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Utilizing Uncertainty Factors in Minimal Risk Levels Derivation

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The Agency for Toxic Substances and Disease Registry (ATSDR) utilizes chemical-specific minimal risk levels (MRLs) to assist in evaluating public health risks associated with exposure to hazardous substances. During MRL derivation, uncertainty factors (UF) are used. Under current ATSDR methodology, default UFs of 10 are applied to extrapolate from a lowest-observed-adverse-effect level (LOAEL) to a no-observed-adverse-effect level (NOAEL), for interspecies extrapolation and for intraspecies variability. However, chemical-specific toxicity information has sometimes made it necessary and appropriate to deviate from using the standard UF of 10. Since its inception in January 1993 until December 1994, ATSDR's Interagency MRL Workgroup has derived 46 inhalation and 67 oral MRLs. When the substance-specific data permitted, the workgroup departed from the default UFs of 10 in 30 specific cases. Specific examples and rationales are presented in this paper.

INTRODUCTION

The mission of the Agency for Toxic Substances and Disease Registry (ATSDR) is to prevent or mitigate adverse human health effects and diminished quality of life resulting from exposure to hazardous substances in the environment. One of ATSDR's mandates is to develop toxicological profiles for chemicals found at hazardous waste sites. To determine the levels of significant human exposure to a given chemical and associated health effects, the profiles examine and interpret available toxicological and epidemiological data. Using data from the studies compiled in toxicological profiles, ATSDR derives 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, noncancer effects over a specified duration of exposure" (ATSDR, 1992a). 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. 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. MRLs are derived from the most sensitive end point for the exposure period which may be either the highest no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL). Human data are preferred; however, human equivalent levels can be derived based on animal exposure. The formula for derivation of an oral MRL is

$$\text{MRL} = \frac{\text{NOAEL (LOAEL)}}{(\text{UF} \times \text{MF})}$$

MRL = minimal risk level (mg/kg/day)
NOAEL = no-observed-adverse-effect level (mg/kg/day)
LOAEL = lowest-observed-adverse-effect level (mg/kg/day)
UF = uncertainty factor (unitless)
MF = 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. The concept was developed years ago for the acceptable daily intake (ADI) calculations (Dourson and Stara, 1983) and is also used for the reference dose (RfD) derivation by U.S. Environmental Protection Agency (EPA) (Barnes and Dourson, 1988). The basis for the approach have been described and investigated by others (Calabrese, 1985; Calabrese *et al.*, 1992; Calabrese and Gilbert 1993; Dourson *et al.*, 1992; Dourson and Stara, 1983; Hattis *et al.*, 1987; Hartley and Ohanian, 1988; Renwick, 1991; Zielhuis and Vanider Kreek, 1979).

UFs with default values of 10 are usually used for all three of the previously cited categories of extrapola-

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tion. A factor of 10 is used for a LOAEL, if a NOAEL was not identified, to move the dose level of the LOAEL into the range of a plausible NOAEL. This adjustment is supported by analyses of several chemicals for which at least one experimental NOAEL and LOAEL were available. These analyses indicated that dividing the lowest LOAEL by a factor of 10 usually yields a value less than the experimental NOAEL (Dourson and Stara, 1983; EPA, 1989).

Although humans are qualitatively similar to other animals with respect to health outcomes following exposures, interspecies differences do exist. UFs of 10 have been used to compensate for the uncertainties surrounding these differences. Dourson and Stara (1983) supported the use and selection of this uncertainty factor based on empirical evidence in the literature suggesting that the 10-fold reduction in animal dose is sufficient to extrapolate to the corresponding human dose (e.g., a NOAEL).

Conditions that may enhance susceptibility to adverse health effects include age, sex, genetic composition, nutritional status, and preexisting disease conditions. UFs of 10 are usually used to derive MRLs protective of these sensitive subpopulations. Following an extensive literature review, Calabrese (1985) concluded that the commonly used UF of 10 seemed to provide protection for 80 to 95% of the human population.

Combining UFs without further evaluation can lead to overestimation of the actual risk. For example, if 2 factors of 10 are multiplied and each factor encompasses an extrapolation at the 95% level, the product will result in an estimate that is more conservative than the 95% level (i.e., in the direction of the 99% level or greater). To obtain a more reasonable estimate of the ADI associated with exposure to a specific chemical, a departure from the standard approach was introduced if the database allowed the health assessors to do so (Dourson and Stara, 1983). On a case-by-case basis, the EPA Reference Dose Workgroup (Dourson, 1994) and the ATSDR Interagency MRL Workgroup have also deliberated and agreed on certain adjustments to the UFs. Examples of UFs lower than 10 used in special circumstances by ATSDR are discussed in this paper.

RESULTS AND DISCUSSION

UF for "minimal" LOAELs. In the default methodology of ATSDR (1992a), an UF of 1 is used when an MRL is based on a NOAEL. An UF of 10 is applied when an MRL is based on a less-serious LOAEL. A less-serious LOAEL 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 (e.g., focal necrosis or fatty degeneration of the liver, epithelial hyperplasia of the bile duct). Serious LOAELs are defined as effects that prevent the organism from functioning normally (e.g., death, coma, seizures, extensive necrosis, abortion, and skeletovisceral defects of development). MRLs are never based on serious LOAELs (ATSDR, 1995).

