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

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Abstract

The Agency for Toxic Substances and Disease Registry (ATSDR) derives health-based guidance values known as minimal risk levels (MRLs). By definition, an MRL is a substance-specific 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. MRLs are preferentially derived from human studies, if available, or from the most sensitive animal species and the endpoint that is most relevant for humans. To date, the agency has derived 346 MRLs. Fifteen MRLs were derived for 11 different chemicals where the database has identified the immune system as the most sensitive target of toxicity. The chemicals include benzene, chlorfenvinphos, endosulfan, heptachlor, gamma-hexachlorocyclohexane, dibutyl tin, tributyl tin, PCBs, 2,3,4,7,8-pentachlorodibenzofuran, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and 2,4-dichlorophenol. The agency's rationale for classification of immunological endpoints is discussed and a brief description given of the critical studies selected for MRL development using immune system endpoints.
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1. Introduction

Under the Comprehensive Environmental Response, Compensation, and Liability Act, Section 104(3) (Superfund), the Agency for Toxic Substances and Disease Registry (ATSDR) is mandated to address the potential health impact of hazardous substances to human health at hazardous waste sites. One of the tools developed for this purpose are minimal risk level (MRLs), which are derived during the development of the toxicological profiles.[1] MRLs are substance-specific estimates of the daily

human exposure to a hazardous substance that are likely to be without appreciable risk of adverse, noncancer health effect over a specified duration of exposure. They are based on the most sensitive endpoints identified in humans or animals. Toxicological and epidemiological reviews have identified the immune system as the most sensitive organ system for a number of chemicals. At least 100 of the top 275 substances on the CERCLA Priority List Hazardous Substances[2] can be considered to have some effect on parameters related to immunocompetence. Of the 346 MRLs derived by ATSDR, immunological endpoints were used 15 times.[3]

The immune system is comprised of highly specialized cells, tissues, and organs that give the human body the

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ability to fight infection from invading organisms. The immune system is not confined to any one specific area nor to any one organ, but is located throughout the body. This increases the number of potential points of contact with and injury by xenobiotic chemicals (ATSDR, 1994). The role of the immune system is to provide immunity, that is, to maintain the homeostatic condition required by the body to protect it from disease (Burns-Naas et al., 2001). Immunotoxicity refers to any condition which perturbs this homeostasis. Chemically induced effects on the immune system can result in (a) immunosuppression, potentially decreasing resistance to infection or cancer, (b) enhanced immunity, or (c) dysregulation of the immune response, potentially leading to autoimmunity or hypersensitivity. Many other factors such as sunlight, stress, illness, medication use, pregnancy, and nutritional status, can also influence the immune response to xenobiotics (Biagini, 1998).

This paper presents a discussion of the endpoint classification rationale used by ATSDR for deriving MRLs based on immunological endpoints. A brief description of the specific chemicals, MRLs, and the key studies is also provided.

2. Methods

2.1. Derivation of minimal risk levels

MRLs are not regulatory values. Rather, they serve as screening levels to assist health assessors in identifying the substances most critical to the health of exposed populations at hazardous waste sites. The procedure for deriving MRLs has been described in detail elsewhere (Chou et al., 1998; Pohl and Abadin, 1995). The approach is similar to the reference doses and reference concentrations used by the Environmental Protection Agency (EPA) (Barnes and Dourson, 1988; EPA, 1994). Typically, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) is used, although other risk assessment approaches can be used that may better define the point of departure for the dose response (e.g., benchmark dose analysis and pharmacokinetic/pharmacodynamic modeling). MRLs are derived for inhalation and oral routes of exposure, and for acute (1–14 days), intermediate (15–364 days), and chronic (>365 days) durations of exposure and follow the general equation:

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

where MRL is the minimal risk level, NOAEL is no-observed-adverse-effect level, LOAEL is lowest-observed-adverse-effect level, UF is uncertainty factor, MF is the modifying factor.

