# Determining Modes Of Action for Biologically Based Risk Assessments

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# DETERMINING MODES OF ACTION FOR BIOLOGICALLY BASED RISK ASSESSMENTS

This brochure describes what a mode of action is, how it differs from a mechanism of action, and what its role is in the development of biologically based risk assessments for exposure to chemicals. AIHC's position on the level or strength of evidence required to establish a mode of action also is presented. Although the focus of this brochure is on cancer, as described in the US Environmental Protection Agency's (EPA) Proposed Guidelines for Carcinogen Risk Assessment (EPA 1996, 1999), the principles discussed are broadly applicable to all toxicological endpoints. Cancer may be a disease of particular concern, and biological mechanisms may differ between cancer and other diseases; however, there is no reason why the general principles for establishing and using the biological mode of action should depend on the type of effect being considered.

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# AIHC'S POSITION ON THE DEVELOPMENT OF A MODE OF ACTION FOR BIOLOGICALLY BASED RISK ASSESSMENTS

A "mode of action" is a category or class of toxic mechanisms for which the major (but not all) biochemical steps are understood. The "mechanism of action" for a chemical, on the other hand, is a complete and detailed understanding of each and every step in the sequence of events that leads to a toxic outcome. Knowledge about the mechanism of action, however, is rarely available. A chemical's mode of action is much easier to determine than the mechanism. Risk assessments that are based on mode-of-action data rather than default assumptions are more reliable. The American Industrial Health Council (AIHC) strongly supports the use of the mode-of-action approach as a basis for assessing health risks from exposure to chemicals. Consistent with this position, AIHC supports the use of the mode-of-action approach by EPA in its Proposed Guidelines for Carcinogen Risk Assessment (EPA 1996, 1999). The purpose of the mode-of-action approach is to allow valid scientific data to replace corresponding default assumptions whenever they are available, even though these data may not be complete or may not provide absolute certainty.

As discussed in an AIHC brochure on default assumptions in risk assessment (AIHC 1997), many default assumptions currently in use are based on data that are more than 20 years old. It is clearly preferable to draw on both the general advances in biology and toxicology since that time, as well as the data available for a particular agent, than to rely on default assumptions. The great uncertainty inherent in the use of default assumptions should not be disguised by their comfortable familiarity (AIHC 1997).

To guide the development and use of modes of action for toxic agents, AIHC suggests several principles.

- Identifying a mode of action does not require the same amount of scientific data or level of certainty necessary to fully describe the mechanism involved in producing the toxic effect, but should be based on solid scientific observations and knowledge of biological and toxicological processes.

- Prescriptive, "check-box" approaches to determining whether data are adequate to support a mode of action should be avoided. Once a mode of action is established for a particular chemical or class of chemicals, less data will likely be needed to support the existence of that same mode of action for other related chemicals.

- The modified Bradford-Hill criteria presented by EPA (1999) are useful for evaluating the sufficiency of data to support a mode of action.

- When a mode of action is proposed for a specific toxic endpoint induced by a particular chemical, the mode of action should be subject to scientific peer review. Peer review should occur prior to its regulatory application, and the scope of the peer review should be commensurate with the expected impact of the resultant regulations (AIHC 1995).

## WHAT IS A MODE OF ACTION?

The science of toxicology often focuses on understanding the biological processes by which an agent exerts some adverse health effect on an individual. When a toxic agent enters the body, a sequence of biological events begins that may lead to a toxic response. To completely understand the *mechanisms* by which this occurs is to have detailed knowledge and understanding of each and every step in the sequence. Gathering such a body of information for even one agent and a particular disease endpoint is a daunting task. Usually, only partial information is available about the toxic mechanism by which a chemical causes a disease outcome. In fact, only a partial understanding of the toxic mechanism is necessary in most cases to substantially improve the risk assessment.

The objective of a health risk assessment is to characterize and quantify the relationship between the amount of a chemical or physical agent to which a human population is exposed and the probability of any adverse response in that population. Data from experimental animals or human health studies often provide good information on this exposure-response relationship at high levels of exposure. Public health protection at low levels of exposure is more problematic because data are likely to be inconclusive. For example, in the United States, cancer risks from exposures resulting from human activities, particularly commercial chemicals, are generally regulated at a risk level of one additional cancer case in a population of one million individuals over their lifetimes. But experimental studies are usually only sufficient to measure a response rate of one in 10 or, on occasion, one in 100.

