

Chapter 2

Hazard Identification

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Abstract Hazard identification is considered an early step in the risk assessment process. The primary goal of this step is to determine whether exposure to a chemical is likely to cause a specific adverse health effect in humans. The process of hazard identification consists of collecting, evaluating and integrating various sources of data to produce a scientifically-defensible conclusion regarding stressor-induced causation of adverse health effects. The product of data integration is a weight of evidence narrative that characterizes the conditions under which exposure to a chemical is likely to harm human health. This chapter provides a basic introduction to the concept of hazard identification, information critical to this step in risk assessment, and evolving trends in hazard identification.

Keywords Hazard identification · Weight-of-Evidence · Bradford Hill criteria · Database evaluation · Mode of action · Critical effect · Molecular initiating event · Adverse outcome pathway · read-across

Student Learning Objectives

The goals of this chapter are:

- To learn the process that goes into making a judgment regarding the effect(s) caused by an agent of concern

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- To understand how to evaluate the available database to determine if there is evidence of causation
- To define mode of action and critical effect
- To discuss evolving trends in hazard identification

Hazard Identification Definition

Historically, hazard identification has been considered as the first step of a risk assessment (OSTP 1985; NRC 1983). Later permutations of the early steps of the risk assessment process include, in addition to hazard identification, steps of planning and scoping and problem formulation, which are discussed elsewhere in this book (USEPA 1992, 1998, 2003, 2004; NRC 2009).

The goal of the hazard identification step is to make a scientifically defensible judgment about whether exposure of the human population to a given stressor, typically but not limited to a chemical of concern, causes a specific adverse health effect. This process requires a detailed evaluation of available data which is then used to generate a weight-of-evidence analysis that supports or opposes the hypothesis that a stressor is causal of a given adverse health effect in humans (OSTP 1985; NRC 1983). It is also the intention of hazard identification to classify the types of adverse health effects a given stressor may cause. The broad classification(s) assigned to such stressors could include: carcinogen, developmental toxicant, reproductive toxicant, immunotoxicant, neurotoxicant, hepatotoxicant, nephrotoxicant, pulmonary toxicant, cardiotoxicant, dermal toxicant, ocular toxicant, et cetera. A chemical stressor could also be classified as toxic to numerous organ systems, which may be targeted concomitantly upon exposure, at certain concentrations/doses¹, or depend on the route of exposure itself (e.g., inhalation, oral, dermal, ocular) (USEPA 1996, 1998, 2005; NRC 2009).

The Database Evaluation

A key aspect in determining the potential hazard associated with a chemical agent, which will be the stressor discussed from this point forward, is identifying what information is available upon which to draw a conclusion. Data used in hazard identification varies. However, studies conducted in humans or on exposed human populations offer the strongest support that exposure to a given chemical stressor causes an observed adverse health effect. While human studies may offer the most compelling evidence for hazard identification, they are often few in number or weakly informative as they may represent the simple observation that a chemical is

¹ The word concentration depicts exposure via inhalation whereas dose indicates exposure by oral route.

associated with an adverse health effect and lack mode-of action data. Thus, many hazard identifications are conducted using animal data where inherent uncertainties exist regarding the relevance of such data to human health. Recent efforts, particularly those of the International Life Sciences Institute Risk Sciences Institute and the International Programme on Chemical Safety, have generated a variety of frameworks and guidance documents to aid in determination of whether data collected from an animal study is indeed relevant to human health (Boobis et al. 2006, 2008; Meek et al. 2003; Meek 2008; Seed et al. 2005; Sonich-Mullin et al. 2001).

Risk assessors rely on a database of information that has been developed during the assessment of chemical toxicity using laboratory animals or epidemiological studies that consider adverse effects associated with exposure. This data becomes integral to establishing whether or not a chemical agent can cause an adverse effect in humans. As one moves along the process of making this determination, it is important to correctly identify the specific chemical of interest for a risk assessment (WHO 2012). In doing so, one can proceed with identifying what information is available to evaluate the intrinsic hazard associated with the chemical agent in question.

