to this
4 support and are reinforced by observation of similar genetic damage in exposed workers.

5 AO is experimentally observed to be readily absorbed by all routes and rapidly distributed
6 through the body.

7
8 **MODE OF ACTION**

9 All of the available data are strongly supportive of a mutagenic mode of action, with a particular
10 human target in lymphatic and blood-forming tissue. The current scientific consensus is that there is
11 virtually complete correspondence between ability of an agent to cause heritable germ cell mutations, as
12 AO does, and carcinogenicity. All of this points to a mutagenic mode of action and supports assuming
13 linearity of the dose response relationship.

14
15 **NARRATIVE #4**

16 **Bis-benzenamine**

17 **CAS# XXX**

18 **CANCER HAZARD SUMMARY**

19 This chemical is *likely* to be carcinogenic to humans by *all routes* of exposure. Its
20 carcinogenic potential is indicated by (a) tumor and toxicity studies on structural analogues, which
21 demonstrate the ability of the chemical to produce thyroid follicular cell tumors in rats and hepatocellular
22 tumors in mice following ingestion and (b) metabolism and hormonal information on the chemical and its
23 analogues, which contributes to a working mode of action and associates findings in animals with those
24 in exposed humans. In comparison with other agents designated as likely carcinogens, the overall
25 weight of evidence for this chemical places it at the *lower end* of the grouping. This is because there is
26 a lack of tumor response data on this agent itself.

27 Biological information on the compound is contradictory in terms of how to quantitate potential
28 cancer risks. The information on disruption on thyroid-pituitary status argues for using a margin of
29 exposure evaluation. However, the chemical is an aromatic amine, a class of agents that are DNA-
30 reactive and induce gene mutation and chromosome aberrations, which argues for low-dose linearity.
31 Additionally, there is a lack of mode of action information on the mouse liver tumors produced by the
32 structural analogues, also pointing toward a low-dose linear default approach. In recognition of these
33 uncertainties, it is recommended to quantitate tumors using *both nonlinear* (to place a lower bound on
34 the risks) *and linear* (to place an upper bound on the risks) *default approaches*. Given the absence

1 of tumor response data on the chemical per se, it is recommended that tumor data on close analogues
2 be used to possibly develop toxicity equivalent factors or relative potencies.

3 Overall, this chemical is an inferential case for potential human carcinogenicity. The
4 uncertainties associated with this assessment include (1) the lack of carcinogenicity studies on the
5 chemical, (2) the use of tumor data on structural analogues, (3) the lack of definitive information on the
6 relevance of thyroid-pituitary imbalance for human carcinogenicity, and (4) the different potential
7 mechanisms that may influence tumor development and potential risks.

8 9 **SUPPORTING INFORMATION**

10 **Human Data**

11 Worker exposure has not been well characterized or quantified, but recent medical monitoring
12 of workers exposed over a period of several years has uncovered alterations in thyroid-pituitary
13 hormones (a decrease in T3 and T4 and an increase in TSH) and symptoms of hypothyroidism. A
14 urinary metabolite of the chemical has been monitored in workers, with changes in thyroid and pituitary
15 hormones noted, and the changes were similar to those seen in an animal study.

16 17 **Animal Data**

18 The concentration of the urinary metabolite in rats receiving the chemical for 28 days was within
19 twofold of that in exposed workers, a finding associated with comparable changes in thyroid hormones
20 and TSH levels. In addition, the dose of the chemical given to rats in this study was essentially the same
21 as that of an analogue that had produced thyroid and pituitary tumors in rats. The human thyroid
22 responds in the same way as the rodent thyroid following short-term, limited exposure. Although it is
23 not well established that thyroid-pituitary imbalance leads to cancer in humans as it does in rodents,
24 information in animals and in exposed humans suggests similar mechanisms of disrupting thyroid-
25 pituitary function and the potential role of altered TSH levels in leading to thyroid carcinogenesis.

26 27 **Structural Analogue Data**

28 This chemical is an aromatic amine, a member of a class of chemicals that has regularly
29 produced carcinogenic effects in rodents and gene and structural chromosome aberrations in short-term
30 tests. Some aromatic amines have produced cancer in humans.

