Public Health Implications of Environmental Exposures

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The Agency for Toxic Substances and Disease Registry (ATSDR) is a public health agency with responsibility for assessing the public health implications associated with uncontrolled releases of hazardous substances into the environment. The biological effects of low-level exposures are a primary concern in these assessments. One of the tools used by the agency for this purpose is the risk assessment paradigm originally outlined and described by the National Academy of Science in 1983. Because of its design and inherent concepts, risk assessment has been variously employed by a number of environmental and public health agencies and programs as a means to organize information, as a decision support tool, and as a working hypothesis for biologically based inference and extrapolation. Risk assessment has also been the subject of significant critical review. The ATSDR recognizes the utility of both the qualitative and quantitative conclusions provided by traditional risk assessment, but the agency uses such estimates only in the broader context of professional judgment, internal and external peer review, and extensive public review and comment. This multifaceted approach is consistent with the Council on Environmental Quality's description and use of risk analysis as an organizing construct based on sound biomedical and other scientific judgment in concert with risk assessment to define plausible exposure ranges of concern rather than a single numerical estimate that may convey an artificial sense of precision. In this approach biomedical opinion, host factors, mechanistic interpretation, molecular epidemiology, and actual exposure conditions are all critically important in evaluating the significance of environmental exposure to hazardous substances. As such, the ATSDR risk analysis approach is a multidimensional endeavor encompassing not only the components of risk assessment but also the principles of biomedical judgment, risk management, and risk communication. Within this framework of risk analysis, the ATSDR may rely on one or more of a number of interrelated principles and approaches to screen, organize information, set priorities, make decisions, and define future research needs and directions. — *Environ Health Perspect* 106(Suppl 1):369–378 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/369-378derosa/abstract.html*

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Introduction

The Agency for Toxic Substances and Disease Registry (ATSDR) is one of eight agencies of the U.S. Public Health Service within the U.S. Department of Health and Human Services. The ATSDR mission is to prevent exposure and adverse human health effects and diminished quality of life associated with exposure to hazardous substances from waste sites, unplanned releases, and other sources of pollution present in the environment (*1*). Pursuant to its responsibilities mandated by the Comprehensive Environmental Response, Compensation, and Liability Act, also known as Superfund, the agency addresses public health concerns associated with a wide range of low-level exposures to substances found at hazardous waste facilities.

The ATSDR, like other agencies such as the U.S. Environmental Protection Agency (U.S. EPA) and the U.S. Food and Drug Administration (U.S FDA), has relied on risk assessment in its task of assessing the public health implications of low-level exposures to hazardous substances. As defined by the National Academy of Sciences in 1983, risk assessment consists of four interrelated steps or components: hazard identification, dose–response assessment, exposure assessment, and risk characterization (*2*). In the real-life risk assessment process, the fourth step, risk characterization, is actually an integration of the other three components into a qualitative and/or quantitative assessment that characterizes the probability of adverse health effects in an exposed population. The ATSDR approach for risk characterization of noncancer adverse health effects is primarily a qualitative rather than a probabilistic undertaking. Nevertheless, the ATSDR does use minimal risk levels (MRLs), health guidance values developed by the ATSDR specifically to identify levels of exposure thought to be without appreciable risk over specified durations and routes of exposure (*3*). Although the procedures used to derive such health guidance values by agency scientists is operationally straightforward, the judgment that goes into development of these values is guided by a full range of expert judgment in the fields of toxicology, epidemiology, and pathology, as detailed below.

In this paper we discuss the role of health guidance values in public health practice and the application of mechanistic insights in deriving health guidance values. Three questions posed are vital to how regulatory and public health agencies consider the biological effects of low-level exposures (*4*):

- Does the understanding of the mechanisms of toxicity affect how agencies assess risks from exposures to toxic substances?
- Does an understanding of the mechanisms by which the body adapts (e.g., detoxifies, repairs, etc.) to the effects of

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Abbreviations used: ADI, acceptable daily intake; ATSDR, Agency for Toxic Substances and Disease Registry; BMD, benchmark dose; CDD, chlorinated dibenzodioxin; CDF, chlorinated dibenzofuran; CNS, central nervous system; ESADDI, estimated safe and adequate daily intake; ETE, essential trace element(s); LOAEL, lowest observed adverse effect level; MRL, minimal risk level; MTBE, methyl-*t*-butyl ether; NOAEL, no observed adverse effect level; NPL, National Priorities List; PBPK, physiologically based pharmacokinetic; PCE, tetrachloroethylene; ppm, parts per million; RfCs, reference concentrations; RfDs, reference doses; TCA, trichloroacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCE, trichloroethylene; TEF, toxicity equivalency factor(s); UF, uncertainty factor; U.S. EPA, Environmental Protection Agency; U.S. FDA, U.S. Food and Drug Administration; WOE, weight of evidence.

exposures to toxic substances affect how the ATSDR assesses risks from exposures to toxic substances?

- If low doses of toxic agents induce apparently beneficial responses (e.g., enhanced longevity, lower incidence of disease), how does and/or could the ATSDR address this?

We conclude these discussions by outlining some future directions being pursued by the ATSDR to expand and strengthen the practical application of biologically based inference and extrapolation to the risk characterization and analysis of environmental exposures to hazardous wastes.

Risk Analysis as an Organizing Construct to Promote Optimal Decisions

The ATSDR uses risk assessment and associated health guidance values in the context of risk analysis as defined by the Council on Environmental Quality (*5*). Within this framework, risk analysis is an organizing construct based on sound biomedical and other scientific judgment as well as on risk assessment and is used to define plausible ranges of concern rather than single numerical conclusions, which often are misinterpreted. In this approach actual exposure conditions are critically important to the process of evaluating the significance of environmental exposures to hazardous substances (*6*).

