Health Effects Classification and Its Role in the Derivation of Minimal Risk Levels: Developmental Effects

Hana R. Pohl, Cassandra Smith-Simon, and Heraline Hicks

Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia 30333

Received March 23, 1998

Agency for Toxic Substances and Disease Registry (ATSDR) utilizes chemical-specific minimal risk levels (MRLs) to assist in evaluating the public health risk associated with exposure to hazardous substances. The MRLs are derived based on the health effects data compiled from current literature searches and presented in ATSDR’s toxicological profiles. Health effects are categorized according to their degree of severity (e.g., serious, less serious, minimal, and not adverse). This evaluation is important, because each respective category can be assigned a different amount of uncertainty, thus affecting the final value of the calculated MRL. From the total of 272 MRLs derived as of December 1997, 21 were based on developmental effects. ATSDR’s ranking of developmental health effects as described in the Guidance for Developing Toxicological Profiles and specific examples of how the categorized health effects were used in MRL derivations are provided in this paper.

INTRODUCTION

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9604 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99-499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop, among other mandates, toxicological profiles for substances listed on the priority list of hazardous substances found at hazardous waste sites. ATSDR must also establish significant human exposure levels for hazardous substances in the environment and the associated acute, subacute, and chronic health effects (42 U.S.C. 9604 (i) (3)). The ATSDR’s Minimal Risk Levels (MRLs) were developed as an initial response to the mandate. 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. By definition, an MRL is “an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse, noncancer health effects over a specified duration of exposure” (ATSDR, 1996). ATSDR categorizes health effects according to their seriousness [e.g., serious, less serious (with a minimal subcategory), not adverse] (Pohl and Abadin, 1995). Serious effects are defined as effects that prevent the organism from functioning normally. Less serious effects are those that 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. 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 (Pohl and Abadin, 1995). ATSDR uses the highest no-observed-adverse-effect level (NOAEL) or lowest less serious lowest-observed-adverse-effect level (LOAEL) in the database to derive MRLs (ATSDR, 1996a; Pohl and Abadin, 1995). As a policy, MRLs are not based on serious health effects. Categorizing the health effects is important because each respective category can be assigned a different degree of uncertainty, thus affecting the final value of the calculated MRL. From the total of 272 MRLs derived by ATSDR as of December 1997, 21 were based on developmental effects. The purpose of this paper is to present ATSDR’s ranking of developmental effects as described in the unpublished internal Guidance for Developing Toxicological Profiles and to present those MRLs that were based on developmental effects.

DEVELOPMENTAL EFFECTS

This section consists mostly of the revised text on developmental effects as written in the Guidance for Developing Toxicological Profiles (ATSDR, 1995a). The original guidance for ATSDR’s toxicological profiles was jointly developed by a workgroup of employees from ATSDR, the Centers for Disease Control and Prevention (CDC), the Environmental Protection Agency (EPA), and the National Institute of Environmental
Altered growth can be induced at any stage of development or neonate. In general, altered growth seen in conjunction with adverse effects in dams, such as decreased weight gain, has been treated as an adverse effect in the fetus or neonate.

Functional deficiency is defined as alterations or delays in the functional competence of the organism or organ system following exposure to an agent during critical periods of development either prenatally or postnatally. Examples include:

- Immunosuppression (suppression of natural immune responses) in offspring. Immune dysfunction may lead to increased risk of infectious diseases or to development of neoplasia, autoimmune disorders, or allergies.
- Neurobehavioral development in offspring. Many neurobehavioral tests are used in studies of newborns to assess abnormalities in developing offspring. Measurement of the course of development of swimming behavior, for example, is a common technique for the evaluation of neuromotor development. The neuromotor system is the system commonly studied when functional development is being assessed. Evaluation of motor development must be of primary consideration in detecting toxicity because the performance of certain responses is known to be influenced by the testing conditions used and the motivational state of animals. To accurately assess the behavioral effects of any substance, a test battery must use multiple behavioral end points.
- Inborn errors of metabolism. Genetically based inborn errors of metabolism are not usually expressed before birth. They are disorders in which defects of single genes cause clinically significant blocks in metabolic pathways. Clinical effects are mostly a result of either an accumulation of enzyme substrate or a deficiency of the reaction product. Examples of inborn errors of metabolism include disorders of carbohydrate metabolism (e.g., glycogen storage diseases), amino acid metabolism (e.g., phenylketonuria), fatty acid oxidation (e.g., glutaric acidemia), and purine metabolism (e.g., hypoxanthine–guanine phosphoribosyltransferase deficiency).
- Abnormal postnatal reproductive function. Such effects can be detected in long-term multigenerational studies.