31 Close structural analogues produce thyroid follicular cell tumors in rats and hepatocellular
32 tumors in mice following ingestion. The thyroid tumors are associated with known perturbations in
33 thyroid-pituitary functioning. These compounds inhibit the use of iodide by the thyroid gland,
34 apparently due to inhibition of the enzyme that synthesizes the thyroid hormones (T3, T4). Accordingly,

1 blood levels of thyroid hormones decrease, which induce the pituitary gland to produce more TSH, a
2 hormone that stimulates the thyroid to produce more of its hormones. The thyroid gland becomes
3 larger due to increases in the size of individual cells and their proliferation and upon chronic
4 administration, tumors develop. Thus, thyroid tumor development is significantly influenced by
5 disruption in the thyroid-pituitary axis.

6 7 **Other Key Data**

8 The chemical can be absorbed by the oral, inhalation, and dermal routes of exposure.
9

10 **MODE OF ACTION**

11 Data on the chemical and on structural analogues indicate the potential association of
12 carcinogenesis with perturbation of thyroid-pituitary homeostasis. Structural analogues are genotoxic,
13 thus raising the possibility of different mechanisms by which this chemical may influence tumor
14 development.
15

16 **NARRATIVE #5**

17 **Brominated Alkane (BA)**

18 **CAS# XXX**
19

20 **CANCER HAZARD SUMMARY**

21 Brominated alkane (BA) is *likely* to be a human carcinogen by *all routes* of exposure. The
22 weight of evidence for human carcinogenicity is at the *high end* of agents in the "likely" group. Findings
23 are based on very extensive and significant experimental findings that include (a) tumors at multiple sites
24 in both sexes of two rodent species via three routes of administration relevant to human exposure, (b)
25 close structural analogues that produce a spectrum of tumors like BA, (c) significant evidence for the
26 production of reactive BA metabolites that readily bind to DNA and produce gene mutations in many
27 systems including cultured mammalian and human cells, and (d) two null and one positive epidemiologic
28 study; in the positive study, there may have been exposure to BA. These findings support a decision
29 that BA might produce cancer in exposed humans. In comparison to other agents considered likely
30 human carcinogens, the overall weight of evidence for BA puts it near the top of the grouping. Given
31 the agent's mutagenicity, which can influence the carcinogenic process, a linear dose-response
32 extrapolation is recommended.

33 Uncertainties include the lack of adequate information on the mutagenicity of BA in mammals or
34 humans *in vivo*, although such effects would be expected.

SUPPORTING INFORMATION

Human Data

The information on the carcinogenicity of BA from human studies is inadequate. Two studies of production workers have not shown significant increases in cancer from exposure to BA and other chemicals. An increase in lymphatic cancer was reported in a mortality study of grain elevator workers who may have been exposed to BA (and other chemicals).

Animal Data

BA produced tumors in four chronic rodent studies. Tumor increases were noted in males and females of rats and mice following oral dermal and inhalation exposure (rat--oral and two inhalation, mouse--oral and dermal). It produces tumors both at the site of application (e.g., skin with dermal exposure) and at sites distal to the portal of entry into the body (e.g., mammary gland) following exposure from each route. Tumors at the same site were noted in both sexes of a species (blood vessel), both species (forestomach) and via different routes of administration (lung). Some tumors developed after very short latency, metastasized extensively, and produced death, an uncommon findings in rodents. The rodent studies were well designed and conducted except for the oral studies, in which the doses employed caused excessive toxicity and mortality. However, given the other rodent findings, lower doses would also be anticipated to be carcinogenic.

Structural Analogue Data

Several chemicals structurally related to BA are also carcinogenic in rodents. Among four that are closest in structure, tumors like those seen for BA were often noted (e.g., forestomach, mammary, lung), which helps to confirm the findings for BA itself. In sum, all of the tumor findings help to establish animal carcinogenicity and support potential human carcinogenicity for BA.

Other Key Data

BA itself is not reactive, but from its structure it was expected to be metabolized to reactive forms. Extensive metabolism studies have confined this presumption and have demonstrated metabolites that bind to DNA and cause breaks in the DNA chain. These lesions are readily converted to gene mutations in bacteria, fungi, higher plants, insects and mammalian and human cells in culture. There are only a limited number of reports on the induction of chromosome aberrations in mammals and humans; thus far they are negative.