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How then can the level of exposure that would yield a risk of no more than one in one million be estimated when our lowest measure is one in 10? To answer this question, some inference must be made about the relationship between exposure and response at low levels of exposure. Is the relationship linear in shape or nonlinear in shape? If nonlinear, what is the shape of the curve? This relationship is governed by the toxic mechanisms involved. Since a *mode of action* is a category or class of toxic mechanisms for which the major (but not all) steps of the biochemical mechanisms are understood, the shape of the exposure-response relationship at low doses can be inferred from the mode of action.

The mode of action for a specific chemical is determined by the class of toxic mechanism by which the chemical induces a particular disease endpoint such as cancer. Identifying the mode of action for a toxic agent is easier and less resource-intensive than identifying the complete mechanism of cancer development because much less scientific data are needed. For example, a rough estimate of the total expenditure on studies to determine the *mechanism of action* for dioxin (TCDD) alone is at least $4 million per year, and the actual amount is probably higher. Yet despite this vast amount of research, there are still many questions to be answered regarding the mechanism of dioxin toxicity. The point is not to put less money into research, but to put these funds to more effective use by supporting the investigation of other chemicals for which mode-of-action data are lacking and contention exists about the dose-response relationship at low levels of exposure. A mode-of-action approach is a more efficient use of scientific resources because a full elucidation of the mechanism of action is not needed, allowing one to make judgments about what levels of exposure are expected to be safe.

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## BACKGROUND

In 1996, EPA issued its revised Proposed Guidelines for Carcinogen Risk Assessment (EPA 1996). In the guidelines, it is recognized that exposure-response relationships at low doses for different agents will be determined by each agent's mechanism of action. Importantly, the guidelines recognize that a complete understanding of the mechanism of action is not essential. Rather, the guidelines discuss the "mode of action," with the primary distinction being whether the mode of action is linear, nonlinear, or undetermined (in which case both modes are evaluated). If a carcinogen is believed to have a linear mode of action, then a linear low-dose extrapolation must be used to determine regulatory exposure limits.

For a nonlinear mode of action, a margin-of-exposure (MOE) analysis may be used. A dose is estimated at which the risk is expected to be no greater than 10 percent (one in 10), or at which there was no statistically significant observed increase in adverse response. The ratio between this dose and the actual exposure level is defined as the MOE. Thus, an MOE of 1,000 means that the actual exposure is 1,000 times less than the dose at which a 10 percent response is expected. The magnitude of the MOE should be based on what is known about the agent and its intended uses, and it should be large enough that the probability of an adverse effect is negligible. For example, if nothing is known about the variability in response among humans, then the MOE could include an uncertainty factor of 10 to account for this variability. If the differences between humans and animals in sensitivity to the agent are unknown, an additional factor might be used to increase the MOE. On the other hand, a smaller margin of exposure relative to default approaches, could be acceptable if the risk assessment is based on a key mode-of-action event that is subtle or within the normal limits of physiology.

A controversial default assumption for cancer risk assessment in EPA's initial 1986 Guidelines for Carcinogen Risk Assessment (EPA 1986) and again in the 1996 Proposed Guidelines (EPA 1996) is linearity of the exposure-response relationship. To depart from this default, sufficient supporting data must be available to show that the mode of action is nonlinear. The latest version of EPA's revised Carcinogen Risk Assessment Guidelines (EPA 1999) offers a number of criteria and relevant questions to assist the risk assessor with making this decision. The proposed criteria and questions are intended to help risk assessors judge whether the data are sufficient for determining mode of action, establish the relevance of data from experimental animals for humans, and depart from the default assumption of linearity at low doses. These criteria, modified from the Bradford-Hill criteria for scientific analysis, provide reasonable guidance for evaluating mode-of-action data.

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Assuming a linear dose-response relationship at low doses without regard for mode-of-action information and calculating the exposure for which cancer risk would be less than one in one million is not consistent with a sound scientific approach to decision making. In all cases, the default assumption of linearity results in regulated exposure levels that are many orders of magnitude below the background cancer rate (e.g., one in one million compared with one in four). A nonlinear dose-response relationship suggests that cancer risk decreases faster than a linear dose-response relationship as the exposure decreases. Therefore, regulated exposure levels derived from a nonlinear approach frequently result in higher, but equally protective, regulatory exposure limits than would be indicated if a linear approach were used. For this reason, determining which low-dose extrapolation method to use for carcinogens is a matter of great importance.