**Congenital neoplasia.** Fetal cells are highly susceptible to carcinogens because of their high rate of proliferation. Transplacental carcinogens can act either directly or through reactive metabolic intermediates. The latter carcinogens require the fetal enzyme levels to be sufficient to metabolize the original agent to its active form. Many teratologic disorders are associated with neoplasms. Examples in humans include association of aniridia with Wilms’ tumor, trisomy 21 with leukemia and retinoblastoma, 13q syndrome with retinoblastoma, and Fanconi’s anemia with leukemia and squamous cell carcinoma.
Death. Embryonic cells are very sensitive to exposure to toxic chemicals. In early stages of development, when cells differentiate into more specialized cell populations, cell death may result in structural malformations or death of the embryo. In humans, it is estimated that 50–70% of all conceptuses are aborted during the first 3 weeks of pregnancy. As with all experiments in laboratory settings, it is important to evaluate the impact of the toxic substance on death of the embryo in comparison with the control group.

Maternal toxicity. Findings of developmental toxicity in the presence of maternal toxicity (i.e., when adverse developmental effects are produced only at maternally toxic doses) are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. Maternal toxicity (even in the absence of developmental toxicity) is an important end point to evaluate in the context of all available toxicity data. The following are some examples of maternal toxicity end points.

- Mortality
- Gestation length (when allowed to deliver pups)
- Body weight
- Body weight change
- Organ weights (in cases of suspected organ toxicity and when supported by adverse histopathology findings)
- Food and water consumption (where relevant)
- Clinical evaluations, including types and incidence of clinical signs, enzyme markers, and clinical chemistries
- Gross necropsy and histopathology.

Body weight and changes in body weight are viewed collectively as indicators of maternal toxicity for most species. These end points may not be as useful in rabbits, because body weight changes in rabbits are not good indicators of pregnancy status. Changes in maternal body weight corrected for gravid uterine weight at sacrifice may indicate whether the effect is primarily maternal or fetal. For example, there may be a significant reduction in weight gain and in gravid uterine weight throughout gestation but no change in corrected maternal weight gain, which would generally indicate an intrauterine effect. Conversely, a change in corrected weight gain and no change in gravid uterine weight generally suggest maternal toxicity and little or no intrauterine effect.

Because the maternal animal and not the conceptus is usually treated during gestation, developmental toxicity data may be presented as incidence per litter or as number and percentage of litters with particular end points. Some of the end points can be categorized as developmental and/or reproductive end points. Reproductive effects are discussed in detail in the section on reproductive effects of the ATSDR (1995a) guidance.

MRLs BASED ON DEVELOPMENTAL EFFECTS

As of December 1997, ATSDR's MRL Workgroup had derived 21 MRLs based on developmental effects. These MRLs and the specific end points that served as the bases for their derivation are listed in Table 2. Most MRLs were based on developmental effects seen in laboratory animals following exposures in utero or during the early days of postnatal development. Only one MRL, that for methylmercury, was based on effects seen in humans. Animal data used in inhalation studies were converted to human equivalent concentrations by using dosimetry adjustment in accordance with EPA (1990). Standard reference values were obtained from EPA (1988). Uncertainty factors (UFs) used in actual computations of respective MRLs are listed in Table 2. The UFs account for extrapolation from a LOAEL to a NOAEL, for extrapolation from animals to humans (interspecies extrapolation), and for human variability (sensitive populations). ATSDR's practice in utilization of UFs in MRL derivation was described in detail in our previous publication (Pohl and Abadin, 1995).

