Health effects classification and its role in the derivation of minimal risk levels: Hepatic effects

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Received 8 December 2004
Available online 24 May 2005

Abstract

The Agency for Toxic Substances and Disease Registry (ATSDR) derives health based guidance values called minimal risk levels (MRLs) to assist with assessment of risks posed by exposures to hazardous chemicals. Current MRLs are posted on ATSDR’s website (www.atsdr.cdc.gov). From the total 326 MRLs currently posted, 79 MRLs are based on hepatic endpoints. The paper reports on endpoints used for the derivation of these MRLs and the use of uncertainty factors. It also describes the ranking of effects into less serious and serious categories as described in ATSDR’s Guidance for Developing Toxicological Profiles.

Keywords: Hepatic effects; Health guidance values; MRL; RfC; RfD

1. Introduction

Over 600 drugs and numerous environmental chemicals have demonstrated toxicity to the liver in humans or in laboratory animals (Evans and Lake, 1998). The liver is a target organ for toxic chemicals because of its position in the organism, metabolic capabilities, and secretory and excretory functions. Chemicals that enter the organism by oral route first traverse the hepatic portal system before reaching the systemic circulation. Metabolism may change the toxicity (active or inactive metabolites) or the bioavailability (first-pass effect) of the parent chemicals (Bryson, 1997). In fact, most chemicals featured in ATSDR’s toxicological profiles display some hepatotoxic effects. However, hepatotoxicity was the most sensitive target endpoint for only 53 chemicals. Identifying the most sensitive endpoint is important for ATSDR to derive the health-based guidance values minimal risk levels (MRLs). 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, non-cancer effects over a specified duration of exposure” (ATSDR, 1992, 1996a). ATSDR uses MRLs as a screening tool for evaluation of chemicals around hazardous waste sites and their possible impact on human populations living in the vicinity of the sites.

The purpose of this paper is to inform the public about MRLs based on hepatobiliary effects and about the guidance provided for the sections of toxicological profiles describing these health effects and their categorization in ATSDR’s Guidance for Developing Toxicological Profiles (ATSDR, 1996b, 2003). So far, the guidance has served as an internal document. However, parts of the guidance related to neurological, developmental, hematological, and respiratory effects and the respective MRLs were previously published (Abadin et al., 1998; Chou and Williams-Johnson, 1998; Pohl et al., 1998; Wilbur, 1998).

2. Materials and methods

By Congressional mandate, ATSDR develops toxicological profiles for hazardous substances found at
National Priority List (NPL) sites. ATSDR also prepares toxicological profiles for the Department of Defense (DOD) and the Department of Energy (DOE) on substances related to federal sites. So far, about 250 profiles were published as final documents. The profiles focus on health and toxicological information. Toxicological profiles (final and draft documents) can be found on ATSDR’s web site (www.atsdr.cdc.gov). MRLs are an integral part of the toxicological profiles.

MRLs are derived according to current ATSDR methodology (Chou et al., 1998; Pohl and Abadin, 1995). ATSDR uses the highest no-observed-adverse-effect level (NOAEL) or lowest low-observed-adverse-effect level (LOAEL) in the available literature to derive the MRLs. Proper categorization of health effects is, therefore, critical for the MRL derivation. The 79 MRLs related to hepatotoxicity were based on vast databases compiled in the 53 toxicological profiles for the respective chemicals. A list of all current MRLs (updated on May 11, 2004) is available on ATSDR’s web site (www.atsdr.cdc.gov).

3. Results and discussion

3.1. ATSDR’s health effects classification

To determine the levels of significant human exposure to a given chemical and associated health effects, ATSDR’s toxicological profiles examine and interpret available toxicological and epidemiological data. As described in the preceding papers (Chou et al., 1998; Pohl and Abadin, 1995), ATSDR categorizes health effects according to the seriousness as “serious,” “less serious,” or “minimal.” A “less-serious” effect can be defined as changes that will prevent an organ or organ system from functioning in a normal manner but will not necessarily lead to the inability of the whole organism to function normally. “Serious” effects are defined as effects that prevent the organism from functioning normally or that can cause death. Subtle effects that they may be components in the sequence of events that leads to toxicity are usually categorized as “minimal” (Pohl and Abadin, 1995).

