APPENDIX A

ATSDR MINIMAL RISK LEVEL AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 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 jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. 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. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

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MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

Rationale Statement
(Acute-duration oral MRL)

An acute-duration oral MRL for methoxychlor has not been derived. ATSDR has withdrawn the previous MRL of 0.02 mg/kg/day for acute-duration exposure derived in the 1994 Toxicological Profile for Methoxychlor. This MRL was based on precocious vaginal opening (early puberty) observed in rats exposed to 25 mg/kg/day for 59–104 days (Gray et al., 1989). This study did not test doses as low as the current intermediate-duration MRL study (Chapin et al. 1997), which demonstrated the same effect from 5 mg/kg/day administered from gestation day 14 to postnatal day 42. Thus, there is some question as to whether the premature puberty would have occurred at a lower acute dose than the 25 mg/kg/day observed in the Gray et al. (1989) study.

A variety of candidate MRL studies were considered for derivation of an acute-duration oral MRL for methoxychlor. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation. A synopsis of each candidate MRL study and the reasons for not using it follow; these two candidate studies probably bracket the upper and lower bounds of where the true MRL should lie.

Upper Bound
Reference: Gray LE, Otsby J, Ferrell J, et al. 1989. A dose-response analysis of methoxychlor-induced alterations of the reproductive development and function in the rat. Fund Appl Toxicol 12:92-109.

Experimental design: In block 2 of this study, groups of eight immature Long-Evans hooded rats of each sex were exposed to either 0, 25, or 50 mg/kg/day technical grade methoxychlor for 59–104 days beginning at 21 days of age by gavage in corn oil. Females were monitored for onset of vaginal opening, onset of estrus, estrus cyclicity, fertility, litter size, number of implantation sites, organ weights, and ovarian and uterine histology. Males were monitored for preputial separation, testis weight, and sperm count.

Effects noted in study and corresponding doses: Female rats exposed to 25 mg/kg/day or more exhibited younger age at vaginal estrus and vaginal opening after 1 week of exposure. Vaginal opening occurred at an average age of 26 days in rats exposed to 25 mg/kg/day, compared with an average vaginal-opening age of 32–33 days in control rats. Atypical vaginal smears (decreased leukocytes, increased cornification) were noted in females exposed to 50 mg/kg/day. This study identifies an acute oral LOAEL of 25 mg/kg/day for reproductive/developmental effects in female rats.

Lower Bound
Reference: Welshons WV, Nagel SC, Thayer KA, et al. 1999. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 15:12-25.

Experimental design: Adult female pregnant CF-1 mice were administered 0 (n=9), 0.02 (n=6), or 2.0 (n=5) mg methoxychlor/kg/day in corn oil on gestation days 11–17. Pups were weaned at postpartum day 23 and males were housed together until 8.5 months of age. One male from each litter was housed individually for 4 weeks, then killed, and the prostate, seminal vesicles, preputial glands, liver, and adrenals were removed and weighed (seminal vesicles and preputial glands were blotted to remove fluid before weighing).

Effects noted in study and corresponding doses: Prostate weight was statistically significantly increased by 61 and 51% in the 0.02 and 2.0 mg/kg/day groups, respectively; seminal vesicle weight was increased by 20% in the 2.0 mg/kg/day group; and liver weight was decreased by 5% in both treatment groups. Although no positive control was done in this study, the methoxychlor-induced prostate enlargement was greater than the enlargement observed in previous studies by the same investigators examining effects from gestational exposure to other estrogen-receptor ligands (estradiol or DES). There were no statistically significant exposure-related changes in body weight or weights of preputial glands, testes, or adrenals. No histological examinations were performed.

Problems with using this study for risk assessment calculations: This is a hypothesis generating study, not a definitive one. There are several areas in which its design is less than ideal. EPA 1998 Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study (EPA 1998) recommends that for a definitive study, each treatment group has 20 pregnant dams yielding offspring; Welshons et al.(1999) only used 5–6 pregnant dams in the treatment groups. These guidelines do not specify how many offspring from each litter should be measured. In good developmental studies, the litter is considered the unit of measurement for statistical calculations (Tyl 2000), but the measurement of the litter response still needs to be accurate. In many thorough developmental studies, such as the Chapin et al. (1997) study used for intermediate MRL derivation, every animal in the litter is assessed and for sex specific end points, each animal of a given sex is measured. The use of only one male from each litter raises questions about the representativeness of the measurement of the litter response; some type of selection bias might have occurred. In an analysis of a similar study in which only one or two animals out of a litter were measured for effects on prostate size, it was found that only measuring one animal per litter resulted in incorrect conclusions 50% of the time, when compared to a study in which all male offspring in each litter were measured (Elwsick et al. 2000a, 2000b; Janszen et al. 2000).

