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

Hana R. Pohl a,*, Bryan Luukinen b, James S. Holler a

a Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, GA, USA
b Internship Fellow Oak Ridge Institute for Science and Education, US Department of Energy, Oak Ridge, TN, USA

Received 7 February 2005
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. From the total of 338 MRLs currently posted on ATSDR’s web site (www.atsdr.cdc.gov), 14 and 5 MRLs are based on reproductive and endocrine endpoints, respectively. This paper also describes the ranking of effects into less serious and serious categories according to ATSDR’s Guidance for Developing Toxicological Profiles, endpoints used for the MRLs derivation, and the use of uncertainty factors.

Published by Elsevier Inc.

Keywords: Reproductive effects; Health guidance values; MRL; RfC, RfD

1. Introduction

In recent years, numerous studies have suggested that many environmentally persistent chemicals have a potential to disrupt normal functions of the endocrine system. Many chemicals have also serious impact on reproductive and developmental endpoints in humans and animals. Assessment of risks posed by chemicals causing reproductive effects and protection of future generations are important public health tasks.

To determine the levels of significant human exposure to a given chemical and associated health effects, Agency for Toxic Substances and Disease Registry’s (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 the health effects according to their seriousness as “serious,” “less serious,” or “minimal.” A “less-serious” effect is 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 (Pohl and Abadin, 1995). “Serious” effects are effects that prevent the organism from functioning normally or can cause death. “Minimal” effects are those that reduce the capacity of an organ or organ system to absorb additional toxic stress but will not necessarily lead to the inability of the organ or organ system to function normally.

ATSDR uses the highest no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) in the available literature to derive a health-based guidance value called a minimal risk level (MRL). 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, noncancerous effects over a specified duration of exposure” (ATSDR, 1992a,b). MRLs are derived for inhalation and oral exposures. For each route of exposure, MRLs can be derived for acute (up to 14 days), intermediate (15-364 days), and chronic (365 days or more) durations.

* Corresponding author. Fax: +1 770 488 4178.
Email address: hpohl@cdc.gov (H.R. Pohl).

0273-2300/$ - see front matter. Published by Elsevier Inc.
Human data are preferred; however, human equivalent levels can be derived based on animal exposure. The formula for derivation of an oral MRL is

\[ \text{MRL} = \frac{\text{NOAEL}}{(\text{LOAEL} \times \text{MF})} \]

where MRL is the minimal risk level (mg/kg/day), NOAEL is the no-observed-adverse-effect level (mg/kg/day), LOAEL is the lowest-observed-adverse-effect level (mg/kg/day), UF is the uncertainty factor (unitless), and MF is the modifying factor (unitless).

UFs are used to account for uncertainties associated with extrapolation from a LOAEL to a NOAEL and from animal to human data, and with adjustments for intraspecies variability. MFs may be applied to reflect additional scientific judgement on the database. The above concept is also used for the reference dose (RfD) derivation by U.S. Environmental Protection Agency (EPA) (Barnes and Dourson, 1988). MRLs provide health professionals with a concept of exposure levels at which adverse health effects are not expected in human populations living in the vicinity of hazardous waste sites or chemical emissions.

The purpose of this review was to inform the public about MRLs based on reproductive and endocrine effects and about the guidance provided for the sections of toxicological profiles describing these health effects and their categorization in the ATSDR’s Guidance for Developing Toxicological Profile (ATSDR, 2003a,b). So far, the guidance has served as an internal document and has not been released to the public. However, parts of the guidance related to neurological, developmental, and respiratory effects and respective MRLs based on these effects were previously published (Chou and Williams-Johnson, 1998; Pohl et al., 1998; Wilbur, 1998).