As applied by the ATSDR in public health practice, risk analysis is a multidimensional endeavor encompassing the concepts of risk assessment, biomedical judgment, risk management, and risk communication (Figure 1). Its application can be visualized within the broader context of the agency's public health assessment program (Figure 2). As part of its public health mission, the ATSDR develops public health assessments to evaluate the public health implications of all hazardous waste sites on the National Priorities List (NPL) and for selected sites identified by public petition (*1*). The ATSDR's public health assessments are based on three key components: environmental monitoring data, health outcome data, and community health concerns. These three components are then evaluated in the context of what is known about the toxicity of site-related contaminants, the probability of past, present, or future exposures, and the significance of site-specific conditions, including demographic and other parameters of exposure. In these evaluations the ATSDR may use health guidance values

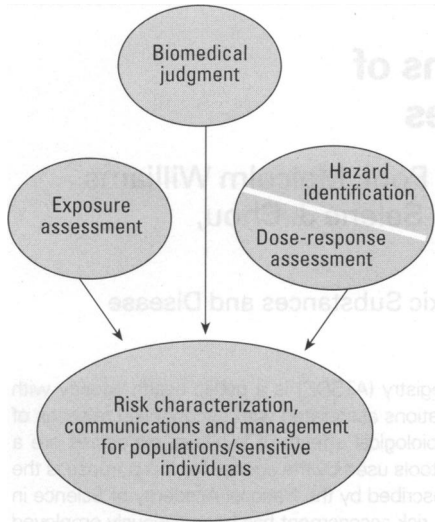

Figure 1. Risk analysis as a multidimensional endeavor, encompassing the components of risk assessment, biomedical judgement, risk management, and risk communication.

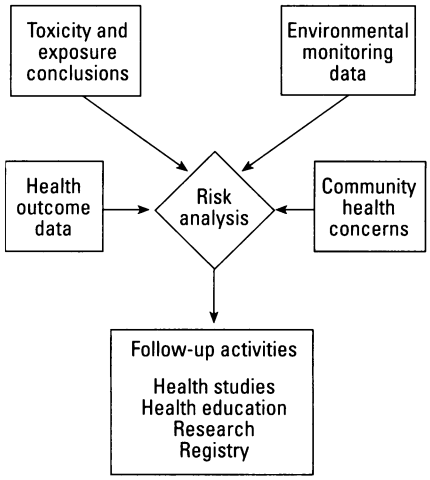

Figure 2. Application of the concept of risk analysis to the ATSDR public health assessment program.

specifically to determine which chemicals should be addressed further in terms of risk posed to exposed communities based on a comparison with environmental monitoring data. Depending on the outcome of the ATSDR analysis, a number of follow-up activities might be considered. Possible follow-up activities may include further health study and/or surveillance, health education, research, and the development of chemical-specific exposure registries.

Derivation of Health Guidance Values

The ATSDR derives chemical-specific health guidance values known as MRLs for oral and inhalation routes of exposure to assist and guide health assessors in evaluating contaminants of concern at hazardous waste sites. An MRL is defined as "an estimate of the daily human exposure to a substance that is likely to be without an appreciable risk of adverse, noncancer effects over a specified duration of exposure" (*3*). Depending on the availability of appropriate data, MRLs are calculated for both oral and inhalation routes of exposure and for three specific durations of exposure: acute (1 to 14 days), intermediate (15 to 364 days), and chronic (365 days or longer).

These MRLs, their supporting databases, and an explanation of factors considered in their derivation are included in toxicological profiles developed by the ATSDR (*7*). Procedurally, the derivation of an MRL is straightforward and analogous to the derivation of acceptable daily intakes (ADIs) and reference doses/reference concentrations (RfDs/RfCs) developed by the U.S. FDA and the U.S. EPA, respectively. Specific internal guidance for derivation of MRLs by the ATSDR has been published (*3*).

Traditionally, health guidance values have been derived from either controlled human clinical studies, human epidemiologic studies (usually retrospective), or controlled studies involving laboratory animals serving as surrogates for human populations. Based on a review of these studies, the lowest dose or exposure level at which an effect considered adverse was observed (LOAEL) and the highest level below which no adverse effect was observed (NOAEL) is similarly determined. Because the population of interest in the health study typically is not the same as a potentially exposed population to be protected by the health guidance value, mathematical adjustments are made to the LOAEL or NOAEL to express uncertainties inherent in the assumptions and database used to calculate the health guidance value.

The uncertainty factors (UFs) proposed by Barnes and Dourson (*8*) and presented in Table 1 provide the basis for the approach and types of mathematical adjustments used by the ATSDR in deriving its MRLs. Each MRL thus has its own area of uncertainty with regard to its derivation based primarily on the study used as the basis for deriving the MRLs. The aggregate UFs can be viewed as loose upper-bound estimates that account for differences in susceptibility between test and target species and for

Table 1. Factors used by the ATSDR in derivation of MRLs.

UFs and MFs[a]	Used to account for[b]
3–10	Extrapolation from animal studies to humans (interspecies differences)
1–10	Differences in sensitivity among humans (intraspecies variability)
3–10	Use of an LOAEL rather than an NOAEL
>0–10	Completeness of database

Abbreviatiom: MF, modifying factor. [a]Maximum factors used to date in MRL derivation is 3000. [b]The ATSDR does not at this time extrapolate between exposure routes.

sensitivity differences within the human population. The overall magnitude of the UF reflects the confidence in the final calculated number and the database supporting that number for a given chemical. As a result the final health guidance value, or MRL, can be viewed as an estimate of a dose that is likely to be without adverse effects in sensitive individuals for a specified duration and route of exposure.