1 **MODE OF ACTION**

2 Human carcinogens often produce cancer in multiple sites of multiple animal species and both
3 sexes and are mutagenic in multiple test systems. BA satisfies these findings. It produces cancer in
4 males and females of rats and mice. It produces gene mutations in cells across all life forms--plants,
5 bacteria and animals--including mammals and humans. Given the mutagenicity of BA exposure and the
6 multiplicity and short latency of BA tumor induction, it is reasonable to use a linear approach for cancer
7 dose-response extrapolation.

8
9 **BRIEFING SUMMARY**

<u>Route(s)</u>	<u>Class</u>	<u>Designation or rationale</u>	<u>Dose Response</u>
all	likely	high end	default-linear

10
11
12
13
14 Basis for classification/dose response

- 15 **1. Human data:** Two studies of production workers show no increase in cancer (one had a small
16 sample size; the other had mixed chemical exposures). An increase in lymphatic cancer is seen
17 among grain elevator workers who may have been exposed to other chemicals.
- 18 **2. Animal data:** BA produces tumors at multiple sites in male and female rats and mice following
19 oral, dermal, and inhalation exposure. Tumors are seen at the site of administration and distally
20 and are often consistent across sex, species, and route of administration; some develop early,
21 metastasize, and cause death.
- 22 **3. Structural analogue data:** Close analogues produce some of the same tumors as are seen with
23 BA.
- 24 **4. Other key data:** BA is metabolized to a reactive chemical that binds DNA and produces gene
25 mutations in essentially every test system including cultured human cells.
- 26 **5. Mode of action:** Like most known human carcinogens, BA is mutagenic in most test systems.
- 27 **6. Hazard classification/uncertainties:** There is a rich database on BA demonstrating its potential
28 ability to cause tumors in humans, including (a) multiple animal tumors, (b) by appropriate routes of
29 exposure, (c) a mode of action relevant to human carcinogenicity, and (d) some information in
30 humans. Together they lead to a designation near the high end of the *likely* human carcinogen
31 class.
- 32 **7. Dose response:** Given the anticipated mode of action, a linear default dose response relationship
33 should be assumed.
34

1 **APPENDIX B**

2
3 This appendix contains responses to the National Academy of Sciences National Research
4 Council report *Science and Judgment in Risk Assessment* (NRC, 1994).

5
6 **Recommendations of the National Academy of Sciences National Research Council**

7 In 1994, the National Academy of Sciences published a report *Science and Judgment in Risk*
8 *Assessment*. The 1994 report was written by a Committee on Risk Assessment of Hazardous Air
9 Pollutants formed under the Academy's Board on Environmental Studies and Toxicology, Commission
10 on Life Sciences, National Research Council. The report was called for under Section 112(o)(1)(A,B)
11 of the Clean Air Act Amendments of 1990, which provided for the EPA to arrange for the Academy to
12 review:

- 13 ! risk assessment methodology used by the EPA to determine the carcinogenic risk
- 14 associated with exposure to hazardous air pollutants from source categories and
- 15 subcategories subject to the requirements of this section and
- 16 ! improvements in such methodology.

17 Under Section 112(o)(2)(A,B), the Academy was to consider the following in its review:

- 18 ! the techniques used for estimating and describing the carcinogenic potency to humans of
- 19 hazardous air pollutants and
- 20 ! the techniques used for estimating exposure to hazardous air pollutants (for hypothetical and
- 21 actual maximally exposed individuals as well as other exposed individuals).

22 To the extent practicable, the Academy was also to review methods of assessing adverse human health
23 effects other than cancer for which safe thresholds of exposure may not exist [Section 112(o)(3)]. The
24 Congress further provided that the EPA Administrator should consider, but need not adopt, the
25 recommendations in the report and the views of the EPA Science Advisory Board with respect to the
26 report. Prior to the promulgation of any standards under Section 112(f), the Administrator is to publish
27 revised guidelines for carcinogenic risk assessment or a detailed explanation of the reasons that any
28 recommendations contained in the report will not be implemented [Section 112(o)(6)].