MRLs for acrylonitrile, di(2-ethylhexyl)phthalate, and ethylenbenzene were based on NOAELs for teratogenic end points. At higher doses, skeletal and/or visceral malformations were detected in exposed animals (e.g., exencephaly, blood vessel abnormalities, fused or branched ribs, fused thoracic vertebrae). If NOAELs had not been established in these studies, the LOAELs would not have been suitable for MRL derivation because of the seriousness of these effects (Table 1). As discussed previously, most teratogenic effects adversely alter survival, development, or function of the affected individual and are, therefore, not suitable for MRL derivation.

Several MRLs were based on LOAELs for neurobehavioral end points that were categorized as less serious. Delayed cognitive function was the end point used in the MRL derivation for chlordane, whereas delayed motor function was used for DDT, xylene, and trichloroethylene. Increased seizure threshold was the lowest LOAEL suitable for MRL derivation in the database of aldrin and chlordane, and hyperactivity was used for the MRL derivation for hexachlorobenzene and tetrachloroethylene. Mild behavioral changes that included alterations in play behavior, displacement, and self-directed behavior in the offspring of monkeys exposed chronically to 2,3,7,8-tetrachlorodibenzo-p-dioxin were used as end points for the MRL derivation for this chemical.

Neurotoxicity is also an important end point for the evaluation of toxic substances. Progression and irreversibility of many neurologic effects add to the long-term implications. Infants and developing fetuses might be more susceptible to neurobehavioral effects (Who, 1986). In addition, effects of exposure may not be apparent until later in life, when functional losses resulting from chemical exposures may be debilitating.
### TABLE 2