There are numerous public databases that can be queried for information regarding chemical-specific toxicity. Examples include government databases (i.e. TOXNET, IRIS, and NTP), peer-reviewed journals, and published books (U. S. EPA 2009). Typically, information is publically available and the content can be easily retrieved or requested from an academic institute. In addition to the sources identified above, there are also proprietary study reports developed by chemical manufacturers. These reports are not always accessible. However, summaries of these studies are available on the Organisation for Economic Co-operation and Development (OECD) eChemPortal website for chemicals sponsored under the OECD SIDS HPV Programme or USEPA High Production Volume Chemicals Program (OECD 2008; USEPA 1990). These reports come in the form of a well-written robust study summary. More recently, the European Chemicals Agency (ECHA) made available on their website physical-chemical, environmental fate, and toxicological data submitted during the process of chemical registration. Unfortunately, the amount of data available varies by chemical. However, the aforementioned databases can be utilized to survey the types of studies available to investigate the chemical of concern. As one moves along the process of identifying and gathering information required for the risk assessment, the intent is to identify studies that are deemed scientifically-defensible meaning they have undergone peer review or conducted according to standardized protocols approved by various regulatory bodies such as the United States Environmental Protection Agency or Food and Drug Administration.

Sources of information available on chemical agents include human clinical or epidemiological studies, *in vivo* or *in vitro* laboratory animal studies, mechanistic or kinetic studies, or computational toxicology (i.e., quantitative structure activity relationship, systems biology) (USEPA 2012). As a general rule, the use of human data is given higher importance than animal studies and most often preferred by a risk assessor (ECETOC 2009). However, before this information can be used in a risk assessment, it must undergo a rigorous review to determine the applicability

to use the data. When considering human data one must assess the appropriateness of the study design, determine the level of exposure information available and the health outcome. The risk assessor needs to clearly identify the appropriateness of the study design in relation to the types of groups used for comparison, time between exposures, adjust for confounding variables when necessary and determine the appropriate use of statistical analysis used to aid in the interpretation of the data (ECETOC 2009). Although there are no standardized protocols available to aid in assessing the integrity of the study design and interpretation for epidemiological studies, recent efforts to develop a systematic approach to yield greater transparency and reproducibility reviewing these types of studies has been proposed (Money 2013). More importantly, not all data obtained from epidemiological or case studies will address the descriptions provided above, therefore it is important to identify additional studies demonstrating some level of causal association related to a particular health outcome to provide the risk assessor with a higher level of confidence that the classification(s) assigned to a chemical of concern are accurate. More detail on assessing epidemiological studies is provided in a later chapter.

Risk assessors, as mentioned earlier, use animal based toxicological studies when human studies (e.g., case or epidemiological studies) are limited. Toxicological studies developed using standard methodologies approved by government agencies such as the United States Environmental Protection Agency (USEPA), Food and Drug Administration (FDA), or European Chemicals Agency (ECHA) are conducted under Good Laboratory Practices (GLP) and deemed of higher quality to be used in a risk assessment. Some considerations are necessary when evaluating these types of studies for use in hazard identification, for instance: validity of the methodology, reproducibility, study reliability, and appropriateness or usefulness of the study for the risk assessment (Bevan and Strother 2012). As for data developed not using standardized methodologies, this will require more effort to become familiar with the methodologies and relevance of the findings when evaluating the quality of this type of information.

One popular approach for evaluating the reliability of a study is the use of the Klimisch Code (also referred to Klimisch Scores). Klimisch et al. (1997) developed criteria to evaluate toxicology and ecotoxicology data. Three components for evaluating a study being considered for use in hazard identification and subsequent risk assessment were defined as: reliability, relevance and adequacy. Reliability of a study report or publication establishes whether or not the information was collected using standardized methodologies with sufficient details of the experimental design that are described in such a way as to provide evidence of the findings in relation to the clarity and plausibility. The extent to which data are appropriate for use in hazard identification or risk assessments relates to the relevance. Adequacy is defined as making a determination on the usefulness of the data to be considered in a risk assessment.