3. Results

3.1. Reproductive effects and related MRLs

Reproductive toxicity is defined as a dysfunction induced by a chemical, physical, and or other agent that affects the process of gametogenesis from its earliest stage to implantation of the conceptus in the endometrium. Reproductive studies were conducted for numerous chemicals. The studies differ not only in their quality, but also in the sensitivity of parameters measured. These include pathological and histopathological changes in organ and cellular structures together with pathophysiological changes in male and female reproductive systems, and reproductive outcomes for both sexes. Examples of reproductive effect end points are listed and classified as less serious or serious according to the ATSDR guidance document (ATSDR, 2003a,b) in Table 1. It is recognized that for some effects, there is a continuum in the severity and the whole clinical

<table>
<thead>
<tr>
<th>Reproductive effect end points</th>
<th>Less serious</th>
<th>Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal semen (morphology, count, motility)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Atrophy</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Decreased fertility</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Decreased litter</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Decreased spermatozoa</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Degeneration of epididymides</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Disrupted spermatogenesis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Female: no reproduction</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Maternal toxicity</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Increased estrus</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Irreversible histological change in testes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ovarian dysfunction</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ovary weight change</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Postimplantation loss</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>50% reduction in number of offspring</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sterility</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Testicular atrophy</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Testicular degeneration</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Granuloma epididymis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tubular degeneration</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tubule edema</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
| *Variability exists among normal/less serious/serious; e.g., a normal human semen specimen has a volume of 3-4 mL, a sperm count of 30 x 10⁶, and 80% morphologically normal and motile spermatozoa.

* The effect can be less serious or serious depending upon the degree.

C This condition can be considered serious because it can lead to progressive fibrosis.
picture has to be evaluated for the correct classification. For example, organ weight changes for ovaries or testes are considered less-serious effects. A definitive organ dysfunction along with the atrophy (weight change) of ovaries or testes is a serious effect.

Although the ovaries and testes have endocrine functions, for reasons of consistency among the toxicological profiles ATSDR’s guidance recommends categorization of effects involving these functions as reproductive effects.

As of May 2004, ATSDR listed 14 MRLs based on reproductive effects. These MRLs and the specific endpoints that served as the bases for their derivation are listed in Table 2. As an example, some MRLs that are no longer valid, because they were supplanted by new MRLs based on updated information, are also listed in the table. They are further discussed in the text.

Because of its noninvasive nature, collection of sperm is one of the easiest ways to evaluate the male reproductive system in humans. Sperm number, structure, maturation, motility, viability, and function (i.e., cervical-mucus penetrations and sperm-oocyte interactions) also are the tests most often performed in laboratory animals. They are, therefore, also the basis for MRL derivation for a number of chemicals (e.g., acrylonitrile, cyanide, di(2-ethyl-hexyl) phthalate, 1,2-dibromo-3-chloropropane, and 1,3-dinitrobenzene).

In some instances, the sperm tests were supplemented with data from evaluation of histopathological changes in testes and epididymis (e.g., acrylonitrile and 1,3-dinitrobenzene). Evaluation of testicular degeneration varied across the studies from crude measurements of testicular and epididymal weights (cyanide), to light-microscopic examination revealing tubular degeneration (1,3-dinitrobenzene), and more advanced electron-microscopic examination describing Leydig cell mitochondrial swelling, focal dilatation, and vesiculation of the smooth endoplasmic reticulum (diethyl phthalate). In addition, hormonal changes served as end points for deriving MRLs for the methoxychlor and 1,2-dibromo-3-chloropropane. In fact, for some chemicals that are known toxicants to the male reproductive system, such as 1,2-dibromo-3-chloropropane, changes in hormonal levels provide early warnings of the chemical-induced toxicity (ATSDR, 1992a,b). MRLs based on endocrine effects other than those pertaining to reproductive hormones changes. MRLs based on changes of reproductive hormones (FSH and prolactin in case of the pituitary gland) are often the subject of investigation of injury caused by endocrine disruptors. Table 3 lists MRLs that were based on thyroid effects. Relevant studies on endocrine effects other than those pertaining to thyroid and reproductive hormones are less frequent. No MRLs were based on these studies. The only notable deviation from applying the default uncertainty factors are the MRLs for iodine. UF of 1 was used for human susceptibility because of the robust database and available studies in children who are considered to be a sensitive population.