**MRLs Based on Developmental Effects**

<table>
<thead>
<tr>
<th>Substance name</th>
<th>Route</th>
<th>Duration</th>
<th>MRL value</th>
<th>UF</th>
<th>End point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile</td>
<td>Oral</td>
<td>Acute Gd 6-15</td>
<td>0.1 mg/kg/day</td>
<td>100</td>
<td>NOAEL in rats; malformation at higher dose</td>
<td>Murray et al., 1978</td>
</tr>
<tr>
<td>Aldrin</td>
<td>Oral</td>
<td>Acute 5-7 d</td>
<td>0.002 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in mice; increased seizure threshold</td>
<td>Al-Hachim, 1971</td>
</tr>
<tr>
<td>Boron</td>
<td>Oral</td>
<td>Intermed Gd 0-20</td>
<td>0.01 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in rats; reduced fetal weight</td>
<td>Heindel et al., 1992</td>
</tr>
<tr>
<td>Chlordane</td>
<td>Oral</td>
<td>Acute 7 d</td>
<td>0.001 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in mice; altered conditioned avoidance response; open field test; electroshock seizure threshold</td>
<td>Al-Hachim and Al-Baker, 1973</td>
</tr>
<tr>
<td>DDT</td>
<td>Oral</td>
<td>Acute Ppd 10</td>
<td>0.0005 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in mice; decrease in cortical muscarinic acetylcholine receptors; decreased environmental habituation assayed by motor activity</td>
<td>Eriksson and Nordberg, 1986; Eriksson et al., 1999a,b, 1992, 1993</td>
</tr>
<tr>
<td>Di(2-ethylhexyl) phthalate</td>
<td>Oral</td>
<td>Intermed Gd 0-17</td>
<td>0.4 mg/kg/day</td>
<td>1000</td>
<td>NOAEL in mice; visceral and skeletal malformations at higher dose</td>
<td>Tyl et al., 1988</td>
</tr>
<tr>
<td>Di-N-butyl phthalate</td>
<td>Oral</td>
<td>Intermed 48 d Gd0-Ld28</td>
<td>0.6 mg/kg/day</td>
<td>1000</td>
<td>NOAEL in rats; decreased pup weight at higher dose</td>
<td>Killinger et al., 1988</td>
</tr>
<tr>
<td>1,4-Dichlorobenzene</td>
<td>Inhalex</td>
<td>Acute Gd 6-18</td>
<td>0.8 ppm</td>
<td>100</td>
<td>NOAEL in rabbits; retroesophageal right subclavian artery at higher dose</td>
<td>Hayes et al., 1985</td>
</tr>
<tr>
<td>Disulfoton</td>
<td>Oral</td>
<td>Intermed F, 15 wks premating</td>
<td>0.00009 mg/kg/day</td>
<td>100</td>
<td>NOAEL in F, rats; significant inhibition of cholinesterase at higher dose</td>
<td>Hiscox and Hathaway, 1986; Andrew et al., 1981</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>Oral</td>
<td>Acute Gd 6-15</td>
<td>2 mg/kg/day</td>
<td>100</td>
<td>NOAEL in mice; malformations at higher dose</td>
<td>Tyl, 1989</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>Oral</td>
<td>Acute 4 d</td>
<td>0.008 mg/kg/day</td>
<td>300</td>
<td>LOAEL in rats; hyperactivity</td>
<td>Goldsey and Taylor, 1992</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>Oral</td>
<td>Chronic F, 130 wks premating</td>
<td>0.00002 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in F, rats; peribiliary lymphocytes and fibrosis of the liver</td>
<td>Arnold et al., 1992, 1995</td>
</tr>
<tr>
<td>Methylmercury</td>
<td>Oral</td>
<td>Chronic</td>
<td>0.00005 mg/kg/day</td>
<td>None</td>
<td>NOAEL in humans; neurobehavioral effects at 19 and 29 months</td>
<td>Davidson et al., 1995</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>Oral</td>
<td>Acute Gd 6-15</td>
<td>0.005 mg/kg/day</td>
<td>1000</td>
<td>LOAEL in rats; delayed ossification of skull</td>
<td>Schwartz et al., 1974; Schants et al., 1992</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzodioxin</td>
<td>Oral</td>
<td>Chronic ~16 months before mating, during gestation and lactation</td>
<td>0.000001 μg/kg/day</td>
<td>90</td>
<td>LOAEL in monkeys; behavioral changes</td>
<td></td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>Oral</td>
<td>Acute Ppd 10-16</td>
<td>0.05 mg/kg/day</td>
<td>100</td>
<td>NOAEL in mice; hyperactivity</td>
<td>Fredriksson et al., 1993, 1995</td>
</tr>
<tr>
<td>Total xylene s</td>
<td>Inhalex</td>
<td>Intermed Gd 4-20</td>
<td>0.7 ppm</td>
<td>300</td>
<td>LOEL in rats; decreased rotated performance</td>
<td>Hass and Jakobsen, 1993</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Oral</td>
<td>Acute Ppd 10-16</td>
<td>0.2 mg/kg/day</td>
<td>300</td>
<td>NOAEL in mice; altered behavior (reduced rearing rate)</td>
<td>Fredriksson et al., 1993, 1995</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>Inhalex</td>
<td>Acute Gd 6-15</td>
<td>0.5 ppm</td>
<td>100</td>
<td>NOAEL in mice; delayed ossification at higher dose</td>
<td>John et al., 1977, 1981</td>
</tr>
</tbody>
</table>

*Note.* d, day; draft, draft for public comments of the toxicological profile for respective chemicals; F0, parental generation; F1, the first generation produced by crossing two parental lines; final, final draft of the toxicological profile for respective chemicals; Gd, gestation day; Ld, lactation day; LOAEL, lowest-observed-adverse-effect level; MRL, minimal risk level; NOAEL, no-observed-adverse-effect level; Ppd, postpartum day; UF, uncertainty factor, wks, weeks.