The Klimisch Codes have become adopted by programs such as the US High Production Volume Program, OECD-SIDS program, and European Union REACH legislation (USEPA 2005; OECD 2008; EU 2006). Another approach that has recently garnered some attention is the use of the ToxRTool (Toxicological data Reliability Assessment Tool). Schneider et al. (2009) developed the tool with the intent

on providing a transparent approach for assessing the reliability of toxicological data. There are two parts of the tool to assess both *in vivo* and *in vitro* studies and key parameters established to aid in the transparency and to harmonize approaches of reliability assessment. In addition, this approach is also useful for identifying the potential sources of variability associated with the evaluation of toxicological studies by various individuals.

Provided that epidemiological and animal studies will be the predominance of information available on a chemical agent at this time, there has been a concerted effort to identify ways to merge this information to aid risk assessments. Adami et al. (2011) recently proposed utilizing the Epid-Tox Framework to describe the strength of association between a toxicological effect and epidemiological information in a scalable form to establish a causal relationship between a chemical agent and an effect. The framework proposes using the following steps:

- a. collect all relevant epidemiological and toxicological studies
- b. assess the quality of each study and assign it to a quality category
- c. evaluate the weight of evidence of the epidemiological and toxicological studies
- d. assign a scalable conclusion to the biological plausibility and epidemiological evidence
- e. determine the placement in a causal relationship grid

One example of the utilization of the Epid-Tox Framework described by the authors related to the adulteration of milk with melamine reported in China (Adami et al. 2011). It has been generally recognized that bladder and kidney toxicity seen in animal studies was considered relevant to humans, but primarily at very high concentrations. However, crystals found in children with melamine exposure in urinary bladder and confirmed deaths provided some corroborating evidence of a mode-of-action (MOA) seen in animal studies at high concentrations (WHO 2009). This type of information provided further support for the biological plausibility regarding human exposure to melamine and concerns with bladder and kidney toxicity. Simpkins et al. (2011) also reported the applicability of this framework by investigating the causal relationship between atrazine exposure and breast cancer in women. They concluded the absence of epidemiological evidence and lack of a plausible MOA associated with mammary tumorigenesis in female Sprague Dawley rats did not support public concerns related to the carcinogenicity of atrazine and was in-line with the previous schemes for the classification of carcinogenic potential of atrazine in humans reviewed by others (USEPA 2003, 2006) and IARC (1999).

In a similar direction and effort, Lavelle et al. (2012) have also proposed a framework aimed at systematically integrating human and animal data with the intent of creating consistency and transparency in the process for the purposes of evaluating and classifying chemical agents. Please refer to Fig. 2.1 for an illustrative example regarding the application of this framework to be used for a chemical risk assessment. As seen from this example, the integration of data from available studies enables a conclusion to be drawn regarding the causal relationship between a chemical agent and an adverse effect.

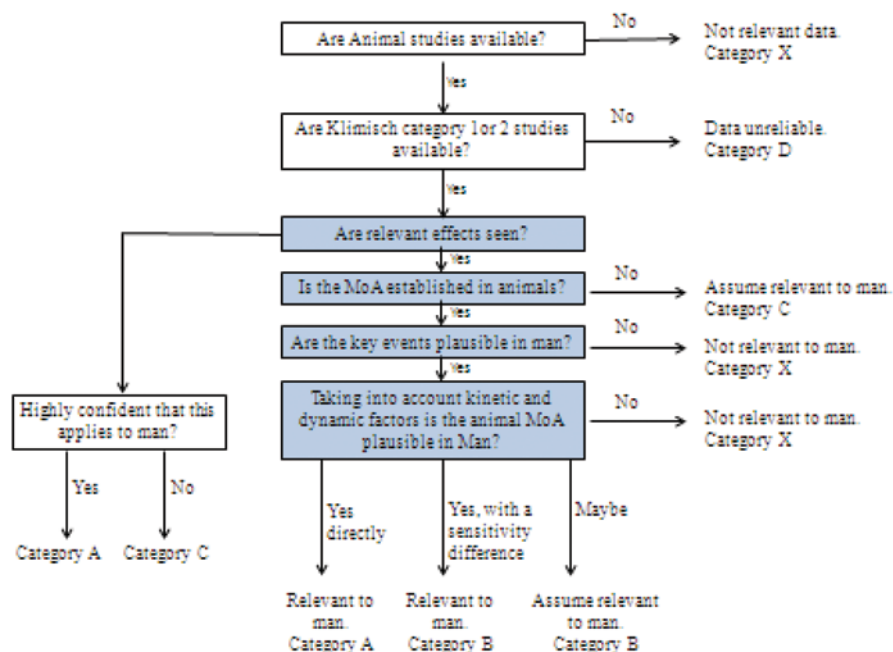


Fig. 2.1 Illustrates an approach to categorize animal data to determine the relevance in human risk assessment. (Reprinted from Regul. Toxicol. Pharmacol, 62/2, Lavelle et al. 2012, with permission from Elsevier)