Default UFs are used to account for uncertainties in the database. They have been in practice for many years and are also used by health and regulatory organizations such as EPA, IPCS, and Health Canada. They have been described and discussed in various papers (Calabrese et al., 1992; Calabrese and Gilbert, 1993; Dourson et al., 1996; Renwick, 1991). ATSDR uses three default UFs when deriving MRLs. These are used to account for use of LOAELs, human variability, and animal to human differences. When a NOAEL is not identified in the critical study, a default UF of 10 is used to account for uncertainties associated with extrapolation from the lowest LOAEL to a NOAEL. ATSDR on occasion will deviate from the default UF if the effect is considered minimal. A minimal effect is defined as an effect that reduces 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). In these cases, ATSDR has reduced the UF to 3. An UF of 100 (10 for extrapolation from animals to humans and 10 to

address uncertainties associated with human variability) is also used. This UF covers the toxicokinetic and toxicodynamic interspecies and interhuman differences. Renwick (1993) suggested that these components be subdivided to take into consideration known chemical-specific information. The concept of chemical-specific adjustment factors (CSAFs) has been presented to better describe the available information in the risk assessment process (WHO, 2006). ATSDR has also used additional UFs to extrapolate across durations of exposure if doing so is determined to be appropriate and modifying factors to adjust for any concerns about the database (Chou et al., 1998; Pohl and Abadin, 1995).

2.2. The immune system and adverse health effect classification

Endpoint classification is an important aspect of deriving health-based guidance values. The agency has developed guidance to define the relevance for MRLs of organ- or system-specific endpoints identified in the critical studies. These have been published elsewhere, most recently by Chou and Pohl (2005) and Pohl and Chou (2005) for renal and hepatic endpoints, respectively. For endpoints affecting the immune system, ATSDR distinguishes between morphological and functional changes. Morphological changes involving lymphatic tissues such as the lymph nodes, spleen, and thymus are designated as lymphoreticular effects. Cells that mature or reside in these tissues, such as various populations of lymphocytes and nonlymphoid cells (phagocytes), participate in immune responses. Nevertheless, lesions involving these tissues *may or may not* be associated with functional changes in the immune response. Examples of lymphoreticular effects are lymphoid aplasia of the thymus, lymphoid hypoplasia of the lymph nodes, lymphoid hyperplasia of the spleen, hemosiderosis of the spleen, and lymph node histiocytosis. In contrast, immunological effects are functional changes in the immune response. These include a broad spectrum of effects, such as anaphylaxis, decreased cell-mediated immunity, autoimmunity, altered complement activity, altered T-cell activity, decreased mitogen response, and increased susceptibility to infection (Table 1).

3. Results

3.1. MRLs based on immunological/lymphoreticular effects

3.1.1. Benzene

An acute inhalation MRL of 0.009 ppm was derived from a mouse study that identified a LOAEL of 10.2 ppm (Rozen et al., 1984). In this study, C57BL/6 mice were exposed to benzene 6 h/day for 6 days. Exposure at 10.2 ppm resulted in depressed circulating lymphocyte counts and mitogen-induced blastogenesis of femoral B-cell and T-cell lymphocytes. A continuation of this line of studies from 6 days to 23 weeks at 300 ppm showed continued decreases in numbers of mature B- and T-lymphocytes produced in the bone marrow, spleen, and thymus (Rozen and Snyder, 1985).

An intermediate inhalation MRL of 0.006 ppm was derived from a LOAEL value of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated *in vitro* following the exposure of male C57Bl/6 mice to benzene vapors 6 h/day, 5 days/week for 20 exposure days (Rosenthal and Snyder, 1987). The chronic inhalation MRL of 0.003 ppm was based on decreased counts of B-lymphocytes in benzene-exposed workers of shoe manufacturing industries in Tianjin, China, using a benchmark dose (BMD) analysis (Lan et al., 2004).

Table 1
Immunological and lymphoreticular effect endpoints

Effect	Less serious	Serious
Altered complement activity	+	
Altered macrophage activity	+	
Altered resistance to tumor susceptibility by sarcoma virus	+	
Altered T-cell activity	+	
Chronic urticaria	+	
Decrease in lymph node cortical lymphoid cells[a]	+	
Decreased mitogen response	+	
Decreased skin graft survival time	+	
Degeneration or necrosis in immunologic components		+
Delayed rejection time of foreign skin graft (non-self)	+	
Enhanced natural killer cell (NKC) activity	+	
Altered Humoral or cell-mediated immune response to SRBC	+	
Increased susceptibility to infection	+	
Pemphigus vulgaris		+
Reduced delayed-type hypersensitivity	+	
Reduced humoral antibody (IgG) production	+[b]	+
Severe reduction in cell-mediated immune response		+
Suppression of lymphoproliferative response to T-cell mitogens	+	
Thymus or spleen lymphoid atrophy[a]		+
Lymphoid hyperplasia of lymph node or spleen[a]	+	
Histiocytosis of lymph node or spleen[a]	+	

[a] These changes are lymphoreticular effects because they represent morphological alterations in affected tissues; immunological effects imply a functional change in the immune response. Lymphoreticular effects may or may not be associated with functional changes in the immune response. If morphological changes are present in lymphoreticular tissues, the possible association with functional alterations should be addressed.