29 The following discussion addresses the recommendations of the 1994 report that are pertinent
30 to the EPA cancer risk assessment guidelines. Guidelines for assessment of exposure, of mixtures, and
31 of other health effects are separate EPA publications. Many of the recommendations were related to
32 practices specific to the exposure assessment of hazardous air pollutants, which are not covered in
33 cancer assessment guidelines. Recommendations about these other guidelines or practices are not
34 addressed here.

1 **Hazard Classification**

2 The 1994 report contains the following recommendation about classifying cancer hazard:

3 ! The EPA should develop a two-part scheme for classifying evidence on carcinogenicity that
4 would incorporate both a simple classification and a narrative evaluation. At a minimum,
5 both parts should include the strength (quality) of the evidence, the relevance of the animal
6 model and results to humans, and the relevance of the experimental exposures (route, dose,
7 timing, and duration) to those likely to be encountered by humans.

8 The report also presented a possible matrix of 24 boxes that would array weights of evidence against
9 low, medium, or high relevance, resulting in 24 codes for expressing the weight and relevance.

10 These guidelines adopt a set of descriptors and subdescriptors of weight of evidence in three
11 categories: “known/likely,” “cannot be determined,” and “not likely,” and a narrative for presentation of
12 the weight of evidence findings. The descriptors are used within the narrative. There is no matrix of
13 alphanumerical weight of evidence boxes.

14 The issue of an animal model that is not relevant to humans has been dealt with by not including
15 an irrelevant response in the weighing of evidence, rather than by creating a weight of evidence then
16 appending a discounting factor as the NRC scheme would do. The issue is more complex than the
17 NRC matrix makes apparent. Often the question of relevance of the animal model applies to a single
18 tumor response, but one encounters situations in which there are more tumor responses in animals than
19 the questioned one. Dealing with this complexity is more straightforward if it is done during the
20 weighing of evidence rather than after as in the NRC scheme. Moreover, the same experimental data
21 are involved in deciding on the weight of evidence and the relevance of a response. It would be
22 awkward to go over the same data twice.

23 In recommending that the relevance of circumstances of human exposure also be taken into
24 account, the NRC appears to assume that all of the actual conditions of human exposure will be known
25 when the classification is done. This is not the case. More often than not, the hazard assessment is
26 applied to assessment of risks associated with exposure to different media or environments at different
27 times. In some cases, there is no priority to obtaining exposure data until the hazard assessment has
28 been done. The approach of these guidelines is to characterize hazards as to whether their expression
29 is intrinsically limited by route of exposure or by reaching a particular dose range based strictly on
30 toxicological and other biological features of the agent. Both the use of descriptors and the narrative
31 specifically capture this information. Other aspects of appropriate application of the hazard and dose
32 response assessment to particular human exposure scenarios are dealt with in the characterization of the
33 dose response assessment, e.g., the applicability of the dose response assessment to scenarios with
34 differing frequencies and durations.

1 The NRC scheme apparently intended that the evidence would be weighed, then given a low,
2 medium, or high code for some combination of relevance of the animal response, route of exposure,
3 timing, duration, or frequency. The 24 codes contain none of this specific information, and in fact, do
4 not communicate what the conclusion is about. To make the codes communicate the information
5 apparently intended would require some multiple of the 24 in the NRC scheme. As the number of
6 codes increases, their utility for communication decreases.

7 Another reason for declining to use codes is that they tend to become outdated as research
8 reveals new information that was not contemplated when they were adopted. This has been the case
9 with the classification system under the EPA, 1986 guidelines.

10 Even though these guidelines do not adopt a matrix of codes, the method they provide of using
11 descriptors and narratives captures the information the NRC recommended as the most important, and
12 in the EPA's view, in a more transparent manner.