Once all data have been identified for conducting a risk assessment, the next step in the process is to determine what critical effect is associated with the chemical of concern. Although in principle this may seem a fairly straight forward process, in actuality there are a number of factors described below that need to be considered and understood before drawing a conclusion. For example, expert judgment in evaluating the quality of studies and suitability for hazard identification to be used in risk assessments are important factors to consider. In addition, identifying an effect seen in animal studies between controls versus treatment groups, establishing if there is clear evidence of a dose response observed with the treatment groups, assessing whether the effect is adverse, and the biological significance of the reported effect are all important for the risk assessor to take into consideration (Dorato and Engelhardt 2005; Lewis et al. 2002). To gain a better appreciation for these types of challenges, the reader is encouraged to follow up with the work submitted by Lewis et al. 2002. The authors provide a comprehensive approach by outlining criteria for establishing whether the observed effect is treatment-related and whether the effect seen in animal studies is adverse.

For toxicological studies, dose-related responses identified as statistically different from the control group are evaluated as potentially adverse. The portion of the dose response where control and exposed organisms are not different is commonly referred to as a No-Observed-Adverse-Effect (NOAEL). It is an important determinant in establishing whether there is a concern related to an observed target organ effect (USEPA 2012). There have been numerous definitions provided by

various regulatory bodies or organizations describing the NOAEL, but generally speaking it is the highest concentration of a chemical of concern not shown to cause an adverse effect such as: alteration in morphology, functional capacity, growth, or developmental life span determined by experimental design or observation (WHO and WHO 1996). As the risk assessor, one needs to have a clear understanding of what constitutes an adverse effect so that a determination can be made about the relevance of that observed effect to human health.

One of the most frequent toxicological effects reported in animal studies and relevance to humans is described as α_{2u} -globulin and nephropathy seen in male rats (USEPA 1991; Swenberg 1993). This commonly reported effect observed in male rats in association with renal carcinogenesis has little or no human relevance. Another commonly reported adverse effect seen in toxicological studies related to exposure to chemicals that induce hepatic enzymes is liver hypertrophy. Chemical-induced hepatic (liver) hypertrophy is well-documented in rodent studies. However, the significance of this observed effect has been questioned. Liver hypertrophy as defined by toxicologists can have various meanings such as; increase in liver weight (liver hypertrophy), increase in average size of hepatocytes (hepatocellular hypertrophy), and hepatic enzyme induction (work hypertrophy) (Hall et al. 2012).

Recently, the European Society of Toxicologic Pathology (ETSP) convened an expert opinion group to discuss the significance of hepatocellular hypertrophy in rodents to establish whether this was an adaptive or adverse response (Hall et al. 2012). The opinion reached by the expert group was that hepatomegaly (enlarged liver) in the absence of histopathological or clinical pathology changes associated with liver toxicity was considered to be an adaptive response and should be reached using a weight of evidence approach. The expert group also stated that hepatocellular hypertrophy associated with the increase in liver metabolizing enzymes can be considered fully reversible and not expected to compromise the viability or functional integrity of the organism. The examples provided above emphasize the importance for identifying the mode-of-action (MOA) of a chemical stressor to characterize what adverse outcomes are associated with a molecular initiating event (MIE), and these concepts will be addressed later in this chapter.