[b] The effect can be less serious or serious depending upon the degree.

3.1.2. Chlorfenvinphos

An MRL of 0.002 mg/kg/day was derived for intermediate oral exposure to chlorfenvinphos. The MRL is based on a LOAEL in mice of 1.5 mg/kg/day (Kowalczyk-Bronisz et al., 1992). In this study, male and female inbred C57BL/6 mice and (C57BL/6 × DBA/2)F1 (BDF1/Iiw) hybrid mice (6–8 weeks old) were orally dosed with chlorfenvinphos (1.5, 3, or 6 mg/kg chlorfenvinphos in 1% methylcellulose solution) daily for 90 days. Mice were immunized by intraperitoneal injections of 0.2 mL of 10% sheep red blood cells (SRBC). IgM–plaque-forming cell numbers were evaluated from spleen cell suspensions at postimmunization day 4 and again after exposure to chlorfenvinphos ceased.

Spleen cell colonies were stimulated, as evidenced by a 190% increase of endogenous spleen colonies and a 162% increase in exogenous spleen colonies at the 1.5 mg/kg/day dose. In addition, a 50% reduction in thymus weight and marked involution of the thymus was observed at this dose. Other effects included a decrease in the number of plaque-forming and E-rosette-forming cells, increase in interleukin-1 activity, and a delayed-type (24 h postchallenge) hypersensitivity (DTH) reaction.

3.1.3. PCBs (Aroclor 1254)

A chronic oral MRL of 0.02 μg/kg/day was derived for PCBs based on a study of female rhesus monkeys that self-ingested capsules equivalent to 0, 0.005, 0.02, 0.04, or 0.08 mg/kg/day doses of Aroclor 1254 for 27 and 55 months (Tryphonas et al., 1989, 1991). Comprehensive immunological evaluations showed that IgM (all doses except 0.02 mg/kg/day) and IgG (all doses) antibody levels to SRBC were significantly reduced compared with controls after 27 months. Secondary challenge with SRBC after 55 months resulted in dose-related decreasing trends in the IgM and IgG anamnestic responses, although only IgM was significantly lower than controls at all dose levels. Other immunologic effects included changes in numbers of lymphocyte T-cell subsets at 27 months (significantly decreased ratio of T-inducer/helper cells to T-cytotoxic/suppressor cells) at the highest dose, although this was the only dose tested. In addition, dose-related trends for several endpoints were observed after 55 months (e.g., decreasing lymphocyte proliferation in response to mitogenic stimulation and decreasing phagocytic activity of peripheral blood monocytes). The MRL was derived from the low dose of 0.005 mg/kg/day, which was considered to be a LOAEL for decreased antibody response. Interpretation of this effect is complicated by the lack of significant alterations in other immunological endpoints. However, because resistance to infection was not tested with live bacteria and in the interest of protecting public health, the effect was considered to be adverse.

3.1.4. Endosulfan

A dietary study in rats was used to derive an intermediate oral MRL of 0.005 mg/kg/day for endosulfan (Banerjee and Hussain, 1986). In this study, male Wistar rats received technical-grade endosulfan (α- and β-endosulfan in the ratio of 7:3) in their diets at dietary levels of 0, 5, 10, or 20 ppm, equivalent to 0, 0.45, 0.9, and 1.8 mg/kg/day. Groups of rats were immunized with tetanus toxin/Freund's adjuvant and sacrificed 20 days later (at weeks 8, 12, 18, and 22). Rats in the 10-ppm dose group had significantly decreased serum IgG levels at weeks 12, 18, and 22. These rats also had significantly decreased antibody titer to tetanus toxin at weeks 8, 12, 18, and 22. Leukocyte and macrophage migration were also inhibited at weeks 8, 12, 18, and 22. The magnitude of the differences between the 10 ppm rats and the controls increased at each later time point. These rats also had an increased albumin-to-globulin ratio at week 22. Rats in the 20 ppm dose group exhibited similar changes, but to a greater degree. In addition, at weeks 2, 18, and 22, these rats showed an increased albumin-to-globulin ratio, and at 22 weeks, these rats showed a decrease in relative spleen weight. The MRL was derived from the NOAEL value of 0.45 mg/kg/day calculated from the 5 ppm group.