For toxic endpoints other than cancer, the default assumption has usually been that a threshold exists at low doses (i.e., a dose exists below which there is no risk of toxic effects). A threshold dose-response curve is a highly nonlinear dose-response curve. Although there has been debate over regulatory levels for some noncarcinogens, the issues are not typically focused on linearity versus nonlinearity. Using laboratory animal data, the approach in these cases is to estimate a dose at which the risk is no greater than 10 percent (like the MOE approach), and divide this dose by a series of "uncertainty factors" to arrive at an acceptable exposure level. While technically different from the MOE approach, this approach is similar to the MOE approach from a practical perspective in that both approaches assume nonlinearity. The approaches differ in that the MOE approach does not prescribe an acceptable margin between the 10 percent response level and a safe level, while the traditional noncancer approach prescribes this margin by explicit use of uncertainty factors.

A recent exception to this "rule" of nonlinearity for noncarcinogens is being considered for endocrine-active compounds (e.g., environmental estrogens). In particular, it has been proposed that chemicals that exert their effect by direct activation of hormone receptors, i.e., those with a *receptor-mediated* mode of action, may be assumed to have linear low-dose-response relationships. AIHC disagrees with this assumption. Nevertheless, this example illustrates that establishing modes of action for noncancer endpoints is becoming more important in determining the shape of the dose-response curve. Daston (1993) provides a good discussion of the existence of thresholds for developmental effects (i.e., a nonlinear relationship). Many of the issues raised by Daston (1993) are more broadly applicable.

Current science is at a crossroads with these two divergent approaches to risk assessment for cancer and noncancer endpoints. Advances in the biochemical and molecular understanding of modes of action of toxicants now allow the identification of noncancer endpoints that are precursor steps

on the path to cancer. Thus, a growing view exists that a "unified" approach to dose-response assessment that encompasses cancer and noncancer effects should be developed, and that the general principles outlined in EPA's Proposed Carcinogen Risk Assessment Guidelines (EPA 1996, 1999) should be applied to noncancer risk assessment as well. In that spirit, the following discussion is relevant to both cancer and noncancer endpoints.

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AIHC believes that the general approach to risk assessment should not be prescriptive but should be uniform in the principles applied across toxic materials and endpoints. A nonprescriptive approach to risk assessment would facilitate the harmonization of cancer and noncancer risk assessment practices. AIHC further suggests that the principles for using the available science on a chemical and disease endpoint to develop a risk assessment should apply equally to cancer and noncancer endpoints, and that a general consistency in the methodology should exist. Specific approaches to low-dose extrapolation should be guided by mode-of-action research. For example, the method used to scale doses from test animals to humans should be based on consistent principles and the mode of action, regardless of toxic endpoint. Barton et al. (1998) discuss the case for harmonization of cancer and noncancer risk assessment methods in more detail.

## SCIENTIFIC EVIDENCE FOR A MODE OF ACTION

As scientific understanding of toxicology grows, there will be more opportunities to establish the mode of action from actual data. For this to happen with any regularity, it is important to have a general understanding of the level of scientific evidence necessary to justify a decision regarding a particular mode of action.

EPA's Proposed Carcinogen Risk Assessment Guidelines (1996, 1999) raise particular questions that should be addressed in determining the mode of action:

- Is there a supporting body of scientific data?
- Has the mode of action been published in peer-reviewed literature, and has it gained general scientific acceptance?
- Is the mode of action consistent with generally agreed-upon principles of toxicology?
- Is there evidence, or can it be reasonably assumed, that the mode of action operates in humans as well as in experimental animals in which the mode of action was established?
- Are humans more or less sensitive to the mode of action than animals?

Although these questions provide some general guidance, the level of "supporting scientific data" that is sufficient to establish a mode of action has not been defined. This point is very much in need of clarification and is addressed in this brochure.

The first step in identifying the mode of action is to show that it is part of the sequence of events leading to the response, i.e., the intermediate step is temporally related to the ultimate

response. This can be accomplished either by directly observing that the events occur as a result of exposure or by showing that when the event is blocked from occurring, the toxic response also is blocked.

An example of an event that contributes to cancer risk and that can be observed directly is the induction of cell division or proliferation (Butterworth et al., 1995; Cohen and Ellwein, 1996; Gaylor and Zheng, 1996). If induced cell proliferation is observed to precede cancer induction in the tissue and cell types with observable carcinogenic lesions, then one can conclude that it is a likely mode of action. The key point of this example is that one can make a reasonable conclusion based on certain observations, even though these observations do not provide absolute *proof* of the conclusion.