4. Endocrine effects and related MRLs

As of May 2004, ATSDR’s MRL Workgroup derived five MRLs based on endocrine effects (excluding reproductive hormones changes). MRLs based on changes of reproductive hormones (FSH and prolactin in case of the pituitary gland) are often the subject of investigation of injury caused by endocrine disruptors. Table 3 lists MRLs that were based on thyroid effects. Relevant studies on endocrine effects other than those pertaining to thyroid and reproductive hormones are less frequent. No MRLs were based on these studies. The only notable deviation from applying the default uncertainty factors are the MRLs for iodine. UF of 1 was used for human susceptibility because of the robust database and available studies in children who are considered to be a sensitive population.

5. Discussion and conclusion

In recent years, attention has focused on the potential for a wide range of xenobiotic chemicals to interact with


ATSDR (Agency for Toxic Substances and Disease Registry), 2003b. FR 60058. Revised priority list of hazardous substances that will be the subject of toxicological profiles. U.S. Public Health Service, Department of Health and Human Services, Atlanta.


The mechanisms by which chemicals cause reproductive effects, it is necessary to have a basic understanding of the physiologic interrelationships of the various reproductive systems. The endocrine system, the multiple control mechanisms, and the pathology (gross and histological) of the targeted organs is easy to make an erroneous conclusion because the reproductive effect based on limited observations at a single time point. Therefore, a battery of tests often is recommended (WHO, 1984).

All MRLs presented in this paper, except those for iodine, were based on effects observed in animals. Even for chemicals well known to target human reproductive system, such as 1,2-dibromo-3-chloropropane, a health-based guidance value related to observed effects in human is difficult to derive. Most available data came from occupational settings, in case of 1,2-dibromo-3-chloropropane, from cohorts of workers exposed to this pesticide during production at factories (Peatman et al., 1978; Werthorn et al., 1979), and from cohorts of applicators and farmers (Glass et al., 1979; Sandifer et al., 1979) and actual levels of exposure were not established. Animal studies intended to investigate reproductive effects should follow certain protocols established by EPA (1991). It is important that the effects characterized in reproductive effects, in this report, are limited to the endpoints described here. The limitations, assumptions, and uncertainties inherent in health risk assessment are addressed in the National Academy of Sciences report “Science and Judgment in Risk Assessment” (EPA, 1994). Therefore, the Academy states that “uncertainty analysis should be an iterative process, moving from the identification of generic uncertainties” to more refined analyses of uncertainties. Further uncertainties include differences in bioavailability of chemicals, background exposures, background prevalence of diseases (health effects), possible misclassification of effects, etc. Some examples of uncertainties and limitations are discussed here.

To understand the mechanisms by which chemicals cause reproductive effects, it is necessary to have a basic understanding of the physiologic interrelationships of the various reproductive systems. The endocrine system, the multiple control mechanisms, and the pathology (gross and histological) of the targeted organs is easy to make an erroneous conclusion because the reproductive effect based on limited observations at a single time point. Therefore, a battery of tests often is recommended (WHO, 1984).

All MRLs presented in this paper, except those for iodine, were based on effects observed in animals. Even for chemicals well known to target human reproductive system, such as 1,2-dibromo-3-chloropropane, a health-based guidance value related to observed effects in human is difficult to derive. Most available data came from occupational settings, in case of 1,2-dibromo-3-chloropropane, from cohorts of workers exposed to this pesticide during production at factories (Peatman et al., 1978; Werthorn et al., 1979), and from cohorts of applicators and farmers (Glass et al., 1979; Sandifer et al., 1979) and actual levels of exposure were not established. Animal studies intended to investigate reproductive effects should follow certain protocols established by EPA (1991). It is important that the effects characterized in reproductive effects, in this report, are limited to the endpoints described here. The limitations, assumptions, and uncertainties inherent in health risk assessment are addressed in the National Academy of Sciences report “Science and Judgment in Risk Assessment” (EPA, 1994). Therefore, the Academy states that “uncertainty analysis should be an iterative process, moving from the identification of generic uncertainties” to more refined analyses of uncertainties. Further uncertainties include differences in bioavailability of chemicals, background exposures, background prevalence of diseases (health effects), possible misclassification of effects, etc. Some examples of uncertainties and limitations are discussed here.