Measuring only one male out of each litter for a characteristic known to vary between litter members may not be a good experimental design strategy. Prostate size in untreated rodents normally varies between males in a litter depending on how they are positioned relative to the females in the litter; males positioned between two females are exposed to more estrogen and consequently have larger prostates than males positioned between two other males (Timms et al. 1999). It is unknown whether treatment with an exogenous estrogen-like compound would decrease the variability of prostate weight within a litter.

As mentioned above, *in utero* exposure to estrogens is one factor that influences prostate weight and some exposure to estrogen may result from the intrauterine position of male fetuses in relation to females. This is a natural consequence of the physiology of multiparous animals. The magnitude of the prostate weight differences resulting from intrauterine position has not been precisely measured; data on this topic would facilitate comparisons with the magnitude of effects produced by methoxychlor. It would be interesting to have data on prostate weights in adult males whose intrauterine position was known via observation after caesarian section delivery. Timms et al. (1999) did measure cross-sectional areas of prostate histology sections, and lengths of prostate buds and estimated prostate volume in rats with a computer model. Data on differences between cross sectional *area* in prostates from males positioned between two other males versus males positioned between two females is presented in Figure 2 of the publication, but no direct comparisons are made of *volume* or weight. It appears from the figure that budding areas of certain parts of the prostate can vary by a factor of about 2-fold as a function of intrauterine position.

Another problem with the Welshons et al. (1999) study is its lack of appropriate positive controls; ideally, one of these would have included various doses of estradiol. Vom Saal et al. (1997) includes data on the prostate weight effects of *in utero* exposure to estradiol continuously delivered from implanted silastic capsules, but this method differs from the once a day dosing of methoxychlor in the Welshons et al. (1999) paper. The prostate effects of the extremely potent synthetic estrogen DES administered by the

same methods as Welshons et al. (1999) have been reported from experiments done at a different time (vom Saal et al. 1997). Although lower doses of DES produced the same increased prostate weight effect in these experiments as methoxychlor did in the Welshons et al. (1999) study, big differences in the magnitude of the response and less than expected differences in the effectiveness of DES and methoxychlor in producing this response raise some questions about how exactly reproducible the results of this experimental protocol are. As can be seen in the table below, the maximum percent increase in prostate weight produced by methoxychlor is 61.5% while that produced by DES is only 29%. Also, there is only a 100-fold difference between the doses of methoxychlor and DES producing the maximal prostate weight increase; a greater fold difference would have been expected based on the fact that DES is an extremely potent estrogen receptor agonist while methoxychlor is a weak one (Dodge et al. 1996; Kuiper et al. 1998; Ousterhout et al. 1981).

Welshons et al. 1999		vom Saal et al. 1997	
Methoxychlor µg/kg/day	mg prostate weight adjusted by ANCOVA for body weight (percent increase from controls)	DES µg/kg/day	mg prostate weight thought to be adjusted by ANCOVA for body weight (percent increase from controls)
0	40.0 (–)	0	41.5 (–)
20	64.5 (61%)	0.002	40.0 (-4%)
2000	60.3 (51%)	0.02	48.0 (20%)
		0.2	55.0 (38%)
		2.0	49.0 (21%)
		20	47.0 (19%)
		200	32.0 (-20%)

It has been shown that low levels of estradiol (0.32 pg/mL serum, a 50% increase in free-serum estradiol over the endogenous level, released continuously from a silastic implant) and DES (0.02, 0.2, and 2.0 µg/kg/day) administered to pregnant mice during gestation produces increased prostate weight in adult male offspring, while higher and lower exposure levels of estradiol (0.21 and 0.56 pg/mL and above) and DES (0.002 and 200 µg/kg/day) resulted in a decrease in prostate weight (vom Saal et al. 1997). This results in an inverted U-shaped dose-response curve. Gestational exposure to low levels of methoxychlor (0.02–2.0 mg/kg/day) have also been shown to result in increased prostate weight (Welshons et al. 1999), while exposure of weanling to adult rats to high levels (100–1,400 mg/kg/day) of methoxychlor have been shown to result in decreased prostate weight (Shain et al. 1977; Tullner and Edgcomb 1962), as well as testicular atrophy (Bal 1984; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962). Adult mice exposed to 60 mg/kg/day methoxychlor developed testicular degeneration (Wenda-Rozewicka 1983).