13 14 **Dose Response**

15 The 1994 report contains the following recommendations about dose response issues:

- 16 ! EPA should continue to explore, and when scientifically appropriate, incorporate
17 toxicokinetic models of the link between exposure and biologically effective dose (i.e., dose
18 reaching the target tissue).
- 19 ! Despite the advantages of developing consistent risk assessments between agencies by
20 using common assumptions (e.g., replacing surface area with body weight to the 0.75
21 power), EPA should indicate other methods, if any, that would be more accurate.
- 22 ! EPA should continue to use the linearized multistage model as a default option but should
23 develop criteria for determining when information is sufficient to use an alternative
24 extrapolation model.
- 25 ! EPA should continue to use as one of its risk characterization metrics upper-bound potency
26 estimates of the probability of developing cancer due to lifetime exposure. Whenever
27 possible, this metric should be supplemented with other descriptions of cancer potency that
28 might more adequately reflect the uncertainty associated with the estimates.
- 29 ! EPA should adopt a default assumption for differences in susceptibility among humans in
30 estimating individual risks.
- 31 ! In the analysis of animal bioassay data on the occurrence of multiple tumor types, the cancer
32 potencies should be estimated for each relevant tumor type that is related to exposure and
33 the individual potencies should be summed for those tumors.

1 The use of toxicokinetic models is encouraged in these guidelines with discussion of appropriate
2 considerations for their use. When there are questions as to whether such a model is more accurate in
3 a particular case than the default method for estimating the human equivalent dose, both alternatives
4 may be used. It should be noted that the default method for inhalation exposure is a toxicokinetic
5 model.

6 The rationale for adopting the oral scaling factor of body weight to the 0.75 power has been
7 discussed above in the explanation of major defaults. The empirical basis is further explored in U.S.
8 EPA, 1992b. The more accurate approach is to use a toxicokinetic model when data become
9 available or to modify the default when data are available as encouraged under these guidelines. As the
10 U.S. EPA, 1992b discussion explores in depth, data on the differences among animals in response to
11 toxic agents are basically consistent with using a power of 1.0, 0.75, or 0.66. The Federal agencies
12 chose the power of 0.75 for the scientific reasons given in the previous discussion of major defaults;
13 these were not addressed specifically in the NRC report. It was also considered appropriate, as a
14 matter of policy, for the agencies to agree on one factor. Again, the default for inhalation exposure is a
15 model that is constructed to become better as more agent-specific data become available.

16 The EPA proposes not to use a computer model such as the linearized multistage model as a
17 default for extrapolation below the observed range. The reason is that the basis for default
18 extrapolation is a theoretical projection of the likely shape of the curve considering mode of action. For
19 this purpose, a computer model looks more sophisticated than a straight line extrapolation, but is not.
20 The extrapolation will be by straight line as explained in the explanation of major defaults. This was
21 also recommended by workshop reviewers of a previous draft of these guidelines (U.S. EPA, 1994b).
22 In addition, a margin of exposure analysis is proposed to be used in cases in which the curve is thought
23 to be nonlinear, based on mode of action. In both cases, the observed range of data will be modeled
24 by curve fitting in the absence of supporting data for a biologically based or case-specific model.

25 The result of using straight line extrapolation is thought to be an upper bound on low-dose
26 potency to the human population in most cases, but as discussed in the major defaults section, it may
27 not always be. Exploration and discussion of uncertainty of parameters in curve-fitting a model of the
28 observed data or in using a biologically based or case-specific model is called for in the dose response
29 assessment and characterization sections of these guidelines.

30 The issue of a default assumption for human differences in susceptibility has been addressed
31 under the major defaults discussion in section 1.3 with respect to margin of exposure analysis. The
32 EPA has considered but decided not to adopt a quantitative default factor for human differences in
33 susceptibility when a linear extrapolation is used. In general, the EPA believes that the linear
34 extrapolation is sufficiently conservative to protect public health. Linear approaches (both LMS and

1 straight line extrapolation) from animal data are consistent with linear extrapolation on the same agents
2 from human data (Goodman and Wilson, 1991; Hoel and Portier, 1994). If actual data on human
3 variability in sensitivity are available they will, of course, be used.