To understand the mechanisms by which chemicals cause reproductive effects, it is necessary to have a basic understanding of the physiologic interrelationships of the various reproductive systems. The endocrine system, the multiple control mechanisms, and the pathology (gross and histological) of the targeted organs is easy to make an erroneous conclusion because the reproductive effect based on limited observations at a single time point. Therefore, a battery of tests often is recommended (WHO, 1984).

All MRLs presented in this paper, except those for iodine, were based on effects observed in animals. Even for chemicals well known to target human reproductive system, such as 1,2-dibromo-3-chloropropane, a health-based guidance value related to observed effects in human is difficult to derive. Most available data came from occupational settings, in case of 1,2-dibromo-3-chloropropane, from cohorts of workers exposed to this pesticide during production at factories (Peatman et al., 1978; Werthorn et al., 1979), and from cohorts of applicators and farmers (Glass et al., 1979; Sandifer et al., 1979) and actual levels of exposure were not established. Animal studies intended to investigate reproductive effects should follow certain protocols established by EPA (1991). It is important that the effects characterized in reproductive effects, in this report, are limited to the endpoints described here. The limitations, assumptions, and uncertainties inherent in health risk assessment are addressed in the National Academy of Sciences report “Science and Judgment in Risk Assessment” (EPA, 1994). Therefore, the Academy states that “uncertainty analysis should be an iterative process, moving from the identification of generic uncertainties” to more refined analyses of uncertainties. Further uncertainties include differences in bioavailability of chemicals, background exposures, background prevalence of diseases (health effects), possible misclassification of effects, etc. Some examples of uncertainties and limitations are discussed here.

To understand the mechanisms by which chemicals cause reproductive effects, it is necessary to have a basic understanding of the physiologic interrelationships of the various reproductive systems. The endocrine system, the multiple control mechanisms, and the pathology (gross and histological) of the targeted organs is easy to make an erroneous conclusion because the reproductive effect based on limited observations at a single time point. Therefore, a battery of tests often is recommended (WHO, 1984).

All MRLs presented in this paper, except those for iodine, were based on effects observed in animals. Even for chemicals well known to target human reproductive system, such as 1,2-dibromo-3-chloropropane, a health-based guidance value related to observed effects in human is difficult to derive. Most available data came from occupational settings, in case of 1,2-dibromo-3-chloropropane, from cohorts of workers exposed to this pesticide during production at factories (Peatman et al., 1978; Werthorn et al., 1979), and from cohorts of applicators and farmers (Glass et al., 1979; Sandifer et al., 1979) and actual levels of exposure were not established. Animal studies intended to investigate reproductive effects should follow certain protocols established by EPA (1991). It is important that the effects characterized in reproductive effects, in this report, are limited to the endpoints described here. The limitations, assumptions, and uncertainties inherent in health risk assessment are addressed in the National Academy of Sciences report “Science and Judgment in Risk Assessment” (EPA, 1994). Therefore, the Academy states that “uncertainty analysis should be an iterative process, moving from the identification of generic uncertainties” to more refined analyses of uncertainties. Further uncertainties include differences in bioavailability of chemicals, background exposures, background prevalence of diseases (health effects), possible misclassification of effects, etc. Some examples of uncertainties and limitations are discussed here.
(p < 0.10) observed in rats at higher doses. Another study with chlorophenols indicated an increased trend in percentage of preimplantation loss in rats. Although no data on reproductive effects of chlorophenols in humans are available in public health agencies, a chronic oral MRL for 2,3,7,8-tetrachlorodibenzodioxin (TCDD) can illustrate deliberations to select a proper end point (ATSDR, 1998a,b). TCDD exposure in rats and mice enhanced surgically induced precocious puberty. Significant increases in the diameter of the endometriotic site and an acceleration of growth were observed in rats (Cummings et al., 1996) and mice (Cummings et al., 1996; Johnson et al., 1997), respectively. However, Foster et al. (1997) noted that prepubescence to TCDD resulted in endometriosis development due to immune suppression rather than an estrogen-resistant disease. Rier et al. (1993) found a dose-dependent decrease in fertility rate of endometriosis in monkeys chronically exposed to TCDD in a diet. However, monkeys appear to be more susceptible to endometriosis, based on a 30% background incidence of endometriosis in monkeys (Rier et al., 1993) compared with a 10% background incidence of endometriosis in humans (Wheeler, 1992). Thus, if the derivation of a chronic oral MRL was based on endometriosis, it would be more sensitive and not account for the unique insensitivity of laboratory animals to humans in regard to sensitivity to chemicals reflected as implantation losses were discussed above. Although this relative insensitivity of laboratory animals may have contributed to overall databases for individual chemicals, other end points proved to be more sensitive indicators of toxicity. This, in turn, accounts for fewer ATSDR’s MRLs and EPA’s RfC and RIDs based on reproductive effects. Nevertheless, careful evaluation of the whole database for each chemical, enabled ATSDR to derive several MRLs on the basis of reproductive and endocrine effects. The MRLs were based on solid scientific evidence derived from methodology of noncancer end point assessment. They are subject to change if new information becomes available (ATSDR, 1996, 2003a,b; EPA, 1998).