Mode of Action Evaluation and Identification of Critical Effect

Chemical stressors may cause a plethora of responses in exposed organisms. The range of effects is often highly variable and driven by the manner in which exposure occurred, the duration of exposure, the dose, inter-organismal variability, and concomitant exposures. Often, one of the most dominant determinants driving the outcome of exposure is the dose to which the organism is exposed. Dose in this context not only refers to the concentration of chemical measured in a given exposure media such as air, soil, or water, but it also refers to the dose at a given target tissue inducing an adverse effect. It is important to consider chemical's characteristics (physical and chemical properties), which may affect its ability to be absorbed

into the body. Furthermore, it is important to consider what happens to the chemical upon absorption. The term toxicokinetics broadly refers to how a chemical is absorbed, what happens to it while it is in the body (i.e., distribution, metabolism), and ultimately how it is removed from the body via excretion. Data regarding these issues is more common to well characterized chemicals where a significant number of studies have been conducted to evaluate toxicokinetic properties. After absorption, the effects of exposure are often described as a series or continuum of effects that are manifest in dose- and duration of exposure-dependent manner. From this perspective, the response to a given exposure may escalate from mild physiological adaptations, to compensatory stress response, then progress to the induction of an apical effect, and finally to the manifestation of an adverse effect (Dourson et al. 2013). This process may be referred to collectively as toxicodynamics.

The sequence of molecular key events that occur prior to the manifestation of an adverse effect is called the chemical's mode-of-action (MOA). It is the identification of the apical effect that is relevant to human health that is crucial during hazard identification. The apical effect is the key event that happens immediately prior to the adverse effect, making it a molecular gate keeper of sorts. Thus, during the evaluation of chemical-specific toxicity data, priority should be given to data collected in humans. When quality data is not available in humans, animal studies may be a source of information. However, it is not the data collected in the most sensitive animal species that matters most. Rather, it is the health effects that are relevant to humans that should be considered to be of greatest concern. When the relevance of the adverse or apical effect to human health is unknown, data collected in the most sensitive animal species may be chosen as a means of conservative scientific judgment.

When considering whether or not an effect induced by chemical exposure is indeed adverse, it is important to define what an adverse effect is. There are several committees and organizations that have attempted to define adverse effect and a general consensus is that an adverse effect is:

- A change in morphology, histology, organ function, growth, reproduction, survival, longevity of a cell, development, of a tissue, organ system, or organism
- This change reduces the organism's ability to function, reduces the ability to respond to other stressors, increases susceptibility for disease or other dysfunction, and decreases the long-term chances of survival (Dorato and Engelhardt 2005; Keller et al. 2012; Lewis et al. 2002; NRC 2007; USEPA 1994).

An adverse effect is distinguishable from an adaptive response in that the change(s) constituting an adverse effect decreases survival of the organisms whereas an adaptive response enables the organism to respond to the stressor such that function is not reduced and survival chances are increased (Lewis et al. 2002; NRC 2007; Williams and Iatropoulos 2002).

Among the available sources of information regarding chemical-specific toxicity, it is important to identify possible adverse effect(s) caused by exposure and potential mechanisms driving those effects. Available data may be insufficient to identify the mechanism of action governing all observed adverse effects induced by chemical exposure. However, the generation and incorporation of more

high-throughput, molecular data is enabling a better characterization of the cellular pathways involved in both homeostatic or stress responses and the induction of dysfunction and damage. When highly detailed data are not available to fully characterize the chemical-specific mechanism(s) of action, the identification of chemical-specific MOA may be possible. MOA is distinguished from mechanism of action in that it is a less detailed description of the key molecular events that precede the manifestation of an adverse effect. The application of MOA is somewhat different than the mechanism of action. Where the mechanism of action is used to fully characterize the molecular events that occur to cause an adverse effect, the MOA utilizes a simplified scheme of events that are critical to the adverse effect (Fig. 2.2). A risk assessor benefits most from the MOA in a sense that it requires less data to generate and is part of the evaluation of dose-response that may lead to the genesis of toxicity factors to be utilized in regulation (Dellarco and Baetcke 2005).