4 In analyzing animal bioassay data on the occurrence of multiple tumor types, these guidelines
5 outline a number of biological and other factors to consider. The objective is to use these factors to
6 select response data (including nontumor data as appropriate) that best represent the biology observed.
7 As stated in section 3 of the guidelines, appropriate options include use of a single data set, combining
8 data from different experiments, showing a range of results from more than one data set, showing
9 results from analysis of more than one tumor response based on differing modes of action, representing
10 total response in a single experiment by combining animals with tumors, or a combination of these
11 options. The approach judged to best represent the data is presented with the rationale for the
12 judgment, including the biological and statistical considerations involved. The EPA has considered the
13 approach of summing tumor incidences and decided not to adopt it. While multiple tumors may be
14 independent, in the sense of not arising from metastases of a single malignancy, it is not clear that they
15 can be assumed to represent different effects of the agent on cancer processes. In this connection, it is
16 not clear that summing incidences provides a better representation of the underlying mode(s) of action
17 of the agent than combining animals with tumors or using another of the several options noted above.
18 Summing incidences would result in a higher risk estimate, a step that appears unnecessary without
19 more reason.

21 **Risk Characterization**

22 ! When EPA reports estimates of risk to decisionmakers and the public, it should present not
23 only point estimates of risk, but also the sources and magnitudes of uncertainty associated
24 with these estimates.

25 ! Risk managers should be given characterizations of risk that are both qualitative and
26 quantitative, i.e., both descriptive and mathematical.

27 ! EPA should consider in its risk assessments the limits of scientific knowledge, the remaining
28 uncertainties, and the desire to identify errors of either overestimation or underestimation.

29 In part as a response to these recommendations, the Administrator of EPA issued guidelines for
30 risk characterization and required implementation plans from all programs in EPA (U.S. EPA, 1995).
31 The Administrator's guidance is followed in these cancer guidelines. The assessments of hazard, dose
32 response, and exposure will all have accompanying technical characterizations covering issues of
33 strengths and limitations of data and current scientific understanding, identification of defaults utilized in

- 1 the face of gaps in the former, discussions of controversial issues, and discussions of uncertainties in
- 2 both their qualitative, and as practicable, their quantitative aspects.

APPENDIX C

OVERVIEW OF CANCER PROCESSES

The following picture is changing as research reveals more about carcinogenic processes. Nevertheless, it is apparent that several general modes of action are being elucidated from direct reaction with DNA to hormonal or other growth-signaling processes. While the exact mechanism of action of an agent at the molecular level may not be clear from existing data, the available data will often provide support for deducing the general mode of action. Under these guidelines, using all of the available data to arrive at a view of the mode of action supports both characterization of human hazard potential and assessment of dose response relationships.

Cancers are diseases of somatic mutation affecting cell growth and differentiation. The genes that control cell growth, programmed cell death, and cell differentiation are critical to normal development of tissues from embryo to adult metazoan organisms. These genes continue to be critical to maintenance of form and function of tissues in the adult (e.g., Meyn, 1993) and changes in them are essential elements of carcinogenesis (Hsu et al., 1991; Kakizuka et al., 1991; Bottaro et al., 1991; Sidransky et al., 1991; Salomon et al., 1990; Srivastava et al., 1990). The genes involved are among the most highly conserved in evolution as evidenced by the great homology of many of them in DNA sequence and function in organisms as phylogenetically distant as worms, insects, and mammals (Auger et al., 1989a, b; Hollstein et al., 1991; Herschman, 1991; Strausfeld et al., 1991; Forsburg and Nurse, 1991).

Mutations affecting three general categories of genes have been implicated in carcinogenesis. Over 100 oncogenes have been found in human and animal tumors that act as dominant alleles, whereas there are about 10 known tumor suppressor genes that are recessive in action. The normal alleles of these genes are involved with control of cell division and differentiation; mutated alleles lead to a disruption in these functions. The third class are mutator genes that predispose the genome to enhanced mutagenic events that contribute further to the carcinogenic process.

Adult tissues, even those that are composed of rapidly replicating cells, maintain a constant size and cell number (Nunez et al., 1991) by balancing three cell fates: (1) continued replication, (2) differentiation to take on specialized functions, or (3) programmed cell death (apoptosis) (Raff, 1992; Maller, 1991; Naeve et al., 1991; Schneider et al., 1991; Harris, 1990). Neoplastic growth through clonal expansion can result from somatic mutations that inactivate control over cell fate (Kakizuka et al., 1991; deThe et al., 1991; Sidransky et al., 1992; Nowell, 1976).

Cancers may also be thought of as diseases of the cell cycle. For example, genetic diseases that cause failure of cells to repair DNA damage prior to cell replication predispose people to cancer.