Certain effects are considered physiological (e.g., glutathione depletion is not a toxic effect, but rather a biochemical event in the physiological pathway of biodegradation of toxic chemicals), adaptive (e.g., liver enlargement may result from stimulation in the activity of hepatic mixed-function oxidases and from the *de novo* protein synthesis in the smooth endoplastic reticulum), or otherwise not adverse (e.g., decreased body weight may be caused by reduced food consumption resulting from unpalatability of the chemical mixed in food). Although some biochemical changes are not toxic effects per se, they may be components in the sequence of events that leads to toxicity. In recognition of these subtle differences, the ATSDR MRL Workgroup adopted a minimal adverse effects category. 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 (Durkin, 1995). For example, slight methemoglobinemia will reduce the oxygen carrying capacity of the blood, but will not necessarily lead to the inability of the blood to supply sufficient oxygen to the body.

The workgroup conducts a careful evaluation of the adversity or nonadversity of each effect. It is, however, recognized that a NOAEL will depend on the sensitivity of the technique used in the particular study. New methods enable detection of subtle changes that were not recognized in older studies. Thus, effects in newer studies are often well below a previously identified NOAEL, and additional scientific judgment is needed to determine their adversity, or lack of adversity.

Since January 1993, the ATSDR Interagency MRL Workgroup has derived MRLs based on LOAELs for minimal health effects for inhalation exposures of acute (acetone, carbon disulfide, carbon tetrachloride, toluene), intermediate (1,1,2,2-tetrachloroethane, toluene), and chronic (titanium tetrachloride, toluene, carbon disulfide) durations. Oral MRLs based on minimal health effects were derived for acute (carbon tetrachloride, dioctylphthalate, toluene, hexachlorobenzene, diethyl phthalate) and intermediate (dichloroethane, toluene, zinc, lindane, diethyl phthalate, MTBE, hexachlorobenzene) duration exposures. Following are examples of effects classified as minimal (Table 1).

Neurological/neurobehavioral effects. The nervous system has been recognized as a target of toxicity for many chemicals. In recent years, an effort has been made to detect early signs of toxic effects, and many new methods have been developed to assess the health

TABLE 1
Specific Examples Where the ATSDR MRL Workgroup Deviated from Default UFs

Chemical	MRL	Duration	Route of Exposure	UF	Total UF
		LOAEL to NOAEL extrapolation			
Toluene	0.02 mg/kg/day	Intermediate	Oral	3	300
Carbon tetrachloride	0.02 mg/kg/day	Acute	Oral	3	300
Naphthalene[a]	0.02 mg/kg/day	Intermediate	Oral	3	300
Hexachlorobenzene[a]	0.008 mg/kg/day	Acute	Oral	3	300
Zinc	0.3 mg/kg/day	Intermediate	Oral	3	3
Toluene	3 ppm	Acute	Inhalation	3	30
Toluene	1 ppm	Chronic	Inhalation	3	30
Carbon disulfide[a]	0.3 ppm	Chronic	Inhalation	3	30
Carbon tetrachloride	0.2 ppm	Acute	Inhalation	3	300
		Interspecies extrapolation			
PCBs	0.02 μg/kg/day	Chronic	Oral	3	300
HMX[a]	0.05 mg/kg/day	Intermediate	Oral	100	1000
Titanium tetrachloride[a]	0.0003 mg/m^3	Chronic	Inhalation	3	90
		Intraspecies extrapolation			
Inorganic arsenic	0.0003 mg/kg/day	Chronic	Oral	3	3
Metallic mercury	20 ng/m^3	Acute	Inhalation	1	100

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

risks associated with exposure. However, because of the vast number of methods available, the comparability of results is sometimes difficult (WHO, 1986a). Adverse health effects induced by chemicals in nervous system function or behavioral performance are identified in animal studies by means of behavioral testing (e.g., respondent and operant behavior), clinical/neurophysiological assessment (e.g., EMG, EEG, EKG), morphological examination (e.g., light and electron microscopy), and biochemical and neuroendocrinological testing (e.g., neurotransmitters, DNA, RNA, protein synthesis). Human studies, especially in occupational settings, however, must rely heavily on noninvasive methods to evaluate health effects. Therefore, subjective reports of adverse health effects are often the only indication of toxicity caused by chemicals in the workplace.

An acute inhalation MRL of 3 ppm for toluene was based on data from an occupational study (Baelum *et al.*, 1985). In this study, a group of workers with past exposure to solvents and a matched control group of workers were used to study neurological effects of toluene. Both groups of subjects were exposed either to 100 ppm toluene for 6 hr or to clean air. All exposed individuals were subjected to a neurobehavioral test battery and the results were compared with the results obtained from the same individuals following exposure to clean air. No difference in results was found between the group with past exposure to solvents and the controls. However, acute exposure to toluene caused subjective symptoms in all individuals (increased fatigue, sleepiness, headache, mucous membrane irritation) and decreased performances on 4 of the 10 neurobehavioral tests. Three of the tests pertained to visual perseverance. The fourth test affected was the simple peg board test of visuomotor function, where the effect was noted in the occupationally preexposed workers to a greater extent than the controls. The effects were considered by the ATSDR MRL workgroup to be minimal primarily because of their subjectivity and reversibility.