Essential to hazard identification is a MOA evaluation (USEPA 2005). There are many MOAs that are the underpinnings of various adverse effects. This step is not only important for determining key events upon which to base a point of departure, but also critical in evaluating the human relevance of an observed MOA and subsequent adverse effect. Recent efforts have attempted to describe a framework to integrate MOA and human relevance together to allow for concomitant evaluation. The unifying element of this approach is to utilize Bradford Hill criteria for causation,

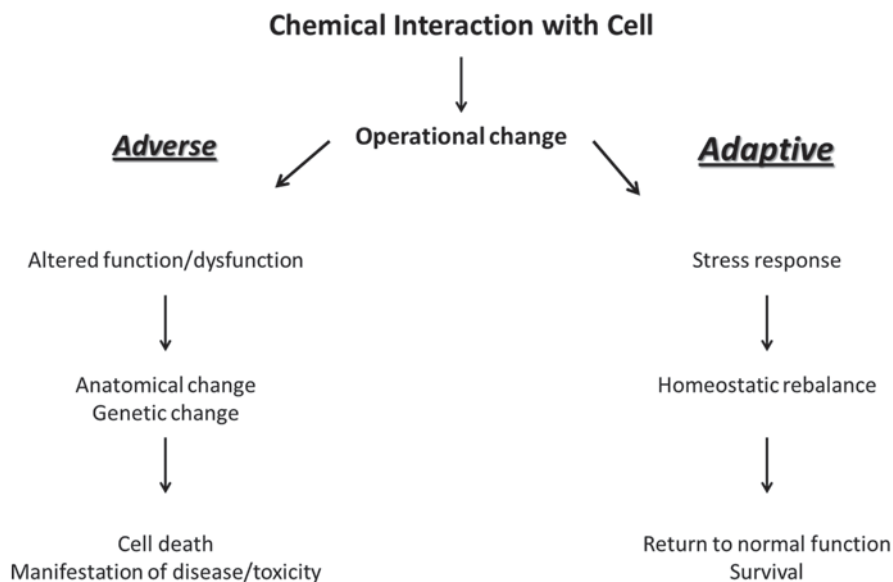


Fig. 2.2 Illustrates some of the key differences between potential cellular responses that may occur following chemical exposure. Responses in this figure may be characterized as either adaptive or adverse and both are part of the chemical-specific MOA. It is important to distinguish adaptive effects from adverse effects during risk assessment process as this distinction is the basis upon which the hazard identification and dose-response assessment are built

which are discussed later in this chapter, to determine whether the available data are adequate to develop a putative MOA and if that MOA is relevant to human health (Meek et al. 2003; WHO 2006; Sonich-Mullin et al. 2001). For data rich chemicals, additional details such as toxicokinetic and toxicodynamic data may be used to further inform the risk assessment using data rather than standard default approaches. Such information may also go beyond the scope of MOA evaluation and also aid in identification of subpopulations at greater risk (Meek 2008).

Evidence Based Evaluation of Available Database

The determination of causation is no simple thing. Epidemiological studies can be misleading by revealing an association between a chemical present in the environment and an adverse effect or disease when the observed effect is due to confounding or poor study design. Similarly, animal studies may indicate that chemical is, for example, a carcinogen when in fact the mechanism of carcinogenesis in the study animal species is not pertinent to human physiology. The hazard identification stage of a risk assessment is dominated by uncertainty regarding the cause and effect relationship that exists between exposure and adverse health effect. This uncertainty is centered around the concern of misclassifying a chemical agent or coming to an incorrect conclusion regarding causation.

To guide consistent decision making, guidelines are useful for facilitating the identification of causation. One such set of guidelines are called the Hill Criteria (Hill 1965):

- **Strength:** refers to how strongly the chemical of concern associates with the adverse effect or disease (*e.g.*, large relative risks or mortality ratios, high tumor incidence)
- **Consistency:** a chemical exposure that is observed to occur concurrent with the manifestation of a given disease or adverse effect in a number of independent studies is considered to be consistently associated
- **Specificity:** an adverse effect or disease is particularly associated with an exposure to a certain chemical and not with other types of exposure
- **Temporality:** the adverse effect of disease is observed after exposure to a chemical of concern
- **Dose-Response:** the magnitude and frequency of the adverse effect or disease is heightened when the exposure is increased
- **Plausibility:** indicates that a proposed mechanism for how a given stressor causes an observed adverse effect or disease is reasonable and biologically possible
- **Coherence:** based on what is known, the chemical of concern causes a given adverse effect or disease; no conflicting data
- **Experimental Evidence:** research in different models or types of experiments indicate that the chemical of concern can cause an observed adverse effect
- **Analogy:** various model systems or structurally related chemicals cause the same effect

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