3.1.5. Heptachlor

In a study by Smialowicz et al. (2001), groups of pregnant Sprague–Dawley rats were gavaged with 0, 0.03, 0.3,

or 3 mg/kg/day heptachlor in corn oil from gestational day 12 through postnatal day 7; pups were then exposed directly until postnatal day 42. Rats were subdivided into three sets (6/dose/sex) and immunological assessments were performed at various intervals beginning at 2 weeks postexposure ($\geqslant 8$ weeks of age). The immunological assessments included splenic lymphoproliferative (LP) responses to T-cell mitogens (e.g., concanavalin A [ConA], phytohemagglutinin [PHA]) and to allogeneic cells in a mixed lymphocyte reaction, primary IgM antibody response to SRBC, examination of splenic lymphocytes subpopulations, and delayed-type and contact hypersensitivity. A dose-related suppression of primary IgM antibody response to SRBC was found in the 21-week-old males at the 0.3 mg/kg/day dose. A second immunization with SRBCs 4 weeks later resulted in a significant reduction in IgG antibody response in males at all dose levels. A decrease in the OX12+OX19− (i.e., B-/plasma cells) population was also found in the spleen of the high-dose group (males only). The intermediate duration MRL of 0.0001 mg/kg/day was derived from the low dose of 0.03 mg/kg/day, which ATSDR interpreted to be minimally adverse for developmental immunotoxicity.

3.1.6. Hexachlorocyclohexane, gamma (HCCH) (Lindane)

The intermediate oral MRL of 0.00001 mg/kg/day for lindane was derived using data from a feeding study in female Swiss mice (Meera et al., 1992). Mice received doses of 0, 0.012, 0.12, or 1.2 mg/kg/day for up to 24 weeks. Endpoints that were evaluated throughout the study included DTH reaction to SRBC, lymphoproliferative response to mitogenic stimulation by concavalin A, mixed lymphocyte reactions, response of IgM antibody forming cells in spleen (plaque formation) to SRBC or lipopolysaccharide (LPS), and peritoneal macrophage phagocytic activity in response to LPS or *Staphylococcus aureus*. Histology of the thymus, peripheral lymph nodes, and spleen was evaluated at 4, 12, and 24 weeks post-treatment.

Cell-mediated and humoral components of the immune system showed a biphasic response, characterized initially by stimulation followed by suppression in a dose-dependent manner with no no-observed-effect level (NOEL) identified. Effects observed at the 0.012 mg/kg/day dose included a DTH reaction to SRBC (increased at 4–12 weeks and decreased at 12–24 weeks), IgM plaque formation to SRBC (increased at 4–8 weeks and decreased at 12–24 weeks), and plaque formation to LPS–SRBC (increased at 4 weeks at $\geqslant 0.12$ mg/kg/day and decreased at 8–24 weeks at $\geqslant 0.012$ mg/kg/day). Histological examinations revealed decreased lymphocyte populations in the thymus and lymph nodes and a reduction in overall cellularity in the spleen and necrosis of the thymus at 1.2 mg/kg/day.

3.1.7. 2,3,4,7,8-Pentachlorodibenzofuran (pentaCDF)

The acute oral MRL of 0.000001 mg/kg/day was based on a LOAEL for mild thymic lymphoid hypoplasia identi-

fied in male Hartley guinea pigs. The animals were dosed by single gavage with pentaCDF concentrations of 0, 1, 3, 10, or 30 μg/kg in corn oil and observed for 30 days (Moore et al., 1979). At concentrations $\geqslant 3$ μg/kg, bone marrow hypocellularity, lymphoid elements in spleen, and Peyer's patches were observed. Histological examinations were not performed on the low-dose group, and the 3-μg/kg/day dose was selected as a LOAEL.