Similarly, subjective symptoms, including fatigue, recent short-term memory problems, concentration difficulties, and mood lability, were reported by printers chronically exposed to toluene (Orbaek and Nise, 1989). However, the results of psychometric tests were confounded by differences between the exposed and control groups on the synonyms' test performance and by alcohol consumption rates. Because of these confounders, the reported effects were classified as minimal and an UF of 3 for a LOAEL to NOAEL extrapolation was used to derive a chronic duration inhalation MRL of 1 ppm.

A chronic duration inhalation MRL of 0.3 ppm for carbon disulfide (CS_2) was based on the Johnson *et al.* (1983) study. Exposed workers were divided into one of three exposure groups having median exposure levels of 1.0, 4.1, or 7.6 ppm CS_2 (average CS_2 exposure concentration for all of the exposed workers was 7.3 ppm). Results of electrodiagnostic tests (nerve conduction velocity measurements) were compared between a comparison group (median CS_2, 0.2 ppm) and the total CS_2 exposure group as well as to each individual exposure group. A statistically significant reduction in nerve conduction velocities (NCVs) was found in the high-exposure group and in the exposed group as a whole when compared to controls. The results were indicative of a potential trend; however, the NCVs were still within the range of clinically normal values. There-

fore, the MRL workgroup considered the effect minimal and applied an UF of 3 to the LOAEL of 7.6 ppm for LOAEL to NOAEL extrapolation, rather than the default value of 10.

Another type of nervous system effect is neurotransmission-associated toxicity. The evaluation of nerve terminal function is rather complex and may include neurotransmitter synthesis and degradation, transport and release, binding, and regulation by ion channels and cyclic nucleotides. However, most chemically induced effects on neurotransmitters are considered short-term interactions that are readily reversible, although exceptions have been reported (Anthony and Graham, 1991).

An intermediate duration oral MRL of 0.02 mg/kg/day for toluene was derived based on a 28-day drinking water study in mice (Hsieh *et al.,* 1990). At a dose level of 5 mg/kg/day, the authors observed increases in the levels of dopamine, norepinephrine, and serotonin in the hypothalamus. Other areas of the brain were affected at higher exposure levels. Effects on mood and emotional state and, possibly, on aggressive behavior were expected. However, no clinical effects were reported in exposed animals. Because of the low clinical significance and potential ease in reversibility, these effects were interpreted as minimal and an UF of 3 was used for the MRL derivation.

Hepatic effects. A vast number of techniques have been developed to detect adverse health effects on the liver, the most common target organ of toxic chemicals after oral exposure. The effects may vary from clinical signs of functional failure to histopathological evidence of distinct changes to more subtle enzymatic and subcellular changes. There is a fine line between effects considered not adverse and those that might be considered minimal, according to the definition cited previously. Following are examples of the workgroup decisions on these issues.

An acute duration inhalation MRL of 0.2 ppm for carbon tetrachloride was derived using a LOAEL of 50 ppm, which described elevated serum alanine transferase (ALT) levels in rats exposed for 4 days, 6 hr/day (David *et al.,* 1981). This LOAEL was supported by histopathology in another experiment described in the David *et al.* (1981) paper: rats were exposed to 100 ppm carbon tetrachloride or more for 4 weeks (intermediate duration). The changes noted included altered liver glycogen distribution, hepatocyte steatosis, hydropic degeneration, and mild focal necrosis. The LOAEL of 50 ppm was also supported in general by the acute duration database for carbon tetrachloride which reported hepatic LOAELs in the range of 100–180 ppm (ATSDR, 1994a). Because the changes could be classified as mild, even after prolonged exposure to higher levels, the workgroup decided to assign an UF of 3 for LOAEL to NOAEL extrapolation.

An UF of 3 was also used to derive an acute duration oral MRL of 0.02 mg/kg/day for carbon tetrachloride. When rats were exposed by gavage to 5 mg/kg/day carbon tetrachloride for 10 days, centrilobular vacuolar degeneration was observed (Smialowicz *et al.,* 1991). No biochemical changes indicative of liver damage were detected at this exposure level. However, hepatotoxicity increased in a dose-related manner. Hepatocellular necrosis accompanied by elevated serum alanine and aspartate aminotransferases was found at the mid-level doses. Cytoplasmic vacuoles frequently appear in hepatocytes following assault from hepatotoxic chemicals. They represent early signs of toxicity indicative of inhibitory effects of these chemicals on protein synthesis. Because of the mild character of changes described in the Smialowicz *et al.* (1991) study, but mindful of the potential for further damage, the workgroup used an UF of 3 for a minimal LOAEL to derive the acute oral MRL.