Mathematically the derivation of the MRL can be expressed as follows:

$$\mathrm{MRL} = \frac{\text{the NOAEL or LOAEL}}{\mathrm{UF} \times \mathrm{MF}}$$

where MF = a modifying factor and the NOAEL, the LOAEL, and the UF are as previously defined.

This formula provides a computational method for determining a reference value for a particular substance and a particular route and duration of exposure. MRLs thus derived provide route-specific guidance that would protect all potentially exposed populations. As such, they also provide a basis for determining screening or trigger levels for chemical-specific exposures of concern on a site-specific basis. Health guidance values such as MRLs, RfDs/RfCs, and ADIs can all be used in specific exposure scenarios to estimate levels of a substance in a particular environmental medium that can serve as a basis to determine whether further evaluation is warranted. Such health guidance values as used by the ATSDR are intended to be used only as screening tools. They are not intended to be interpreted as precise values or used as action levels but as indicators of whether further evaluation is warranted for a particular exposure scenario. Moreover, because of the specific exposure route, exposure duration, and toxicity end point associated with each health guidance value, the values can also be used to alert health care providers about what outcomes they should be concerned with in a particular locality or site vicinity.

The Role of Biomedical Judgment in Derivation of Health Guidance Values

Although procedures used by agency scientists to derive health guidance values are operationally straightforward, the judgment used in developing these values is guided by a full range of expertise in the fields of toxicology, epidemiology, and pathology, as detailed below.

Application of Mechanistic Insights in the Derivation of Health Guidance Values

With regard to the first question posed in "Introduction," an understanding of the mechanism of toxicity affects how the ATSDR assesses risks from exposure to toxic substances. Because reactions between toxic chemicals and organisms are interactive processes, several issues must be considered when evaluating mechanisms of toxicity for risk-assessment purposes. First, how does the organism affect the chemical being evaluated in terms of toxicokinetic mechanisms (i.e., absorption, distribution, metabolism, and excretion)? Differences in the toxicokinetics of various chemicals, and similarly, interspecies differences in the toxicokinetics of the same chemical, may account for significant variations in toxicity. Second, how does the chemical affect the organism in terms of toxicodynamic mechanisms (e.g., interacting with specific receptors, enzymes, membrane sites, or other proteins)? In addition, after initial chemical insult, further chemical exposure and subsequent biochemical reactions may lead to toxicologically significant morphological changes. The ATSDR's multifaceted risk-assessment approach requires full consideration of such mechanistically based differences and similarities in the pathogenesis of disease. These considerations are especially important to the process of evaluating and selecting appropriate toxicity end points for the derivation of MRLs.

Toxicokinetic Mechanisms. Several examples in which the ATSDR used a knowledge of chemical-specific toxicokinetic mechanisms in derivation of its MRLs are listed and discussed below. These examples are summarized in Table 2.

Absorption/Bioavailability. 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD). The ATSDR has developed a proposed acute oral MRL for TCDD based on a study in mice exposed to 14 daily doses of TCDD by gavage in oil vehicle (*9*). An important issue considered during derivation of this MRL was the bioavailability of TCDD (*10*): It has been well documented in animal studies that adsorption of TCDD from the gastrointestinal tract depends largely on the carrier vehicle (*11*). Absorption of TCDD from both soil and feed has been estimated to be 20 to 60%, whereas the adsorption of TCDD from a corn oil vehicle has been estimated to be as much as 85%. Because the likely means of environmental exposure to TCDD is through ingestion of contaminated food or soil but the definitive animal TCDD toxicity study used corn oil gavage, MRL derivation

Table 2. Mechanistic insights affecting derivation of MRLs: toxicokinetics.

Chemical	Toxicokinetic mechanism	Description
TCDD	Absorption	Differences in absorption using soil, feed, or oil vehicle; reflected in the use of MF
Cyanides	Metabolism	Interspecies differences in tissue levels of rhodanese[a]; very low in dogs
PCE	Metabolism	Interspecies differences in metabolism of PCE to TCA; more TCA in mice than in humans
TCE	Distribution/excretion	Not readily eliminated, accumulation in adipose tissue; adjustment for continuous exposure
1,1,1,-Trichloroethane	Distribution/excretion	Rapidly eliminated, blood levels plateau after 2 to 3 hr; no adjustment for continuous exposure
Hexachlorethane	Distribution/excretion	Rapid turnover in tissues; no adjustment for continuous exposure
Vinyl chloride	Distribution/excretion	Rapidly metabolized and excreted, readily reaches steady state; no adjustment for continuous exposure

Abbreviations: PCE, tetrachloroethylene; TCA, trichloroacetic acid; TCE, trichloroethylene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. [a]An enzyme used in the conversion of cyanide into inactive thiocyanate.

was divided by an additional modifying factor of 0.5 to adjust for the approximate 2-fold greater bioavailability from corn oil compared to that from food or soil.

Metabolism. Many toxic chemicals are metabolized by similar metabolic pathways across species. However, interspecies differences are also well documented (*12*). These differences often are attributed to lack of particular enzymes or differences in the relative importance of particular routes of metabolism. Cyanides and tetrachloroethylene (PCE) are examples of chemicals with specific mechanistic differences in metabolism that affected derivation of the ATSDR MRL.