3.1.8. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Immune system endpoints were used to derive both acute and intermediate MRLs for TCDD. In the acute study, female B6C3F1 mice were administered a single gavage dose of 0, 0.001, 0.005, 0.01, 0.05, or 0.1 μg/kg of TCDD in corn oil and challenged intranasally with influenza A virus 7 days later (Burleson et al., 1996). In a separate experiment, mice received single gavage doses of 0, 0.001, 0.01, or 0.1 μg TCDD/kg and were infected 7 days later with influenza A virus at a dose not known to cause mortality, or were sham-infected. Body weight, thymus weight, and wet lung weights were measured 3, 9, or 12 days post-infection. Pulmonary virus titers were determined in groups of 72 mice exposed to 0, 0.001, 0.01, or 0.01 μg TCDD/kg and infected with influenza A virus 7 days later. For the virus titer study, groups of mice were killed 2 h, 1, 4, 6, 7, 8, 9, 10, and 11 days postinfection.

Increased mortality was observed at doses $\geqslant 0.01$ μg/kg in the TCDD-exposed animals. However, no between-group differences in mortality were observed at these dose levels. The concentrations of 0.005 and 0.01 μg/kg were selected as the NOAEL and LOAEL for impaired resistance, respectively. The lack of dose-response in mortality and the lack of effect on the relative lung weight, thymus weight, and viral titers prompted the authors to suggest that TCDD might be exerting an effect via an indirect mechanism, such as through an effect on cytokines.

The intermediate MRL of 0.0002 μg/kg/day for TCDD was derived from a 90-day feeding study in weanling Hartley guinea pigs (DeCaprio et al., 1986). Diets provided an average TCDD dose of 0, 0.0001, 0.0007, 0.005, or 0.028 μg. The animals were sacrificed at the end of the dosing period, and clinical chemistries, hematology, organ weights, and histopathology examinations performed. The recovery following treatment was studied in groups of 10 guinea pigs fed a diet containing the TCDD concentration of 0.028 μg/kg/day 11, 21, or 35 days and allowed to recover for 79, 69, or 55 additional days, respectively.

The highest dietary level of TCDD caused net body weight loss and mortality. Gross lesions were observed only in the highest dose group and included thymic atrophy, depletion of body fat, and liver enlargement. Significant changes in organ weights included a decrease in absolute kidney weight and in absolute and relative thymus weight in males dosed with 0.005 μg/kg/day, increase in relative liver weight in males and females at the 0.005 μg/kg/day dose, and increase in relative brain weight in males at 0.005 μg/kg/day. Organ weights from high-dose animals

were not monitored. Treatment-related histological altera-tions were seen only in the two higher-dose groups and consisted of hepatocellular cytoplasmic inclusion bodies and atrophy of the thymic cortex. In the recovery study, there was 10% mortality in the groups treated for 11 and 21 days and 70% mortality in the group treated for 35 days. Surviving animals in all groups showed markedly reduced body weight gain. The dose of 0.0007 μg/kg/day was select-ed as the NOAEL for the derivation of the intermediate MRL of 0.00002 μg/kg/day, based on the decreased thy-mus weight that occurred at the next higher level of 0.005 μg/kg/day.

3.1.9. 2,4-Dichlorophenol (2,4-DCP)

An intermediate MRL was derived from a study in which Sprague–Dawley rats were exposed via drinking water to calculated DCP doses of 0, 0.3, 3, and 30 mg/kg/day (Exon et al., 1984; Exon and Koller, 1985). The animals were dosed from weaning through breeding at 90 days, parturi-tion, and weaning of pups. Increased relative spleen and liv-er weights were recorded at a dose of 30 mg/kg/day. No histopathology was observed in the liver, spleen, and thy-mus. Examination of immune functions showed a signifi-cant decrease ($p < 0.05$) in delayed type hypersensitivity response to bovine serum albumin in Freund's complete adjuvant at $\geqslant 3$ mg/kg/day. Antibody production (in response to keyhole limpet hemocyanin) was significantly increased only at the 3 mg/kg/day dose. The low dose of 0.3 mg/kg/day was identified as a NOAEL and used to derive the intermediate MRL of 0.003 mg/kg/day.