3.2. ATSDR’s guidance document

The guidance document (ATSDR, 2003) provides instructions on classification of some endpoints that may be controversial as to the seriousness of the effects. As noted in the guidance document, “exposure to many substances may result in adaptive changes in the liver that are characterized by induction of the mixed function oxidase enzyme system and proliferation of smooth endoplasmic reticulum. Modifications occurring in the mixed function oxidase system as a consequence of the adaptive response may potentiate or inhibit toxic responses to other exogenous substances. Agents that induce chemical metabolizing enzyme systems (e.g., acetone) generally tend to potentiate hepatic injury produced by compounds such as chloroform, carbon tetrachloride, or halothane. For ATSDR, this is an especially important concept to consider because, in addition to the specific chemical causing adaptive changes, there is the potential for exposure to many other substances at NPL sites” (ATSDR, 1996b, 2003).

“The borderline between adaptive physiology and toxicity (functional impairment) is not always well delineated. The following guidance provides general direction for assessing hepatic adaptive responses; although this guidance is appropriate in most cases, there may be exceptions. However, for the purpose of assessing the biological significance of adaptive responses in the liver, the following criteria should be used: biochemical changes characterized by induction of enzymes of the mixed function oxidase system along with morphologic changes of hepatocellular hypertrophy and proliferation of smooth endoplasmic reticulum should be considered potentially adverse and should be classified as a less serious LOAEL. Other supportive changes that may be observed include increased organ weight, hepatic enlargement, and accentuated cytoplasmic eosinophilia. To maximize the accuracy of assessing hepatic (or other) adaptive responses, in addition to the guidance given here, this interpretative process is accompanied by insightful case-by-case analysis” (ATSDR, 1996b, 2003).

Similarly, the whole clinical picture has to be evaluated for effects classified as less serious LOAELs versus serious LOAELs. In animal studies, normal ranges are often not well established and statistical increase in liver enzymes is frequently classified as a less serious effect; however, when the increases are combined with other effects showing a threat to the organism from a serious damage to the liver, they would be classified as a serious LOAEL. In contrast, normal ranges and clinically defined pathological levels are used to identify LOAELs in human studies. Other instructions in the guidance document pertain to a table with examples of hepatic health effects classification (Table 1). Some effects need further evaluation as

<table>
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<tr>
<th>Table 1 Hepatic effect endpoints</th>
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<tr>
<td><strong>Effect</strong></td>
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<tr>
<td><strong>Less serious</strong></td>
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<tr>
<td><strong>Serious</strong></td>
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<tr>
<td>Altered liver enzymes +</td>
</tr>
<tr>
<td>Hepatomegaly (enlargement of the liver) +</td>
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<tr>
<td>Porphyria (disturbance of porphyrin metabolism) +</td>
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<tr>
<td>Hepatocyte vacuolisation +</td>
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<td>Congestion of liver +</td>
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<td>Jaundice +</td>
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<td>Gall bladder effects +</td>
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<td>Fatty changes in liver +</td>
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<td>Hepatocellular degeneration +</td>
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to the seriousness based on information provided in the study the effects were described in (i.e., they can be serious or less serious).

3.3. Hepatobiliary effects and related MRLs

MRLs based on specific hepatic and biliary endpoints are those that are related to liver weight and hepatobiliary function. Hence they are the most commonly used endpoints as crude indicators of hepatotoxicity in animal studies. MRLs based on the hepatobiliary endpoints include MRLs for hexachlorocyclohexane, hexachloroethane, dieldrin, 1,2-dichloropropane, 1,2,3-trichloropropane, and 1,2-dichloroethane. Historically, these effects were used to provide a NOAEL (an increase in liver size if the liver weight of individuals without an increase in cell numbers) or hyperplasia (an increase in liver size as a result of an increase in cell numbers) (Evans and Lake, 1998). MRLs based on the LOAELS for these endpoints include MRLs for aflatoxin B1, 3,4-trinitro-l-nitrosoaniline, and 1,2-dichloroethane.

Clinical chemistry is an important tool for detecting hepatobiliary effects; and serum enzymes are the markers most often used to detect the injury. Increased levels of enzymes such as sorbitol dehydrogenase (SOD), ornithine carbamoyltransferase (OCT), and alanine transaminase (ALT) are used, but they are not specific to hepatic injury and may be increased following injury to other organs (e.g., kidneys) and muscles. MRLs based on the LOAELS for these endpoints include MRLs for chloroform, chloromethane, xylene, 4,4'-methylenedioxyanisole, 4,4'-methylenediamine, and 1,2-dichloroethane. Clinical pathology includes MRLs for isophorone and 1,2-dichloroethane.

As an example, the effects of carbon tetrachloride and ethanol were described in (i.e., they can be serious or less serious) the effects were confirmed as hypertrophy (an increase in size of hepatic cells), steatosis (fatty liver), and fibrosis (scarring). MRLs based on the NOAEL for carbon tetrachloride was derived for rats for dose-related, statistically significant liver cellular effects until recent years.