CYANIDES. Rhodanese is an enzyme used in the conversion of cyanide into inactive thiocyanate. Dog tissues have low levels of rhodanese compared with levels in other species; therefore dogs are extremely susceptible to cyanide poisoning. As a result, cyanide metabolism in a species such as the rat more accurately reflects cyanide metabolism in the human than does cyanide metabolism in the dog. Because of this, the ATSDR based its intermediate-duration oral MRL on a study in rats even though a similar study in dogs reported much lower toxic effect levels (*13*).

TETRACHLOROETHYLENE. During the ATSDR derivation of MRLs for intermediate-duration inhalation and oral exposures to PCE, it was noted that the proposed toxic end point reported for mice (peroxisomal proliferative response in the liver) might not be relevant to humans. Several differences in PCE metabolism exist between mice and humans. The rate of metabolism of PCE to trichloroacetic acid (TCA)—the reactive metabolite in the hepatic toxicity—is much greater in mice than in humans (*14*). Moreover, mice and rats also respond to TCA and many other chemicals by induction of hepatocellular peroxisomes, whereas humans either are much less responsive to peroxisome proliferators or do not respond at all to doses that cause marked responses in rodents. Because humans produce little TCA following PCE exposure and because peroxisomal proliferation in humans is minimal compared to that in rodents, liver hypertrophy and tumor development as observed in mice may not occur by the same mechanism in humans or may be much less likely to occur. Therefore, the ATSDR did not derive intermediate-duration MRLs for PCE from these rodent studies but instead deferred derivation until the significance of these mechanistic differences between humans and rodents is more fully understood.

Distribution and Excretion. According to the ATSDR MRL methodology (*3*), if a study serving as the base for an MRL derivation does not involve continuous dosing over the entire exposure period, an adjustment can be made to correct for intermittent exposure. Generally, the intermittent exposure dose is multiplied by correction factors to adjust for a full 24 hr/day and 7 days/week of exposure.

More specifically, if study results indicate that a toxic end point depends directly on the duration of exposure (e.g., if metabolism and excretion is moderate to slow or the study results indicate that the toxic end point is a cumulative effect), adjustments to correct for intermittent exposure may be appropriate. Conversely, if the critical effects are considered to depend mainly on exposure concentration rather than length of exposure and if the substance being tested is rapidly metabolized and/or eliminated, dose adjustment for intermittent exposure generally is considered inappropriate. A number of examples are presented below in which the ATSDR considered the appropriateness of dose adjustment for intermittent exposure in derivation of MRLs.

TRICHLOROETHYLENE. An acute-duration inhalation MRL was derived for trichloroethylene (TCE) based on neurologic effects observed in human volunteers (*15*). An intermediate-duration inhalation MRL was also derived from a study in which neurological effects were seen in rats (*16*). Because the toxicokinetic data indicate that TCE is not readily eliminated and can accumulate in adipose tissue (*17*), both MRLs were adjusted for intermittent exposure.

1,1,1-TRICHLOROETHANE. An acute-duration inhalation MRL was derived for 1,1,1-trichloroethane based on an LOAEL for reduced performance on psychomotor tests reported in humans (*18*). However, 1,1,1-trichloroethane is rapidly eliminated in the expired air and blood levels of 1,1,1-trichloroethane plateau after 2 to 3 hr (*18*). These findings suggest that longer exposure would not lead to higher body burdens. Therefore, a correction for intermittent exposure was considered inappropriate for the acute-duration inhalation MRL for 1,1,1-trichloroethane (*19*).

HEXACHLOROETHANE. An acute-duration inhalation MRL was derived for hexachloroethane based on an NOAEL for neurological effects reported in pregnant rats exposed to hexachloroethane (*20*). Laboratory data showed that effects depend on blood levels, which directly depend on concentration, not duration of treatment (*21*). Moreover, the toxicokinetic data also showed a rapid turnover in the tissues. Therefore, no adjustment was made to the MRL to correct for intermittent exposure.

VINYL CHLORIDE. An acute-duration inhalation MRL was derived for vinyl chloride based on developmental effects in offspring of mice exposed during gestation (*22,23*). An intermediate-duration inhalation MRL was also derived based on hepatic effects in rats (*24*). A review of the toxicokinetic data indicated that vinyl chloride readily reaches steady state, is rapidly metabolized and excreted, and neither it nor its metabolites readily accumulate in tissue (*25*). Therefore, no adjustment for intermittent exposure was made in the derivation of either the acute or the intermediate-duration inhalation MRL for vinyl chloride.

Toxicodynamic Mechanisms. Chemical interactions with specific receptors or enzymes are examples of toxicodynamic mechanisms. Such mechanisms and their associated interspecies differences may play critical roles in the expression of toxicity as well as in deliberations about risk assessment for toxic chemicals. Two specific examples of how understanding such toxicodynamic mechanisms has affected the ATSDR derivation of MRLs are discussed below.

Chlorinated Dibenzodioxins and Dibenzofurans. Chlorinated dibenzodioxins (CDDs) and chlorinated dibenzofurans (CDFs) are groups of halogenated aromatic hydrocarbons that are closely related chemically and often occur together in the environment (*26*). To estimate the risk associated with exposure to chemical congeners of these two closely related chemical classes, the ATSDR has adopted the U.S. EPA toxicity equivalency factors (TEF) method, which is based on congener-specific data and the assumption that *Ah* receptor-mediated toxicity is common to dioxinlike congeners and is additive (*27*). The TEF scheme compares the relative toxicity of individual CDD and CDF congeners to that of 2,3,7,8-TCDD, the most toxic and extensively studied of these halogenated aromatic hydrocarbons. The TEF for 2,3,7,8-TCDD is defined as unity, whereas TEF values for all other CDD and CDF congeners are less than 1 (0 has been assigned to all non-2,3,7,8-substituted congeners); this reflects the lower toxic potency of most CDD and CDF congeners. A description of the ATSDR MRL values for a number of CDDs and CDFs using the TEF approach was recently published in an article that also points out a number of uncertainties associated with this method (*28*).