3.1.10. Dibutyl tin (DBT)

Male and female weanling Wistar rats were fed diets of dibutyl tin dichloride at estimated dibutyl tin doses of 5 and 15 mg/kg/day for 4–6 weeks (Seinen et al., 1977). Humoral immune response was assessed by measuring the levels of antibodies against SRBC and *Escherichia coli* lipopolysaccharide. The cellular immune response was assessed by examining allograft rejection (rats were grafted at week 7). Final body weight after 4 weeks of exposure was not significantly altered relative to controls, but it was 28% lower than controls in the high-dose group after 6 weeks of exposure. Allograft rejection time was signifi-cantly delayed in the high-dose group. In the tests for humoral response, the number of antibody-producing cells per million spleen cells was not affected, but the number per whole spleen was decreased in a dose-related manner. This response was associated with a decreased hemaggluti-nation titer in the high-dose group. The antibody titers against *Escherichia coli* lipopolysaccharide were slightly but not significantly lower in treated groups than in con-trols. The dose of 5 mg/kg/day was used to derive the inter-mediate MRL of 0.005 mg/kg/day for dibutyl tin.

3.1.11. Tributyl tin (TBT)

The study by Vos et al. (1990) was used to derive both an intermediate and a chronic MRL for tributyl tin. In this study, Wistar rats were fed diets providing approximately 0.025, 0.25, and 2.5 mg/kg/day of tribuytl tin oxide for 4.5–18 months. Parameters of specific resistance evaluated included IgM and IgG response to ovalbumin, DTH response to ovalbumin and tuberculin, resistance to *Trich-inella spiralis* infection, mitogenic response of thymus and spleen cells, and surface marker analysis of mesenteric lymph nodes. Parameters of nonspecific resistance exam-ined included clearance of injected *Listeria monocytogenes* from the spleen and natural cell-mediated cytotoxicity of spleen and peritoneal cells.

The study NOAEL of 0.025 mg/kg/day was used to derive the intermediate and chronic MRLs of 0.0003 mg/kg/day. Effects observed at higher doses included a 17% reduction in thymus weight in the high-dose group at 4.5 months, dose-related suppression of immunoglobulin E (IgE) response to *Trichinella spiralis* at 4.5 and 16 months (as determined by the passive cutaneous anaphylaxis reac-tion), increase in the number of *Trichinella spiralis* larvae in muscle after injection at 5.5 and 16.5 months of exposure, reduction in the relative count of T-lymphocytes and an increase in the percentage of B-lymphocytes in the mid- and high-dose groups after 6 and 18 months (as revealed by surface marker analysis of mesenteric lymph node cells), and impaired *in vivo* clearance of *Listeria monocytogenes* in the high-dose group after 5 and 17 months of treatment (Vos et al., 1990).

4. Discussion and conclusion

The field of immunotoxicology and its role in risk assessment has grown considerably in recent years. This has been due, in part, to the standardization and validation of assays that serve as biomarkers of immunological alter-ations in animals and humans (Dean et al., 2001). For example, in an effort to standardize and validate immuno-toxicity testing, the National Toxicology Program devel-oped a battery utilizing a tiered approach (Luster et al., 1988). Tier I consisted of screening tests; tier II included more comprehensive assays to better describe the mecha-nisms of immunotoxicity. Subsequent reports described the results of this testing battery for over 50 chemicals and the relationships between immune function tests and host resistance (Luster et al., 1992, 1993). In 1992, CDC/ATSDR convened a workshop to propose field test batter-ies to be used to evaluate the impact of environmental tox-icants on the immune systems of populations living near hazardous waste sites (ATSDR, 1994). In the area of phar-maceuticals, a standardized approach for immunotoxicity guidance has been recommended by the International Con-ference on Harmonisation (Guidance on S8 Immunotoxic-ity Studies for Human Pharmaceuticals) (FDA, 2006).