An intermediate duration MRL of 0.02 mg/kg/day for naphthalene was derived using data from a 90-day study in which mice were exposed by gavage once daily (7 days/week) (Shopp *et al.,* 1984). Serum chemistry data indicated a dose-related decrease in blood urea nitrogen (BUN) in females at all doses tested (5.3, 53, and 133 mg/kg/day). The authors noted that although this effect was dose-related, the BUN values were within the normal range for mice. In addition, a dose-dependent decrease in benzo[*a*]pyrene hydroxylase activity was observed in both males and females. Depressed BUN values can result from overhydration, pregnancy, or liver damage. However, since neither overhydration nor pregnancy were relevant factors in these study animals, the workgroup classified decreased BUN as a minimal LOAEL for hepatotoxicity and used an UF of 3 to derive an MRL. This decision was based on the subtle nature of the effects; although the BUN values were within normal limits, they were nonetheless significantly elevated from the control values, perhaps indicating that the 5.3 mg/kg/day dose was near a threshold for hepatic effects.

Developmental effects. Main categories of developmental toxicity include embryotoxicity (death), fetotoxicity (altered growth), and teratogenicity (malformations and functional deficits) (Haddad and Winchester, 1990). In the latter category, behavioral teratogenicity is a relatively new research field. Behavioral tests are designed to evaluate behaviors that are functions of sensory, motor, and integrative processes of the nervous system. Behavioral changes may be very sensitive indicators of chemical exposure during nervous system development (in humans, both prenatally and postnatally). However, some authors caution that not enough evidence has accumulated to assure that the manifestation of effects in animals and humans is similar (WHO, 1984). Inadequate testing or interpretation of

data, interspecies differences in behavioral patterns, and other variables may confound the results. An example of utilizing an UF lower than 10 is the derivation of an MRL for hexachlorobenzene.

When female rats were exposed by gavage to 2.5 mg/kg/day hexachlorobenzene in oil for 4 days, 2 weeks prior to mating, neurobehavioral effects were detected in their offspring (Goldey and Taylor, 1992). The effects included increased negative geotaxis reflex (Postnatal Days 6 and 8), increased olfactory discrimination/homing (Postnatal Days 9, 10, and 11), and increased exploratory behavior (Postnatal Days 19 and 20, but not Days 15, 16, 17, and 18). Negative results were obtained in other tests (e.g., acoustic startle reflex). The authors speculated that the results were indicative of hexachlorobenzene-induced (through transplacental exposure) hyperactivity in young pups. However, concurrent exposure through maternal milk may have resulted in higher actual doses (Goldey, 1991). Later studies in offspring (Postnatal Days 40 and 50) showed that the effects were reversible; no significant impact on learning ability or motor activity was detected. Therefore, the workgroup used an UF of 3 for minimal LOAEL adjustment to derive an acute duration oral MRL of 0.008 mg/kg/day for hexachlorobenzene.

Hematological effects. Blood, together with organs of hematopoiesis, is a multifunctional system; exposure to toxic chemicals may affect its morphology and/or function. Some of the effects in blood caused by toxic chemicals include depressed cell formation, increased cell destruction, desaturation of hemoglobin, and impairment of coagulation. Modern laboratory methods can reveal subtle changes that previously were unnoticed.

An intermediate duration oral MRL of 0.3 mg/kg/day for zinc was based on the Yadrick *et al.* (1989) study that evaluated women taking daily dietary zinc supplements. A reduction (47%) in erythrocyte superoxide dismutase (ESOD) levels (compared to preexposure levels) was detected in women taking 50 mg/day of the zinc supplement for 10 weeks. This decline was considered to be biologically significant and similar to that seen after experimental copper depletion. In sheep, copper is added to the ESOD apoenzyme at the time of erythropoiesis (Andreawartha and Caple, 1980). Yadrick *et al.* (1989) proposed that if this was the case for humans, only newly formed erythrocytes would reflect a copper deficiency; therefore, a decline in ESOD activity would become apparent only after sufficient erythrocyte turnover. The authors suggested that a copper deficiency may have occurred in the study group prior to actual detection in the total erythrocyte population. The results, suggestive of copper–zinc interactions, are supported by other reports of decreased copper levels in individuals taking zinc supplements and were considered minimal effects (ATSDR, 1993a). Therefore, an UF of 3 was used for minimal LOAEL to NOAEL adjustment. Consideration of the essentiality of zinc in a diet also played a role in the workgroup's deliberations.

UF for interspecies differences. Ideally, evidence of adverse effects resulting from exposure to hazardous substances should be obtained from human evaluations. For obvious reasons, human toxicological studies are not often available. Although a number of epidemiological studies are available that identify adverse effects in a defined human population, these studies often suffer from a number of uncertainties, including unknown exposure levels, confounding risk factors, and concomitant exposure to other chemicals. As a result, toxicologists have had to rely heavily on animal studies to assess the potential health risks to humans. When using animal studies for MRL derivation, ATSDR selects the most sensitive species as presented in the database.