METHYL-*T*-BUTYL ETHER. An MRL for chronic-duration inhalation exposure to methyl-*t*-butyl ether (MTBE) was derived from an NOAEL for chronic progressive nephropathy in female rats (*29*). Chronic progressive nephropathy is an age-related spontaneous disorder of rats that is more severe in males than in females and affects certain strains more than others (*30*). Chronic exposure of male rats to α2μ-globulin-inducing agents results in aggravation of chronic progressive nephropathy, which is characterized by increased severity and earlier onset of the disease. Based on these considerations, the ATSDR concluded that the higher incidence and greater severity of chronic progressive nephropathy observed at lower MTBE exposure concentrations in male rats compared with that in female rats may have been due to exacerbation of this syndrome by the accumulation of α2μ-globulin or another unknown protein unique to male rats. As a result this end point was considered unsuitable for MRL derivation. However, because female rats also exhibited enhanced chronic progressive nephropathy but at higher exposure levels, the NOAEL for this end point in female rats was used (instead of the NOAEL observed in male rats) to derive the chronic-duration inhalation MRL for MTBE.

Assessment of Adaptive Responses in the Derivation of Health Guidance Values

In response to the second question posed in "Introduction," understanding the mechanisms by which the body adapts to the effects of exposure to toxic substances affects how the ATSDR assesses risk from exposure to toxic substances. Many potentially toxic chemicals cause changes in an organism that may be considered adaptive. In other words, the organism responds to the chemical exposure in a way that maintains homeostasis.

Adaptive responses within organisms may involve biochemical or structural alterations. Examples of adaptive biochemical responses include induction of the cytochrome P450 mixed-function oxidase system in the liver or other organs as well as glutathione depletion and synthesis in the liver. Examples of structural adaptive responses within tissues include atrophy, hypertrophy, hyperplasia, and metaplasia. Adaptive responses generally are considered to enhance an organism's performance or its ability to withstand challenge. Actually, however, some alterations that typically are classified as adaptive responses may have potentially harmful (i.e., adverse) effects on the host in some cases. An adverse effect is defined as "any effect that reduces the capacity of an organism or a component of the organism to function in a normal manner or diminishes the ability to withstand further stress" (*31*).

Classification of biologic changes in tissues often is challenging. For example, the borders between hyperplasia and neoplasia and between benign and malignant neoplasia often are indistinct. Similarly, the boundary between adaptive and toxic responses often is not well delineated. As a result biologic assessment of adaptive responses as adverse or not adverse is a matter of judgment and sometimes is controversial. Hypertrophy of skeletal muscle in response to increased workload is an example of an adaptive change that might prove beneficial to the host. However, hypertrophy of the left heart ventricle because of arterial hypertension, even though it allows the heart to function against an increased workload, will result in decreased cardiac ability to compensate for additional stress. Metaplasia also typically is considered an adaptive response but the predictive value for lesion progression and secondary effects is not always clear. If metaplasia occurs in the pancreas (e.g., squamous metaplasia of pancreatic ducts associated with exposure to a test substance), this process generally does not interfere with pancreatic function. However, if squamous metaplasia occurs in the tracheal epithelium it may interfere with normal respiratory defense function (mucociliary escalator).

A common biochemical adaptive response is induction of the cytochrome P450 mixed-function oxidase system (*32–34*). Many chemicals evaluated by the ATSDR (e.g., aldrin, chloroform, DDT, PCE) can induce the cytochrome P450 system in the liver or other organs. This induction leads to stimulation of protein synthesis and proliferation of smooth endoplasmic reticulum. Depending on the inducing agent, one or more isoenzymes of P450 may be induced, each of which has different affinities for a variety of other substances. The P450 system plays a critical role in the metabolism of both endogenous and exogenous compounds. In some cases, as in acetaminophen or carbon tetrachloride, this can be viewed as a toxification reaction (i.e., the parent compound is metabolized to a more toxic species). In other cases, as with ethanol or phenobarbital, the cytochrome P450 system detoxifies the parent compound, which results in less reactive metabolites. However, once induction of the mixed-function oxidase system has occurred (no matter what chemical was the initiater), this adaptive response has significant implications in future chemical exposures. This adaptive modification may potentiate or inhibit toxic responses to subsequent chemical exposures. The enhancement or inhibition of a compound's metabolism can lead to toxicological interactions that may be important in site-specific risk assessments (*35*). This concept is particularly significant to the ATSDR in its assessment of NPL sites, where multichemical exposures are much more likely than exposure to a single chemical.

Within the framework of human health risk assessment, hepatic cytochrome P450 induction generally should be classified as an adverse effect. This induction alters the normal functioning of the organ (i.e., the organ is able to metabolize compounds at a greater rate). Even though this effect may be beneficial for some compounds it may be detrimental for others. Additionally, the designation of P450 induction as an adverse effect reflects the judgment that involuntary environmental exposure to chemicals should not be at levels that alter the normal state of the organism.

Glutathione depletion in the liver is another example of a biochemical adaptive response. The hepatic metabolism of chemicals such as acetaminophen can result in such depletion. Acetaminophen is metabolized by cytochrome P450 to a reactive intermediate that is detoxified by conjugation with glutathione (*36*). As long as levels of glutathione are not depleted, no hepatotoxic effects are induced. However, high doses of acetaminophen result in glutathione depletion, after which overt toxic effects are likely in the liver. Such chemical-induced depletion of liver glutathione is considered an adverse effect because it diminishes the normal capacity of the liver to respond to other chemical agents.