Twenty-three substances for which ATSDR has devel-oped a toxicological profile have been shown to exert effects on the immune system (ATSDR, 2005). Despite this, only 11 of these substances have MRLs based on immuno-logical/lymphoreticular endpoints (Table 2). As discussed

Table 2
MRLs based on Immunological/Lymphoreticular effects

Substance	Duration[a]	MRL	LN	AH	HS	MF	Endpoint used for MRL derivation	Critical study
		Inhalation (ppm)						
Benzene[b]	Acute	0.009	10	3	10		LOAEL: decreased peripheral lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes	Rozen et al. (1984)
	Intermediate	0.006	10	3	10		LOAEL: delayed splenic lymphocyte reaction to foreign antigens	Rosenthal and Snyder (1987)
	Chronic	0.003			10		BMD: decreased counts of B-lymphocytes in workers	Lan et al. (2004)
		Oral (mg/kg/day)						
Aroclor 1254	Chronic	0.00002	10	3	10		LOAEL: decreased IgM and IgG levels in response to sheep RBCs	Tryphonas et al. (1989)
Chlorfenvinphos	Intermediate	0.002	10	10	10		LOAEL: 190% increase of spleen endogenous colonies; 162% increase of spleen exogenous colonies; 50% reduction in thymus weight	Kowalczyk-Bronisz et al. (1992)
Endosulfan	Intermediate	0.005		10	10		NOAEL: at higher doses: decreased humoral and cell-mediated response	Banerjee and Hussain (1986)
Heptachlor[b]	Intermediate	0.0001	3	10	10		Minimal LOAEL: decreased response to SRBC in offspring	Smialowicz et al. (2001)
HCCH, gamma	Intermediate	0.00001	10	10	10		LOAEL: biphasic changes in cell- and humoral-mediated immunity to SRBC	Meera et al. (1992)
2,3,4,7,8-Pentachlorodibenzofuran	Acute	0.000001	10	10	10	3[c]	LOAEL: mild, thymic lymphoid hypoplasia	Moore et al. (1979)
2,3,7,8-TCDD	Acute	0.0000002		3	10	0.7[d]	NOAEL: at higher doses: decreased influenza virus host resistance	Burleson et al. (1996)
2,3,7,8-TCDD	Intermediate	0.00000002		3	10		NOAEL: at higher doses: decreased thymus weights	DeCaprio et al. (1986)
2,4-Dichlorophenol	Intermediate	0.003		10	10		NOAEL: at higher doses: decreased delayed-type hypersensitivity	Exon and Koller (1985); Exon et al. (1984)
Tin, Dibutyl	Intermediate	0.005	10	10	10		LOAEL: reduced humoral response to SRBC.	Seinen et al. (1977)
Tin, Tributyl	Intermediate	0.0003		10	10		NOAEL: at higher doses: depression of IgE titers and increased T. spiralis in muscle	Vos et al. (1990)
	Chronic	0.0003		10	10		NOAEL: at higher doses: depression of IgE titers and increased T. spiralis in muscle	Vos et al. (1990)

AH, animal to human; BMD, benchmark dose; Hb, hemoglobin; HS, human susceptibility; Ht, hematocrit; LOAEL, lowest-observed-adverse-effect level; LN, LOAEL to NOAEL; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mg/kg/day, milligram per kilogram of body weight per day; MRL, minimal risk level; MtHb, methemoglobin; NOAEL, no-observed-adverse-effect level; ppm, parts per million; RBC, red blood cell; SRBC, sheep red blood cells; UF/MF, uncertainty factor/modifying factor.

[a] By ATSDR definition: acute = 14 days or less; intermediate = 15–364 days; chronic = 365 days or more.

[b] MRL subject to change pending release of final profile.

[c] For lack of neurological studies.

[d] To adjust for difference in bioavailability from oil gavage administration.

here, this comprises 15 of the 346 MRLs derived to date. MRLs are derived from the most sensitive endpoints, although they do not protect for allergic sensitization. This suggests that the database for these substances did not identify the immune system as the most sensitive system for the remaining 12 of 23 substances. This may be explained by the lack of in-depth immunological studies that might identify more specific disruptions in the immune system at lower exposure doses than are observed in general toxicological studies. Or it may simply be that immune system effects are not the most sensitive endpoints for these particular chemicals.

The task of relating human health effects to specific immunotoxic parameters in laboratory animals is difficult due to the complexities of the immune system, although much has been accomplished in recent years to improve and standardize testing guidelines. However, immunotoxicity is an important area for human risk assessment (Descotes, 2004; Taylor and Pauels, 2006). Increased knowledge and improved methodologies to better detect adverse immune system responses from exposures to xenobiotic chemicals is a continual need. This will allow the identification of environmental toxicants that can potentially impact the immune system of exposed individuals and help to clarify the association between immune responses in animal models and clinical disease in humans.

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