Interfering with a key event often proves useful in demonstrating the role of the key event in a mode of action. Blocking receptor activation is a good example. If the toxic response does not occur when the receptor activation step is blocked or when the receptor is genetically deleted, then one can say with certainty that receptor activation is a necessary step in the process.

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Cell proliferation can be an important mode of action for cancer. Increased cell division can result when a tissue is healing in response to cell death or when agents directly induce cells to divide. If an agent causes an increase in cell division, and is *not* genotoxic, leading to a tumorigenic response, then the agent can be considered to have a nonlinear mode of action for risk assessment.

Demonstrating chemical-induced gene mutation also can be important in determining a mode of action, but at the same time may not be sufficient. The scientific community generally recognizes that, in order to cause an increase in cancer risk,

an agent must cause either increased genetic damage to cells or an increase in the rate at which cells divide. Increasing cell division causes an increase in spontaneous mutations. According to the Proposed Carcinogen Risk Assessment Guidelines (EPA 1996, 1999), "A default assumption of linearity is appropriate when the evidence supports a mode of action of gene mutation due to DNA reactivity...." It should be noted that some types of genetic damage, such as damage to and rearrangement of chromosomes, are believed to have nonlinear dose-response curves (Tucker and Preston, 1996). Since various types of genetic alterations result in different toxic outcomes, as well as different dose-response curves at low doses, AIHC strongly believes that demonstration of chemically induced genetic alterations alone is *not* an adequate basis for grouping materials into a common mode of action. In fact, it is reasonable to expect some cases in which DNA reactivity will be demonstrated but will not be a relevant mode of action at actual human exposure levels. Therefore, in the practical application of mode-of-action principles, scientific judgment always should be used to determine which data are relevant and to interpret the observations.

Finally, there are some modes of action that exist in animal models but that do not apply to humans. Consequently, they should not be used as the basis for human health risk assessment. One such mode of action that has received considerable attention is the induction of kidney tumors in male rats through a mechanism involving a male rat-specific protein (Borghoff et al., 1990; EPA, 1991; Hard et al., 1993). This chemical-binding protein is not found in humans. Consequently, chemicals that induce cancer only by this mode of action likely do not present a carcinogenic risk to humans. The observation of kidney tumors formed through this mode of action is not considered relevant to humans.

*Use of mode-of-action information should always take precedence over use of a default assumption.*

## CONCLUSIONS

A mode of action is a category or class of biological mechanisms of toxicity for which the major steps (but not all) of the biochemical processes are understood, allowing the shape of the dose-response curve to be inferred at low levels of risk and low levels of exposure. Identifying a mode of action requires solid scientific information and inference but requires less scientific data and is considerably more feasible than elucidation of the full set of biological mechanisms by which a toxic agent exerts its effect. For this reason, the use of a mode of action rather than the mechanism of action can more often replace the use of default assumptions about the shape of the dose-response curve and the relative sensitivity of humans compared with laboratory animals in risk assessment. Since mode-of-action descriptions are based on a much greater body of knowledge about biology and toxicology than current default assumptions, use of mode-of-action information should always take precedence over use of a default assumption.

In general, establishing a mode of action involves logic, inference, and good scientific judgment. It is impossible to unambiguously *prove* the entire mechanism involved in producing a given response. The purpose of the mode-of-action approach, however, is to use readily available scientific information without requiring the full effort and significant resources needed to prove a mechanism. Although some may view the use of scientific judgment as arbitrary and uncertain, the implementation of scientific peer review for any proposed use of modes of action for risk assessment purposes can offset this concern.

AIHC recognizes that modes of action are subject to some uncertainty by their very nature. The value of this method, however, arises from the fact that this uncertainty should be far less than that of default methods. Further,

encouraging use of biologically based approaches (based on a mode of action) will provide incentives for the development of new scientific data so that regulations continue to be refined and improved. AIHC endorses the use of the mode-of-action approach whenever it is supported by good scientific data and judgment, and urges research scientists, risk assessors, and regulators to view this approach as a useful tool to better characterize human health risks.

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**AIHC's mission is to promote the sound use of scientific principles and procedures in public policy for the assessment and regulation of risks associated with human health effects and ecological effects. Although AIHC does not act as an advocate for any product or substance, its generic positions affect the scope and impact of individual regulatory decisions.**

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