Clearly, P450 induction and glutathione depletion are not frank toxic effects. At the whole-organism or whole-organ levels no damage may be apparent. Consequently, adaptive or compensatory changes such as P450 induction or hepatic glutathione depletion are most appropriately classified as minimal adverse effects. If exposures causing such effects are associated with more severe responses, the nature of these responses rather than the associated adaptive changes should be used to evaluate toxicity at that level of exposure.

To be consistent with the ATSDR's public health mission of preventing and

mitigating adverse health effects caused by exposure to hazardous substances, it is important to assess adaptive responses to chemical exposures on a case-by-case basis. Within the definitions presented earlier and the general context of risk analyses involving chemical contamination, an adaptive response may be classified as adverse when the adaptive alteration potentially contributes to frank toxic effects, when it diminishes the organism's ability to withstand further stress, or when it compromises an organism's normal ability to dispose of chemical substances.

All these criteria are individually and collectively considered by the ATSDR in assessing the risk posed by exposure to toxic substances. Adaptive response may actually be the basis for selecting the toxic end points the ATSDR uses in its derivation of health guidance values. Moreover, the understanding and interpretation of such adaptive responses may also affect how the agency evaluates multiple chemical exposures at complex waste sites. Clearly, an in-depth understanding of adaptive mechanisms is crucial to the ATSDR's multifaceted risk-assessment process.

Hormesis in the Derivation of Health Guidance Values

Regarding the third question posed in "Introduction," the ATSDR has long been aware of the complexity of issues associated with those toxic agents that also exhibit beneficial effects at low exposure levels. To date, approximately 275 chemical contaminants commonly found at hazardous waste sites have been categorized and ranked by the ATSDR according to toxicity, frequency of occurrence, and potential for human exposure (*37*). Many of these substances have been shown to be toxic at high exposure levels but appear to be beneficial at much lower exposure levels. Such substances include not only dietary essential trace elements such as manganese, fluoride, chromium, zinc, and selenium but also a diverse spectrum of purported hormetic agents such as carbon tetrachloride, chloroform, cyanide, heavy metals, polychlorinated biphenyls, and insecticides (*38,39*). Because many of these substances are common contaminants at a number of toxic waste sites, they present a variety of unique problems for public health assessments at those sites.

As previously mentioned, the ATSDR carefully considers the biological effects of low-level exposures in its human health assessments, particularly in those deliberations involving the derivation of MRLs. Any evidence of hormesis (i.e., the induction of beneficial effects by low doses of otherwise harmful physical or chemical agents) has always been carefully noted and considered in these deliberations. However, even though the concept of hormesis has been the subject of extensive scientific debate in recent years (*40,41*), it still is not known whether hormetic effects occur with most toxicological end points (e.g., carcinogenesis, immunotoxicity, mutagenesis, teratogenesis) or whether hormetic effects occur at the same site of action for both low and high levels of exposure.

Nonetheless, the fact remains that many chemicals on the ATSDR list of hazardous substances known to be toxic at high exposure levels can cause deficiency associated toxicity if exposure levels are extremely low or absent (e.g., chromium, manganese, zinc). For each substance in its MRL deliberations the ATSDR considers not only high-dose toxicity but also any evidence of essentiality or beneficial effects as well as any evidence of deficiency associated toxicity.

The best known and understood chemical substances identified as beneficial at extremely low doses are dietary essential trace element(s) (ETE), i.e., chemical substances that must be present in small quantities in the human diet to maintain normal physiological functions. For each ETE, two ranges of exposure or intake are associated with adverse health effects: intakes that are too low and result in nutritional deficiency and intakes that are too high and cause toxicity. Some examples of the types of information considered by the ATSDR in derivation of oral MRLs for a number of ETEs are discussed below.

Chromium

Chromium enters the environment in several different forms. Cr(III) is an essential nutrient that acts as a cofactor for insulin and helps the body use sugar, protein, and fat (required for normal energy metabolism). Cr(VI), however, generally is considered harmful and is produced only from anthropogenic sources. Usually a substantial margin of safety exists between the amount of Cr(III) normally consumed and the amount considered to have harmful effects. High levels of Cr(VI) have been reported to increase the incidence of bronchial cancer in workers exposed to chromate dusts (*42*) and to cause sensitization, asthma, and dermatitis. Humans cannot oxidize the nontoxic Cr(III) to the potentially carcinogenic Cr(VI); however, Cr(VI) can be reduced to Cr(III) after penetration of biological membranes and in the gastric environment and therefore there has been some difficulty in distinguishing between the effects of the two Cr forms (*43*).

In a number of animal studies severe Cr deficiency in animal studies has resulted in hyperglycemia, decreased weight gain, elevated serum cholesterol levels, aortic plaques, corneal opacities, impaired fertility, and lethality (*43*). For humans the estimated safe and adequate daily intake (ESADDI) is 50 to 200 µg (*44*). In high-dose studies in mice with either chromium sulfate or potassium dichromate, the most sensitive toxic end point observed was decreased spermatogenesis (*45*). However, this end point was classified by the ATSDR as a serious effect and therefore inappropriate for derivation of an MRL (*3,43*). Because of the lack of data appropriate for deriving an MRL, the ATSDR adopted the upper limit of the ESADDI—200 µg Cr/day—as an interim health guidance value for Cr(III) and Cr(aVI). Such guidance was deemed both appropriate and necessary because of the prevalence of Cr at hazardous waste sites, the fairly complete database on Cr, and the fact that Cr is an essential nutrient.