Uncertainty factors have been used in human health risk assessments to adjust for interspecies differences. Dourson and Stara (1983) reviewed a number of studies comparing animal versus human toxicity for several toxicants. They concluded that, in lieu of chemical-specific data, an uncertainty factor of 10 was sufficient for animal to human adjustment. Although humans are considered by some investigators to be more sensitive than animals in terms of milligrams per kilogram of body weight (Dourson and Stara, 1983; Lu, 1983), this is not always the case. Digression from the default UF of 10 is warranted when chemical-specific information is available to support such a change. The MRL Workgroup chose to deviate in several specific cases. Since January 1993, inhalation MRLs for titanium tetrachloride and oral MRLs for 1,2-dichloroethane, HMX, γ-lindane, and PCBs have been derived using UFs other than 10 for interspecies adjustment. Examples of these cases are discussed below (see Table 1).

A chronic oral MRL of 0.02 μg/kg/day for PCBs was based on the Tryphonas *et al.* (1989, 1991a,b) studies. Immunological parameters were altered in rhesus monkeys exposed chronically to doses as low as 5 μg/kg/day. The monkeys were administered sheep red blood cells (SRBCs) at 23 months (Tryphonas *et al.*, 1989) and at 55 months (Tryphonas *et al.*, 1991a). At 23 months, a primary response to SRBCs was evoked. An anamnestic response was elicited at 55 months. In both cases, decreases in IgM and IgG levels were observed, although the IgG response at 55 months was significant only when the overall trend was analyzed. This depressed response to both primary and secondary stimulation was seen as sufficient evidence of an adverse effect of PCBs on the immune system of exposed monkeys. Reduced humoral antibody production is classified as a less-serious or a serious LOAEL depending on the degree of suppression (ATSDR, 1995). In this case, the reduction was categorized as less seri-

ous. Decreased immune or antibody response for a single route/duration is not usually regarded as adequate evidence for identifying the immune system as a target organ system. However, when coupled with other indicators of immune system dysfunction as supportive evidence, these endpoints may be used for MRL derivation. The supporting evidence need not come from the same study (ATSDR, 1995). Although only immunoglobulins were measured at the 5 μg/kg/day level of PCBs exposure in Tryphonas *et al.* (1989), other indicators of immunotoxicity (e.g., decreased phagocytic ability of monocytes) were found at higher doses (Tryphonas *et al.*, 1989, 1991a,b). Furthermore, supportive evidence that PCBs are immunotoxic is provided by a number of other studies cited in the *Toxicological Profile for PCBs* (ATSDR, 1992b). It is believed that, similarly to chlorinated dibenzodioxins (CDDs) and chlorinated dibenzofurans (CDFs), coplanar PCBs act through the Ah receptor mechanism. The antibody response to SRBCs is the only immunological parameter consistently suppressed by halogenated aromatic hydrocarbons (PCBs, CDDs, CDFs) in various species (ATSDR, 1992b, 1994c). In contrast, changes in other immunological parameters are more inconsistent among the studies. The reason for this is not clear.

Monkeys and humans are closely related species that metabolize PCBs by similar pathways. Because monkeys are very sensitive to PCBs-induced immunotoxicity, and suppression of antibody response is consistent among species following halogenated aromatic hydrocarbons exposure, an UF of 3 instead of the default value of 10 was used for MRL derivation to adjust for interspecies differences.

A chronic duration inhalation MRL of 0.0003 mg/m^3 was derived for titanium tetrachloride. Major health effects noted were dose-dependent increases in tracheitis and rhinitis in male and female rats exposed to 0.1 mg/m^3 for 2 years (Lee *et al.*, 1986; EPA, 1986). Tracheitis increased with duration, and to a lesser degree, with concentration. The incidences of tracheitis at the end of the 2 years were 12–20% for the low-dose group (0.1 mg/m^3) and 30–44% for the high-dose group (10 mg/m^3). Gross pathology and histopathology revealed compound-related changes in the lungs and thoracic lymph nodes of the treated animals. The effects observed are considered a consequence of direct contact. This results from the instability of titanium tetrachloride in the presence of water leading to its rapid hydrolysis and subsequent generation of hydrochloric acid (ATSDR, 1994b). The MRL Workgroup concluded that despite the direct contact mechanism, an UF of 3 instead of the default value of 10 was needed to account for possible anatomical differences of the respiratory tract between humans and animals.

An intermediate duration oral MRL of 0.05 mg/kg/day was derived for HMX (cyclotetramethylene–tetranitramine). This was based on a 13-week study in Fischer rats (Everett and Maddock, 1985). A NOAEL of 50 mg/kg/day was identified. Exposure to ≥150 mg/kg/day resulted in hepatic centrilobular cells with pale nuclei and dark cytoplasm. Additional effects observed included focal renal tubule atrophy, dilatation, and increased kidney weights in females at 270 mg/kg/day and elevated BUN at 1500 mg/kg/day. In deliberating the MRL for HMX, the workgroup considered additional information. This included the fact that acute exposure data suggested that neurological effects may be more sensitive endpoints, although neurotoxicity has not been evaluated for intermediate duration exposure. Furthermore, limited evidence suggested that rabbits are a more sensitive species (ATSDR, 1994d). As a result, an additional UF of 10 (total UF = 100 for interspecies extrapolation) was used rather than the default of 10.