* Final toxicological profile peer-reviewed and public reviewed published on date indicated.

b Draft toxicological profile peer-reviewed and released for public comments on date indicated.
In general, neurotoxic effects are due to structural or functional alterations in central and peripheral nervous systems. The underlying defects may include damage of neurons and axons; myelinopathies; effects on neurotransmitters, end plates, and synapses; effects on central nervous system (CNS) metabolism; or circulatory changes (Koestner and Norton, 1991). Batteries of neurobehavioral tests have been developed for evaluating neurotoxicity in humans and laboratory animals (ATSDR, 1995b, 1996b). Some of the changes reported in experimental studies are very subtle; others may be apparent in one test but not in a different test designed to evaluate the same end point. Therefore, classification of NOAELs and less serious or serious LOAELs becomes critical for the development of health guidance values.

Because neurobehavioral toxicity is a recently developed discipline, not enough data are yet available to develop animal models that parallel or convincingly simulate known effects in humans. Evidence of similarities may often be concealed by inadequate testing or interpretation of data, interspecies differences in developmental maturity of the CNS, and differences in behavioral patterns. UF's for interspecies extrapolation used in health risk assessment acknowledge these differences and the reservations associated with them. It is, however, recognized that mild, subclinical behavioral or neurologic changes can be early indicators of an important ongoing disease process (ATSDR, 1995b). As such, these effects must be considered in the thorough health assessment of developing organisms.

Impaired fetal growth and early neonatal growth are nonspecific markers of intruterine insult. They may be associated with maternal toxicity. The outcome of the chemical insult during gestation may be embryolethality, teratogenicity, or growth retardation depending on the chemical, the period of fetal development, and the dose. There are three patterns of dose–response relationship between growth retardation, teratogenicity, and embryolethality (Manson and Wise, 1991). For some chemicals, malformed fetuses often have decreased growth and the effects precede lethality (i.e., teratogenicity is a main manifestation of the primary insult). In other instances, a combination of embryolethality, teratogenic effects, and growth retardation can be seen at the same dose levels (i.e., these are all manifestations of the primary insult). Chemicals may also cause lethality at a higher dose and growth retardation at a lower dose without inducing malformations (i.e., embryolethality is a main manifestation of the primary insult). Embryolethality and teratogenicity are mostly considered as serious effects (Table 1) and are not suitable for MRL derivation (ATSDR, 1995a). Unless the weight decreases are so profound that they could interfere with further proper development and/or survival, they are categorized as less serious. A LOAEL for reduced fetal weight was the basis for the intermediate-duration oral MRL for boron. At higher exposure levels, teratogenic effects (rib cage defects) and embryolethality (high resorptions) were reported (Heidel et al., 1991). For di-N-butylphtalate, the MRL was based on a NOAEL for decreased weight of offspring of exposed rats.

Skeletal development is another important end point in developmental toxicology studies. Delayed ossification can be associated with decreased fetal weight, but can also occur sporadically. Subtle structural changes due to delayed ossification are often considered reversible (Aliverti et al., 1979) and can, therefore, be classified as less serious for the purpose of MRL derivation. Delayed ossification was the end point for MRL derivation in the case of pentachlorophenol exposure. Skeletal anomalies were reported with higher exposure levels, suggesting a progression of severity of skeletal effects with increasing dose.

As described previously, developmental effects can be categorized according to the degree of severity. Because many developmental effects belong to the serious category (Table 1), and ATSDR does not base MRLs on serious effects (ATSDR, 1996a), serious developmental effects observed at the lowest effect levels in the database precluded the derivation of MRLs for several chemicals. For example, an intermediate-duration oral MRL for trichloroethylene was not derived because the most sensitive end point in the database was a development of serious cardiac anomalies in the offspring of exposed rats (Dawson et al., 1993).

CONCLUSION

ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, minimal LOAEL, less serious LOAEL, or serious LOAEL. The distinctions between these categories are important because they help health assessors identify levels of exposure at which major health effects start to appear and place into perspective the possible significance of these effects to human health.

Developmental effects are important because not only is the parental generation affected by the chemical exposure, but there are also consequences to future generations. It is important for ATSDR to properly categorize developmental effects and to recognize possible impacts on human populations.

ACKNOWLEDGMENT

The authors thank Ms. Anne Olin for editorial comments.

REFERENCES