Manganese

Manganese is a constituent of several metalloenzymes (arginase, pyruvate carboxylase, and manganese superoxide dismutase) and an enzyme activator (hydrolases, kinases, decarboxylases, and transferases) (*44,46,47*). It is required for normal brain function. Although Mn is an essential nutrient, exposure to high levels through inhalation or ingestion may cause adverse health effects. Inhalation of high Mn concentrations in dust or fumes, not dietary intake, is the primary exposure route associated with toxicity in humans (*48*). Toxicity in humans can be manifest as a crippling neurologic disorder of the extrapyramidal system, with morphological lesions reminiscent of Parkinson's disease (*49*). In addition to nervous system damage, reproductive and immune dysfunction, nephritis, testicular damage, pancreatitis, lung disease, and hepatic damage can also occur (*49*). In domestic animals iron deficiency has also occurred as a result of the inhibitory effect of Mn on iron absorption (*50*). Conversely, Mn deficiency results in poor reproductive performance, growth retardation, congenital malformations in offspring, abnormal

formation of bone and cartilage, and impaired glucose tolerance (*50*).

The ESADDI for Mn intake for adults is 2 to 5 mg/day (*44*). Because appropriate data are lacking, no oral MRLs have been derived for either acute-, intermediate-, or chronic-duration exposure to Mn. As a result the ESADDI upper limit of 5 mg/kg/day was used to derive an interim health guidance value of 0.07 mg/kg/day until such time that information appropriate for derivation of these MRLs becomes available (*49*). The ATSDR considers such interim guidance both appropriate and necessary because of the prevalence of Mn at hazardous waste sites and the fact that Mn is an essential nutrient.

Zinc

Zinc is an essential element for plants, animals, and humans, and is a component of many enzymes involved in major metabolic pathways (*44,51*). Zn provides a molecular mechanism by which growth hormone can bind to the prolactin receptor (*52*). However, like all essential elements Zn can also be toxic. Small incremental changes in Zn intake can have significant effects on the absorption of both copper and iron in some individuals (*53,54*). Ingestion of excess Zn depresses superoxide dismutase levels in humans (*55*). Supplementation with amounts many times the recommended daily allowance (*5*) resulted in impairment of various immune responses (*56*) and caused a decline in high-density lipoproteins in serum (*57*). Conversely, Zn deficiency causes growth and developmental retardation in humans (*58,59*), reduced immune function (*60*), and reproductive failure and teratogenesis in animals (*51*). Young men with Zn deficiency exhibited hypogonadism with impaired development of secondary sexual characteristics (*58*); general signs and symptoms in humans include loss of appetite, slow wound healing, decreased sense of taste and smell, and dermatological problems.

The ATSDR derived an intermediate oral MRL of 0.3 mg Zn/kg/day based on hematologic effects, including decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase activity, in women given daily supplements of Zn as Zn gluconate for 10 weeks (*54,61*). Normally for derivations of this type a UF of 100 would have been used (10 for sensitive human populations and 10 for the use of an LOAEL instead of an NOAEL) (*3*). However, because this LOAEL was considered to be a minimal LOAEL (*3,61*) and because Zn is an essential nutrient, a total UF of 3 was used instead of the normally applied 100. The 0.3 mg Zn/kg/day MRL thus derived has also been adopted as a chronic oral MRL because of a lack of adequate long-term studies in either humans or animals. This value is expected to be without adverse effects when Zn is consumed at this level on a daily basis over a long period of time and neither induces nutritional deficiency in healthy, nonpregnant adults ingesting the average American diet nor causes toxicity.

Future Directions

In addition to the three mechanistic areas of consideration discussed above, the ATSDR is aware of a number of additional methodological issues that also affect its risk analysis processes. To address these issues the ATSDR is developing and implementing a number of additional programs specifically designed to reduce the uncertainties in its public health assessments, improve the accuracy of its MRL, and provide additional interim health-based guidance values. These efforts include evaluation of the potential impact of physiologically based pharmacokinetic (PBPK) modeling and benchmark dose (BMD) modeling on the development of MRLs. The ATSDR is also assessing specific chemicals and chemical mixtures using an *in vitro* functional screening approach. In addition, the ATSDR is evaluating chemical interactions in the expression of toxicity from exposure to chemical mixtures.

PBPK Modeling

The ATSDR's PBPK modeling approach consists of applying knowledge about biologic mechanisms to the calculation of toxic chemical dosimetry in target tissues from external exposure concentrations (*62*). PBPK modeling recently performed by the ATSDR revealed that when a chemical is rapidly metabolized to a relatively less toxic intermediate, the current ATSDR MRL methodology (*3*) may overestimate the relative human hazard. For example, the ATSDR's acute inhalation MRL of 0.4 parts per million (ppm) for methylene chloride (*63*) was derived from an LOAEL of 300 ppm for central nervous system (CNS) effects (decreased neurobehavioral performance) in humans (*64*) adjusted to continuous exposure and divided by a UF of 100 (10 for use of an LOAEL and 10 for human variability). In comparison, PBPK modeling provided pharmacokinetic dose metrics for methylene chloride in the blood for both peak concentration and area under the concentration curve (*65*). By dividing each of these resulting dose metrics by the same UF of 100, an acute inhalation PBPK-based MRL for methylene chloride was calculated to be either 6 or 0.8 ppm, respectively, depending on the dose metric used. Acute CNS effects for solvents such as methylene chloride display both rapid onset and rapid reversibility and are likely to result from a direct effect of the parent chemical on the CNS. Rapid metabolism of methylene chloride to a less toxic intermediate would tend to reduce acute toxicity by lowering the amount of the parent chemical in the body, thereby leaving less active parent chemical to interact with the CNS. Such information and logic would thus support the higher acute inhalation MRL for methylene chloride derived through PBPK modeling.