Hepatobiliary function includes specific endpoints in the liver, and is characterized by changes in liver weight (including hypertrophy), a decrease in liver weight (including atrophy), or changes in liver structure (such as fibrosis). MRLs for carbon tetrachloride, endosulfan, and 1,2-dichloroethane are based on the NOAEL for these endpoints. Endosulfan is a widely used fungicide, and 1,2-dichloroethane is a solvent used in the manufacture of vinyl chloride.

Hepatobiliary effects include MRLs for 1,2-dimethylhydrazine. An MRL based on the NOAEL for this endpoint was derived for rats and monkeys. H. P. Til, H. R. PoM, C.-H. S. J. Chou I Regulatory Toxicology and Pharmacology 42 (2005) 161–171
foracenaphthene, fluoranthene, and 1,1,2-trichloroethane.
These MRLs correspond with the respective RfDs when adjusted for duration of exposure (i.e., divided by 10). MRLs for pyridine, tetrahydrofuran, and vinyl chloride, styrene, 1,4-dichlorobenzene, and PBDEs differ very slightly. The differences are due to the studies and/or endpoints, and uncertainty factors used for the derivation. The noteworthy difference was between the chronic oral MRL of 2 x 10^{-3} mg/kg/day for vinyl chloride and a corresponding RID of 3 x 10^{-3} mg/kg/day. The RID for vinyl chloride is based on liver cell polymorphism in rats reported in the TII et al. (1983, 1991) studies. EPA used a PBPK model to calculate a human equivalent NOAEL of 0.09 mg/kg/day. An UF of 30 was applied; 3 for animal to human extrapolation and 10 for human variability. A LOAEL of 0.018 mg/kg/day (ATSDR, 2004). This MRL is currently reviewed by the chronic oral MRL for vinyl chloride. A UF of 1000 was applied; 10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 for human variability. As mentioned previously, a recent re-evaluation of the chronic oral MRL for vinyl chloride concluded that liver basophilic foci are generally considered preneoplastic, and that it would be more appropriate to base the vinyl chloride chronic oral MRL on the NOAEL for liver cell polymorphism, which is considered non-neoplastic. The proposed revised chronic oral MRL for vinyl chloride is 3 x 10^{-3} mg/kg/day (ATSDR, 2004). This MRL is currently released for public comment in the draft toxicological profile.

4. Conclusion

As demonstrated here, the hepatobiliary endpoints are important in the derivation of MRLs. Hepatocellular toxicity is seen with many chemicals, and for some the liver is a primary target of their toxicity. Proper classification of effects is crucial for the process of MRL derivation. This requires clear understanding of pathology and pathophysiology. Consistency in classification of similar endpoints across the different studies and chemicals has great significance in MRL derivation. That is why ATSDR has a guidance document to help with the classification and a workgroup of scientists discussing the proper classification of health effects on a case-by-case basis. In reviewing the past decisions regarding classification of hepatic effects, this article strives to bring this issue in focus. ATSDR uses up-to-date methodology for the MRL derivation where biomedical judgment plays an important role (e.g., application of UFs). It is helpful to realize that ATSDR’s results are comparable with other agencies that derive health based guidance values for hazardous chemicals.

ATSDR has searched for alternative methodologies to refine the chemical health risk assessment. New approaches to risk assessment, e.g., benchmark dose calculations, physiologically based pharmacokinetic (PBPK) modeling, and quantitative structure-activity relationships (QSARs) are being explored. Better understanding of toxicokinetics and toxicodynamics of chemicals and their interactions with living organisms on molecular levels will enable the health assessors a better evaluation of the health risks. This understanding will also improve the risk assessment of joint toxic action of environmental chemicals in mixtures, and in combinations with pharmaceuticals, viral agents, and other hepatotoxins.

References


changes (hepatic foci, areas of cellular alteration) in rats was used to derive a chronic oral MRL for methyl chloride. A LOAEL for basophilic foci in rats was used to derive a chronic oral MRL for vinyl chloride. A recent re-evaluation of the chronic oral MRL for vinyl chloride concluded that liver basophilic foci are generally considered preneoplastic, and that it would be more appropriate to base the vinyl chloride chronic oral MRL on the NOAEL for liver cell polymorphism, which is considered non-preneoplastic. In contrast, ATSDR did not derive an oral chronic MRL for decabromodiphenyl ether based on a LOAEL for thrombosis because the LOAEL for thrombosis was also associated with pre-neoplastic nodules in the liver.