1 These changes are also frequently found in tumor cells in sporadic cancers. These changes appear to
2 be particularly involved at points in cell replication called "checkpoints" where DNA synthesis or mitosis
3 is normally stopped until DNA damage is repaired or cell death induced (Tobey, 1975). A cell that
4 bypasses a checkpoint may acquire a heritable growth advantage. Similar effects on the cell cycle
5 occur when mitogens such as hormones or growth factors stimulate cell growth. Rapid replication in
6 response to tissue injury may also lead to unrepaired DNA damage that is a risk factor for
7 carcinogenesis.

8 Normally a cell's fate is determined by a timed sequence of biochemical signals. Signal
9 transduction in the cell involves chemical signals that bind to receptors, generating further signals in a
10 pathway whose target in many cases is control of transcription of a specific set of genes (Hunter, 1991;
11 Cantley et al., 1991; Collum and Alt, 1990). Cells are subject to growth signals from the same and
12 distant tissues, e.g., endocrine tissues (Schuller, 1991). In addition to hormones produced by
13 endocrine tissues, numerous soluble polypeptide growth factors have been identified that control normal
14 growth and differentiation (Cross and Dexter, 1991; Wellstein et al., 1990). The cells responsive to a
15 particular growth factor are those that express transmembrane receptors that specifically bind the
16 growth factor.

17 Solid tumors develop in stages operationally defined as initiation, promotion, and progression
18 (see, for example, Pitot and Dragan, 1991). These terms, which were coined in the context of specific
19 experimental designs, are used for convenience in discussing concepts, but they refer to complex events
20 that are not completely understood. During initiation, the cell acquires a genetic change that confers a
21 potential growth advantage. During promotion, clonal expansion of this altered cell occurs. Later,
22 during progression, a series of genetic and other biological events both enhance the growth advantage
23 of the cells and enlist normal host processes to support tumor development and cells develop the ability
24 to invade locally and metastasize distally, taking on the characteristics of malignancy. Many
25 endogenous and exogenous factors are known to participate in the process as a whole. These include
26 specific genetic predispositions or variations in ability to detoxify agents, medical history (Harris, 1989;
27 Nebreda et al., 1991), infections, exposure to chemicals or ionizing radiation, hormones and growth
28 factors, and immune suppression. Several such risk factors likely work together to cause individual
29 human cancers.

30 A cell that has been transformed, acquiring the potential to establish a line of cells that grow to a
31 tumor, will probably realize that potential only rarely. The process of tumorigenesis in animals and
32 humans is a multistep one (Bouk, 1990; Fearon and Vogelstein, 1990; Hunter, 1991; Kumar et al.,
33 1990; Sukumar, 1989; Sukumar, 1990) and normal physiological processes appear to be arrayed
34 against uncontrolled growth of a transformed cell (Weinberg, 1989). Powerful inhibition by signals

1 from contact with neighboring normal cells is one known barrier (Zhang et al., 1992). Another is the
2 immune system (at least for viral infection). How a cell with tumorigenic potential acquires additional
3 properties that are necessary to enable it to overcome these and other inhibitory processes is a subject
4 of ongoing research. For known human carcinogens studied thus far, there is an often decades-long
5 latency between exposure to carcinogenic agents and development of tumors (Fidler and Radinsky,
6 1990; Tanaka et al., 1991; Thompson et al., 1989). This latency is also typical of tumor development
7 in individuals with genetic diseases that make them prone to cancer (Meyn, 1993; Srivastava et al.,
8 1990).

9 The importance of genetic mutation in the carcinogenic process calls for special attention to
10 assessing agents that cause such mutations. Heritable genetic defects that predispose humans to cancer
11 are well known and the number of identified defects is growing. Examples include xeroderma
12 pigmentosum (DNA repair defect) and Li Fraumeni and retinoblastoma (both are tumor suppressor
13 gene mutations). Much of the screening and testing of agents for carcinogenic potential has been driven
14 by the idea of identifying this mode of action. Cognizance of and emphasis on other modes of action
15 such as ones that act at the level of growth signalling within or between cells, through cell receptors, or
16 that indirectly cause genetic change, comes from more recent research. There are not yet standardized
17 tests for many modes of action, but pertinent information may be available in individual cases.