Table 2 also lists some of the MRLs that have been supplanted by new MRLs based on more recent evaluations of the data and/or that were considered relevant in consideration for human risk assessment. The statistical increase in losses linked to the exposure similarity could be reflected in humans by a further increase in losses above already high background levels.

Although many chemicals are considered endocrine disruptors and/or reproductive toxicants, other end points in their database were more sensitive and appropriate for MRL derivations. A chronic oral MRL for dibenzo-p-dioxins (CDDs), chlorinated dibenzofurans (CDFs), or polychlorinated biphenyls (PCBs) and endometriosis in a clinical study in women; (3) the U.S. Environmental Protection Agency (EPA) (1997) found that “the evidence is strong” supporting the hypothesis that CDDs and PCBs are causally related to human endometriosis via an endocrine-disruption mechanism is very weak.” Therefore, even though information indicates that the endometriosis end point was the most appropriate end point for TCDD exposure to TCDD, the neurobehavioral developmental end point (altered social behavior) reported in the Schantz et al. (1992) study was determined to be the most appropriate end point for deriving an MRL for chronic oral exposure to TCDD.

Toxicological profile for 1,2-dibromo-3-chloropropane. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

References


Army, 1983. Determination of the chronic mammalian toxicological profile for 1,2-dibromo-3-chloropropane was evaluated by both agencies. The RfC of 0.0002 mg/m³ (ppm) is identical to the ATSDR’s derived MRL for intermediate-duration exposure. The end point was evaluated by ATSDR. Of the remaining seven chemicals with RfDs based on reproductive effects, only two corresponding chemicals have MRLs based on reproductive effects. Both agencies have the same value of 0.005 mg/kg/day for methoxychlor. The RID for RDX is 0.003 mg/kg/day, the intermediate-duration oral MRL for RDX was calculated. However, the RID for the value is for chronic exposure and this MRL is for intermediate-duration exposure, the value would be virtually the same after extrapolation across durations other than chronic (ATSDR, 1996). A valid comparison cannot be made.

Although, because of interspecies variability and multifactorial control mechanisms, it is often difficult to predict reproductive toxicity in humans based on animal studies. For example, fertility studies, especially in rodents, are relatively insensitive indicators of chemical toxicity. Normal male rodents produce numbers of sperm that greatly exceed the minimum requirements for fertility studies, while in humans, the sperm counts in many individuals approach the threshold for infertility (Kimmel et al., 1992). In contrast, fertility in rodents remains unchanged, even after a 90% reduction in sperm count (Meistrich, 1982; Robaire et al., 1984). Interspecies differences between the female rodent and human in regard to sensitivity to chemicals reflected as implantation losses were discussed above. Although this relative insensitivity of laboratory animals may have contributed to overall databases for individual chemicals, other end points proved to be more sensitive indicators of toxicity. This, in turn, accounts for fewer ATSDR’s MRLs and EPA’s RfCs and RIDs based on reproductive effects. Nevertheless, careful evaluation of the whole database for each chemical, enabled ATSDR to derive several MRLs on the basis of reproductive and endocrine effects. The MRLs were based on solid scientific evidence derived from methodology of noncancer end point assessment. They are subject to change if new information becomes available (ATSDR, 1996, 2003a,b; EPA, 1998).