3.4. Use of uncertainty factors

Uncertainty factors (UFs) are used in the process of MRL derivation to account for uncertainties associated with extrapolation from a LOAEL to a NOAEL and from animal to human data, and with adjustments for interspecies variability. UFs with default values of 10 are usually used for all three categories of extrapolation. However, in some cases, the uncertainty is decreased, resulting in utilization of a lower UF (Pohlon and Abudin, 1995). Following are examples of the application of UFs other than 10 in the derivation of MRLs based on health effects.

Just a few MRLs were based on the endpoints considered the borderline between adaptive physiology and toxicity (see the section on ATSDR’s guidance document). An oral acute MRL for carbon disulfide was based on a LOAEL in rats (ATSDR, 1996c). Dose-dependent decreases in hepatic micromsomal drug-metabolizing enzymes were detected. Similarly, an intermediate-duration oral MRL for diethyl phthalate was based on a LOAEL in rats that showed peroxisomal proliferation, increased liver weight, and enzyme activities (ATSDR, 1995). The LOAELs were considered minimal, and an UF of 3 was used for NOAEL extrapolation in the derivation of both MRLs. In contrast, the acute oral MRL for 4-chlorophenol was based on a NOAEL for hepatic effects in rats (ATSDR, 1999). At the LOAEL level, foamy cytoplasm, clustering of mitochondria and endoplasmic reticulum were reported. These electron microscopic changes could be considered borderline; however, further evaluation that considered progression of changes with increasing dose in the database caused the endpoints to be classified as LOAEL rather than NOAEL. Other MRLs based on "minimal" LOAELs that warranted the use of UFs of 3 include MRLs for bromodimethoxane, carbon tetrachloride, chloroform, 1,4-dichlorobenzene, 1,1,2,2-tetrachloroethylene, 1,4-dichlorobenzene, and 4,4'-methylene-dianiline. For respective endpoints that were considered "minimal" see descriptions in Table 2. A default UF of 10 was used for most of the animal to human extrapolation of study results. An UF of 3 was used for inhalation exposure route in cases of MRLs derived for carbon tetrachloride, chloroform, hydrazine, JP-4, JP-5, JP-7, JP-8, methylene chloride, 1,1-dimethylylhydrazine, and 1,2-dichloroethane. The change was mostly justified by calculating the NOAEL human equivalent concentration (HEC) (HEC, 1994). Similarly, an UF of 3 for interspecies extrapolation was used for acute inhalation exposure scenarios in cases of chloroform because dosimetry adjustment was made, and an UF of 3 was used to account for toxicodynamic differences.

In some instances, modifying factors (MFs) are used to account for additional uncertainty associated with the guidance value. For example, an MF of 3 was applied for insufficient diagnostic data to determine the seriousness of hepatotoxic effects in a study in exposed workers (Pohlon et al., 1983) used for deriving the intermediate inhalation MRL for chloroform. Similarly, an insufficient database was the reason a MF of 3 was used for the derivation of a chronic inhalation MRL for 1,2-dichloroethane.

3.5. Comparison with other guidance values

Other agencies also derive health-based guidance values. For example, the U.S. Environmental Protection Agency (EPA) derives reference concentrations (RfCs) for chronic duration inhalation exposures and reference doses (RfDs) for chronic exposure. Current RfCs and RfDs are posted on the EPA’s web site (www.epa.gov/iris/). In July 2004, 69 RfCs were posted, including 8 based on hepatic endpoints. From the 358 RfDs posted, 94 were based on slightly elevated, many of the chemicals with RfCs/RfDs derived from hepatic effects were not evaluated by ATSDR as ATSDR evaluates only chemicals on its priority list (Roney et al., 1995). In fact, only four chemicals had both the RfC and the inhalation MRL. These included chloride an MRL of 2 x 10^{-2} mg/m^3 and an RfC of 7 x 10^{-2} mg/m^3), vinyl chloride with an intermediate-duration MRL of 3 x 10^{-2} mg/m^3 and an RfC of 1 x 10^{-2} mg/m^3, 1,1-dichloroethane with an intermediate-duration MRL of 2 x 10^{-2} mg/m^3 and an RfC of 2 x 10^{-2} mg/m^3, and 1,4-dichlorobenzene with a chronic MRL of 6 x 10^{-2} mg/m^3 and an RfC of 8 x 10^{-2} mg/m^3.

For oral exposure, only 14 chemicals had both the RfD and the MRL derived. Chronic duration MRLs were equal in value to RfDs in cases of aldehydes, chloroform, DDT, and dieldrin. ATSDR did not derive chronic MRLs but has intermediate duration MRLs derived.