18 Agents of differing characteristics influence cancer development: inorganic and organic,
19 naturally occurring and synthetic, of inanimate or animate origin, endogenous or exogenous, dietary and
20 nondietary. The means by which these agents act to influence carcinogenesis are variable, and
21 reasoned hazard assessment requires consideration of the multiple ways that chemicals influence cells in
22 experimental systems and in humans. Agents exert mutagenic effects either by interacting directly with
23 DNA or by indirect means through intermediary substances (e.g., reactive oxygen species) or
24 processes. Most DNA-reactive chemicals are electrophilic or can become electrophilic when
25 metabolically activated. Electrophilic molecules may bind covalently to DNA to form adducts, and this
26 may lead to depurination, depyrimidation, or produce DNA strand breaks; such lesions can be
27 converted to mutations with a round of DNA synthesis and cell division. Other DNA-interactive
28 chemicals may cause the same result by intercalation into the DNA helix. Still other chemicals may
29 methylate DNA, changing gene expression. Non-DNA-reactive chemicals produce genotoxic effects
30 by many different processes. They may affect spindle formation or chromosome proteins, interfere with
31 normal growth control mechanisms, or affect enzymes involved with ensuring the fidelity of DNA
32 synthesis (e.g., topoisomerase), recombination, or repair.

33 The "classical" chemical carcinogens in laboratory rodent studies are agents that consistently
34 produce gene mutations and structural chromosome aberrations in short-term tests. A large database

1 reveals that these mutagenic substances commonly produce tumors at multiple sites and in multiple
2 species (Ashby and Tennant, 1991). Most of the carcinogens identified in human studies, aside from
3 hormones, are also gene or structural chromosome mutagens (Tennant and Ashby, 1991). Most of
4 these compounds or their metabolites contain electrophilic moieties that react with DNA.

5 Numerical chromosome aberrations, gene amplification, and the loss of gene heterozygosity are
6 also found in animal and human tumor cells and may arise from initiating events or during progression.
7 There is reason to believe that accumulation of additional genetic changes is favored by selection in the
8 evolution of tumor cells because they confer additional growth advantages (Hartwell and Kastan,
9 1994). Exogenous agents may function at any stage of carcinogenesis (Barrett, 1993). Some
10 aberrations may arise as a consequence of genomic instability arising from tumor suppressor gene
11 mutation, e.g., p53 (Harris and Hollstein, 1993). The frequent observation in tumor cells that both of a
12 pair of homologous chromosomes have identical mutation spectra in tumor suppressor genes suggests
13 an ongoing, endogenous process of gene conversion. Currently, there is a paucity of routine test
14 methods to screen for events such as gene conversion or gene amplification and knowledge regarding
15 the ability of particular agents of environmental interest to induce them is, for the most part, wanting.
16 Work is under way to characterize, measure, and evaluate their significance (Travis et al., 1991).

17 Several kinds of mechanistic studies aid in risk assessment. Comparison of DNA lesions in
18 tumor cells taken from humans with the lesions that a tumorigenic agent causes in experimental systems
19 can permit inferences about the association of exposure to the agent and an observed human effect
20 (Vahakangas et al., 1992; Hollstein et al., 1991; Hayward et al., 1991). An agent that is observed to
21 cause mutations experimentally may be inferred to have potential for carcinogenic activity (U.S. EPA,
22 1991a). If such an agent is shown to be carcinogenic in animals, the inference that its mode of action is
23 through mutagenicity is strong. A carcinogenic agent that is not mutagenic in experimental systems but
24 is mitogenic or affects hormonal levels or causes toxic injury followed by compensatory growth may be
25 inferred to have effects on growth signal transduction or to have secondary carcinogenic effects. The
26 strength of these inferences depends in each case on the nature and extent of all the available data.

27 Differing modes of action at the molecular level have different dose response implications for
28 the activity of agents. The carcinogenic activity of a direct-acting mutagen should be a function of the
29 probability of its reaching and reacting with DNA. The carcinogenic activity of an agent that interferes
30 at the level of signal pathways with many potential receptor targets should be a function of multiple
31 reactions. The carcinogenic activity of an agent that acts by causing cell toxicity followed by
32 compensatory growth should be a function of the toxicity.

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