If toxicity is attributable to a stable metabolite, however, the current ATSDR MRL methodology may actually underestimate the relative human hazard. For example, the ATSDR's current acute oral MRL of 0.5 mg/kg/day for TCE was derived from an LOAEL for developmental effects in mice (*66*) divided by a UF of 100 (10 for use of an LOAEL and 10 for animal to human extrapolation) (*17*). In comparison, PBPK modeling of TCE (*67*) provided dose metrics for the area under the concentration curve for both the parent chemical and the metabolite, TCA. Dividing these dose metrics by a UF of 30—10 for use of an LOAEL and 3 for animal-to-human extrapolation (instead of 10 because of the reduced uncertainty provided by pharmacokinetic modeling)—an acute oral MRL for TCE was calculated to be either 0.02 mg/kg/day based on the TCE dose metric or 0.04 mg/kg/day based on the TCA dose metric. Because the TCA metabolite tends to accumulate in the fetus through an ion-trapping mechanism and acts as a teratogen by coagulating fetal proteins, this would support the application of PBPK modeling to lower the acute oral MRL for TCE.

Benchmark Dose Modeling

The ATSDR is also evaluating the utility of BMD modeling to obtain low-incidence response exposure levels calculated from mathematically fitted dose–response curves as an adjunct to the current NOAEL/LOAEL approach in developing MRLs. A BMD is defined as "a statistical lower confidence limit for a dose that produces a predetermined change in the response rate of an adverse effect compared to background" (*68*). The BMD

approach is not restricted to experimental NOAELs/LOAELs but also makes use of dose–response data and sample size (*69*). Because the purpose of the BMD model is to derive an estimate of dose for a given incidence that is likely to fall within a predetermined experimental dose range, and because the model does not require extrapolation to estimates far below the experimental dose range, the degree of uncertainty in the dose estimates is lessened. Depending on the end point and acceptable response level selected, using the BMD model may obviate the need for a default UF of 10 for LOAEL-to-NOAEL extrapolation (*68,70*).

The ATSDR's current intermediate oral MRL of 0.002 mg/kg/day for inorganic mercury was based on an NOAEL for increased kidney weight in rats divided by a UF of 100 (10 for animal-to-human extrapolation and 10 for human variability) (*71*). In a BMD analysis of inorganic mercury studies recently conducted by the ATSDR, relative kidney weight was used as the response to calculate BMDs from the same study using the Weibull model. Application of 100-fold UF for intra- and interspecies variability yielded the following BMD-based intermediate oral MRL for inorganic mercury: 0.003 mg/kg/day for an estimated 10% risk, 0.002 mg/kg/day for an estimated 5% risk, and 0.0003 mg/kg/day for an estimated 1% risk.

In this case, the BMD-based intermediate oral MRL for an estimated 5% risk was the same as the ATSDR's current MRL.

Functional Toxicology

Through an interagency agreement with the National Institute of Environmental Health Sciences, the ATSDR is pursuing development of functional toxicology assays to screen chemicals. These screens are comprised of human or animal cell lines transfected with a specific receptor gene along with a reporter gene. The purpose of these *in vitro* screens is to provide an assessment of the degree to which a single chemical or chemical mixture might exhibit some specific functional activity (e.g., estrogenic activity or dioxinlike activity). It is hoped that this approach eventually will permit priority chemicals and/or chemical mixtures to be evaluated on the basis of functional activity such as receptor binding, receptor occupancy, or gene activation rather than traditional whole-animal bioassay results (*72*).

Chemical Interactions

The ATSDR, in collaboration with the TNO Nutrition and Food Research Institute, The Netherlands, is conducting studies to evaluate the role of chemical interactions in the expression of toxicity from low-level exposure to combinations of chemicals. A weight-of-evidence (WOE) approach (*73*) is used to predict the toxicity of some simple chemical mixtures based on published literature reports then compare these predictions with test results from actual animal toxicity studies. The WOE evaluation process uses individual chemical dose–response assessments and algorithms that incorporate various assumptions regarding potential chemical interactions.

Qualitative (type of toxicity) and semiquantitative (direction of response not specific magnitude) evaluations have been prepared on binary weight-of-evidence combinations of chemicals included in two four-component mixture studies conducted by the TNO. These evaluations were used to estimate the overall toxicities of the mixtures that were then compared with experimentally determined toxicities (*74,75*). Preliminary analyses of the data indicate that the WOE approach quantitatively accounts for the observed interactions for mixtures of similarly acting renal toxicants, but for dissimilarly acting renal toxicants the method performs less well. This could be attributed to the fact that WOE evaluations are based on dose additivity that postulates that all chemicals in a given mixture act in the same way by the same mechanism and differ only in their potencies. Therefore, although this approach may be inappropriate for evaluating interactions for dissimilar acting agents, it may hold promise as a means to identify agents having different mechanisms of action.

Conclusions

The ATSDR believes that because it is a public health agency it is vitally important to continue to expand and improve its understanding and practical application of mechanistic principles to the risk analysis of hazardous environmental exposures. Mechanistic factors that must be considered in this process include specific chemical speciation, bioavailability, toxicokinetics and toxicodynamics, adaptive responses, hormesis and essentiality, and interactive effects of chemical mixtures. As was previously pointed out in Johnson and Jones (*76*), it is essential to continue to invest in programs that expand our understanding of these mechanisms so that both our "risk assessments and science policies can be adjusted in light of new and better scientific data."

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