UF for intraspecies differences. Similarly to interspecies extrapolation, an UF of 10 is usually used to account for intraspecies differences; (i.e., human variability) in response to toxic chemicals. This UF was introduced to protect sensitive individuals within a population. Age, sex, genetic composition, nutritional status, and preexisting diseases may all alter susceptibility to toxic chemicals.

Data available for some chemicals allowed for departure from this policy. An UF of 1 was used to derive an intermediate duration oral MRL for zinc and an acute duration inhalation MRL for metallic mercury. An UF of 3 was used to derive an acute duration inhalation MRL for acetone, an intermediate duration inhalation MRL for xylenes, an acute duration oral MRL for γ-lindane, and a chronic duration oral MRL for arsenic. The following are examples of issues the MRL Workgroup considered before deriving MRLs for these chemicals (Table 1).

Infants and young children differ from adults in several ways. Larger body surface area in relation to weight, higher metabolic rate and oxygen consumption, greater energy and fluid requirements, etc., may affect the infant or young child's susceptibility to chemically induced effects (WHO, 1986b). Organs such as the liver and kidneys are not functionally developed enough to properly metabolize and excrete toxic chemicals which may lead to chemical accumulation and additional damage. Furthermore, some systems (e.g., immunological, neurological) are not fully developed and are easy targets for chemical injury. The workgroup's reflections on these aspects can be seen in the following example for mercury.

An acute duration inhalation MRL of 20 ng/m^3 for metallic mercury was based on the Fredriksson *et al.* (1992) study. In this study, newborn rats were exposed to 0.05 mg/m^3 metallic mercury for 1 hr a day on Postpartum Days 11 through 17. At the age of 4 months, the exposed rats showed increased locomotor activity

and decreased rearing. At 6 months, the rats needed a longer time to complete a radial arm maze. The authors concluded that exposure to metallic mercury during central nervous system (CNS) development may cause effects later in adult CNS function.

Studies of metallic mercury indicate that it is quickly absorbed by the inhalation route and its half-life in the body is within a range of several weeks (ATSDR, 1993b). Neurotoxicity of metallic mercury has been demonstrated in adults of several species. It is known that an extremely critical period for CNS damage is during the brain's growth spurt, the period investigated in the Fredriksson *et al.* (1992) study. In support of this observation, numerous examples of toxic effects induced in developing versus mature CNS can be found in studies on lead (ATSDR, 1992c). Therefore, the newborn rats were considered as the most sensitive population and an UF of 1 instead of the default value of 10 for intraspecies variability was applied in the MRL calculation.

One of the theories of aging implies that changes in gene expression or gene structure may result in altered biochemistry, physiology, and, eventually, special pathologies of the elderly (WHO, 1993). These changes may have a great impact on susceptibility to a chemical insult. Further, the aged population often has been subjected to previous chemical exposure, either occupationally or in the environment. In that case, further exposure may exceed the natural ability of the organism to withstand chemical injury.

When deliberating the chronic oral MRL of 0.0003 mg/kg/day for inorganic arsenic, the workgroup considered the preceding factors. The MRL was based on the Tseng *et al.* (1968) study in which a large Taiwanese population was exposed to arsenic, mainly through drinking water. An UF of 3 rather than the default value of 10 for human susceptibility was used because the database for human exposure to arsenic is large, thus, reducing the uncertainty (Crump, 1984; Gaylor, 1983). There is a clear agreement of NOAEL and LOAEL values across human studies; in addition, the effects of arsenic increase with age indicating that the sensitive population was taken into account in the aged population of the Taiwanese study.

CONCLUSIONS

The risks to human health from exposure to chemicals in the environment are assessed by the evaluation of substance-specific databases. Identification of NOAELs is useful to the decision-making process by guiding health assessors to levels that potentially threaten human health. The uncertainties surrounding possible dissimilar responses between animals and humans, variation in human susceptibility, and use of LOAELs in lieu of missing NOAELs have traditionally been accounted for by the use of UFs of 10. This approach has been challenged for various reasons outlined by Barnes and Dourson (1988). Criticisms include the fact that the NOAEL–UF approach does not take into consideration the slope of the dose–response curve or sample size of the critical study, and questions surrounding appropriate endpoint selection.

ATSDR as well as other agencies (e.g., EPA) have recognized these deficiencies and contend that the current approach, if used in conjunction with scientific judgment, can be useful (Dourson, 1994). For example, the issue of sample size has been discussed by Gaylor (1983). In two hypothetical experiments of two different sample sizes, he calculated a higher "safe" dose for the experiment with the smaller sample size when it should actually have been lower due to the greater degree of uncertainty in the smaller experiment. The ATSDR MRL Workgroup took this issue into consideration during deliberations for the inorganic arsenic oral MRL. A more plausible MRL was derived using an UF of 3 for intraspecies variation for reasons previously described that included the large study population.

Exposure to minimal LOAELs may not be deleterious in and of itself, but may provide an early warning signal of toxicity. Again, as previously described, the acute duration oral MRL for carbon tetrachloride was derived from a hepatic endpoint (centrilobular vacuolar degeneration). Although this effect may be considered as mild and reversible, hepatic toxicity increased in a dose-related manner as evidenced by centrilobular necrosis that developed following exposure to higher doses.

The standard approach to health risk assessment using MRLs that were derived by the methods as described in this article is intended to present to the public levels of exposure that will not be associated with adverse health effects. However, health assessors are often faced with the problem of people being exposed at a higher level than MRL (or RfD). For most of the environmental contaminants, there is a gap of information about the levels at which the first subtle health effects will occur in humans.

Information used for evaluating public health risks needs to be chemical-specific because dose/severity relationship may differ for each chemical. For some chemicals, the progression from mild to severe effects is steady. For other chemicals, a sudden steep rise in the number and severity of effects may occur due to saturation of the detoxification or elimination pathway. That is why new approaches to risk assessment, e.g., benchmark dose calculation, physiologically based pharmacokinetic (PBPK) modeling, and quantitative structure–activity relationships (QSAR) are being explored. ATSDR has joined the search for alternative methodologies to refine the strategies used in making public health decisions at hazardous waste sites by establishing PBPK and QSAR workgroups. They are intended to provide help in decision making on a case-by-case basis.

REFERENCES

Andreawartha, K. A., and Caple, I. W. (1980). Effects of changes in nutritional copper on erythrocyte superoxide dismutase activity in sheep. *Res. Vet. Sci.* **28,** 101–104.

Anthony, D. C., and Graham, D. G. (1991). Toxic responses of the nervous system. In *Casarett and Doull's Toxicology* (M. O. Amdur, J. Doull, and C. D. Klaassen, Eds.), pp. 407–429, McGraw–Hill, New York.

ATSDR (1992a). *Public Health Assessment Guidance Manual.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA, NTIS PB92-147164.

ATSDR (1992b). *Toxicological Profile for Selected Polychlorinated Biphenyls.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1992c). *Toxicological Profile for Lead.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1993a). *Toxicological Profile for Zinc.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1993b). *Toxicological Profile for Mercury.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1994a). *Toxicological Profile for Carbon Tetrachloride.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1994b). *Toxicological Profile for Titanium Tetrachloride—Draft for Public Comment.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1994c). *Toxicological Profile for Chlorodibenzofurans.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1994d). *Toxicological Profile for HMX—Draft for Public Comment.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1995). *Guidance for Developing Toxicological Profiles.* U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Baelum, J., Anderson, M. D., Gunnar, R., *et al.* (1985). Response of solvent-exposed printers and unexposed controls to six-hour toluene exposure. *Scand. J. Work. Environ. Health* **11,** 271–280.

Barnes, D. G., and Dourson, M. (1988). Reference dose (RfD): Description and use in health risk assessments. *Regul. Toxicol. Pharmacol.* **8,** 471–486.

Calabrese, E. J. (1985). Uncertainty factors and interindividual variation. *Regul. Toxicol. Pharmacol.* **5,** 190–196.

Calabrese, E. J., Beck, B. D., and Chappell, W. R. (1992). Does the animal to human uncertainty factor incorporate interspecies differences in surface area? *Regul. Toxicol. Pharmacol.* **15,** 172–179.

Calabrese, E. J., and Gilbert, C. E. (1993). Lack of total independence of uncertainty factor. *Regul. Toxicol. Pharmacol.* **17,** 44–51.

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Fundam. Appl. Toxicol.* **4,** 854–871.

David, A., Frantik, E., Holusa, R., *et al.* (1981). Role of time and concentration on carbon tetrachloride toxicity in rats. *Int. Arch. Occup. Environ. Health* **48,** 49–60.

Dourson, M. L. (1994). Methods for establishing oral reference doses. In *Risk Assessment of Essential Elements* (Mertz, Abernathy and Olin, Eds.), pp. 51–61, ILSI Press, Washington, DC.

Dourson, M. L., Knauf, L., and Swartout, J. C. (1992). On reference dose (RfD) and its underlying toxicity data base. *Toxicol. Ind. Health* **8,** 171–189.

Dourson, M. L., and Stara, J. F. (1983). Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol.* **3,** 224–238.

Durkin, P. (1995). *Guidelines for Assessing Adverse Health Effects and Using Severity of Effects in Risk Assessment.* (Draft). Prepared by Syracuse Environmental Research Associates, Inc., for ATSDR under ERG Contract 205-93-0641.

EPA (1986). *Two-Year Inhalation Study with Titanium Tetrachloride in Rats.* Final Report. Submitted under TSCA Section 8E, OTS0509697.

EPA (1989). *General Quantitative Risk Assessment Guidelines for Noncancer Health Effects* (draft). ECAO-CIN-538.

Everett, D. J., and Maddock, S. M. (1985). *HMX: 13-Week Toxicity Study in Rats by Dietary Administration.* Ft. Detrick, MD: U.S. Army Medical Research and Development Command, U.S. Army Medical Bioengineering Research and Development Laboratory.

Fredriksson, A., Dahlgren, L., Danielson, B., *et al.* (1992). Behavioral effects of neonatal metallic mercury exposure in rats. *Toxicology* **74**(2–3), 151–160.

Gaylor, D. W. (1983). The use of safety factors for controlling risk. *J. Toxicol. Environ. Health* **11,** 329–336.

Goldey, E. S. (1991). *Maternal Transfer and Behavioral Teratology of Hexachlorobenzene in Rats.* Ph.D. dissertation, Miami University, Oxford, OH.

Goldey, E. S., and Taylor, D. H. (1992). Developmental neurotoxicity following premating maternal exposure to hexachlorobenzene in rats. *Neurotoxicol. Teratol.* **14,** 15–21.

Haddad, L. M., and Winchester, J. F. (1990). *Clinical Management of Poisoning and Drug Overdose.* Saunders, Philadelphia, PA.

Hartley, W. R., and Ohanian, E. V. (1988). The use of short-term toxicity data for prediction of long-term health effects. In *Trace Substances in Environmental Health* (D. D. Hemphill, Ed.), Vol. 22, pp. 3–12. University of Missouri, Columbia, MO.

Hattis, D., Erdreich, L., and Ballew, M. (1987). Human variability in susceptibility to toxic chemicals—A preliminary analysis of pharmacokinetic data from normal volunteers. *Risk Anal.* **7,** 415–426.

Hsieh, G. C., Sharma, R. P., Parker, R. D., *et al.* (1990). Evaluation of toluene exposure via drinking water levels on regional brain biogenic monoamines and their metabolites in CD-1 mice. *Ecotoxicol. Environ. Saf.* **20,** 175–184.

Johnson, B. L., Boyd, J., Burg, J. R., *et al.* (1983). Effects on the peripheral nervous system of workers' exposure to carbon disulfide. *Neurotoxicology* **4**(1), 53–66.

Lee, K. P., Kelly, D. P., Schneider, P. W., and Trochimowicz, H. J. (1986). Inhalation toxicity study on rats exposed to titanium tetrachloride atmospheric hydrolysis products for two years. *Toxicol. Appl. Pharmacol.* **83,** 30–45.

Lu, F. C. (1983). Toxicological evaluations of carcinogens and noncarcinogens: Pros and cons of different approaches. *Regul. Toxicol. Pharmacol.* **3,** 121–132.

Orbaek, P., and Nise, G. (1989). Neurasthenic complaints and psychometric function of toluene-exposed rotogravure printers. *Am. J. Ind. Med.* **16,** 67–77.

Renwick, A. G. (1991). Safety factors and establishment of acceptable daily intake. *Food Addit. Contam.* **8,** 135–150.

Shopp, G. M., White, K. L., Holsapple, M. P., Barnes, D. W., Duke, S. S., Anderson, A. C., Condie, L. W., Hayes, J. R., and Borzelleca, J. F. (1984). Naphthalene toxicity in CD-1 mice: General toxicology and immunotoxicology. *Fundam. Appl. Toxicol.* **4,** 406–419.

Smialowicz, R. J., Simmons, J. E., Luebke, R. W., *et al.* (1991). Immu-

nologic assessment of subacute exposure of rats to carbon tetrachloride with comparison to hepatotoxicity and nephrotoxicity. *Fundam. Appl. Toxicol.* **17,** 186–196.

Tryphonas, H., Hayward, S., O'Grady, L., *et al.* (1989). Immunotoxicity studies of PCB (Aroclor 1254) in the adult rhesus monkey—Preliminary report. *Int. J. Immunopharmacol.* **11,** 199–206.

Tryphonas, H., Luster, M. I., White, K. L., *et al.* (1991a). Effects of PCB (Aroclor 1254) on specific immune parameters in rhesus monkeys. *Int. J. Immunopharmacol.* **13,** 639–648.

Tryphonas, H., Luster, M. I., Schiffman, G., *et al.* (1991b). Effects of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the rhesus monkey. *Fundam. Appl. Toxicol.* **16,** 773–786.

Tseng, W. P., Chu, H. M., How, S. W., *et al.* (1968). Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst.* **40,** 453–463.

WHO (1984). *Environmental Health Criteria 30: Principles for Evaluating Health Risks to Progeny Associated with Exposure to Chemicals during Pregnancy.* World Health Organization, Geneva.

WHO (1986a). *Environmental Health Criteria 59: Principles for Evaluating Health Risks from Chemicals during Infancy and Early Childhood: The Need for a Special Approach.* World Health Organization, Geneva.

WHO (1986b). *Environmental Health Criteria 60: Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals.* World Health Organization, Geneva.

WHO (1993). *Environmental Health Criteria 114: Principles for Evaluating Chemical Effects on the Aged Population.* World Health Organization, Geneva.

Yadrick, M. K., Kenney, M. A., and Winterfeldt, E. A. (1989). Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females. *Am. J. Clin. Nutr.* **49,** 145–50.

Zielhuis, R. L., and van der Kreek, F. W. (1979). The use of a safety factor in setting health based permissible levels for occupational exposure. *Int. Arch. Occup. Environ. Health* **42,** 191–201.