Although the Welshons et al. (1999) study is not definitive enough to be the basis of an MRL, the suggestion that gestational exposure to methoxychlor, and other estrogenic compounds, can increase prostate weight at some dose is worthy of further investigation. This issue has been featured prominently in Section 3.12.2, Identification of Data Needs, of this Toxicological Profile.

A peer review panel was organized by the National Institute of Environmental Health Sciences (NIEHS), National Institute of Health (NIH), National Toxicology Program (NTP) to examine low-dose effects of endocrine disruptors (NTP 2001). The panel examined a number of studies involving estrogen and several estrogenic chemicals (including methoxychlor), androgens, and antiandrogens, as well as biological factors and study design, statistics, and dose-response modeling. The panel's overall conclusions included:

(1) While low-dose effects have been observed in some laboratory animals with certain endocrine disruptors, they are compound- and end point-specific, and in some cases they have not been replicated in studies by other investigators. Additionally, the toxicological significance of some of the end points is not known.

(2) The shape of the dose-response curve may be low-dose linear, threshold appearing, or non-monotonic. The curve shape varies with the end point and dosing regimen.

(3) Previously reported key low-dose findings need to be replicated, and studies are needed to characterize target tissue dosimetry, identify sensitive molecular markers, and determine the long-term health consequences of low-dose effects.

(4) The current testing paradigm for reproductive and developmental toxicity should be revisited to determine if changes need to be made regarding dose selection, animal model selection, age of animals when evaluated, and end points measured.

Conclusions regarding methoxychlor studies included:

(1) Methoxychlor is a weakly estrogenic chemical that can induce uterotrophism in immature rodents.

(2) There is a wide range of changes in estrogen sensitive organs at doses of 5 mg/kg/day and higher.

(3) Some immune effects, which need to be further evaluated to determine their toxicological significance, were seen following exposure to 1 mg/kg/day.

(4) More data are needed on the differences in toxicology of technical grade and pure methoxychlor.

Therefore, ATSDR has concluded that without additional data to confirm the causal relationship between exposure to extremely low doses of methoxychlor and increased prostate weight in adult male offspring (and other estrogen-related effects), derivation of an MRL in the nanogram range is not justified. However, the current data do suggest that low-dose effects of methoxychlor may be real, and more definitive studies are necessary to examine them further. An intermediate oral MRL of 0.005 mg/kg/day has been derived based on data that are well supported by the database and based on an end point that is well-established for methoxychlor (accelerated onset of puberty).

A potential acute-duration oral MRL of 0.00002 mg/kg/day can be derived from the LOAEL of 0.02 mg/kg/day in the Welshons et al. (1999) study dividing by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Other Studies Considered for Use as the Basis for an Acute Oral MRL:

(1) A LOAEL of 0.02 mg/kg/day for increased urine-marking behavior in mice when placed in a new territory (vom Saal et al. 1995). Only two male pups per litter were tested and apparently, each was only tested one time; the accuracy of the assessment of the test was somewhat questionable; it is unknown how reproducible the results are, and there was no indication of what, if any, statistical methods were used to evaluate the results.

(2) A LOAEL of 1.8 mg/kg/day for aggressive behavior (infanticide) in mice toward an unrelated pup (Parmigiani et al. 1998). The strain of mouse used was the "house mouse," and no information was provided on the specifics of the strain or whether they were inbred; only two male pups per litter were tested; the effect was only seen at one middle dose (no dose-response); and no effect was seen with DES, a positive estrogenic control.

(3) A LOAEL of 0.02 mg/kg/day for decreased aggression of young male mice toward male siblings at postpartum day 39, but not at postpartum 54 (Palanza et al. 1999). The effect was transient and there was no dose-response (no effect was seen at 200 or 2,000 mg/kg/day).

(4) A LOAEL of 16.7 mg/kg/day methoxychlor for a 2-fold increase in uterine weight in ovariectomized mice (Tullner 1961). There was little information about the condition of the animals used in the experiments; the estrogenic activity of methoxychlor was discovered serendipitously following the dusting of the mice (being used for an experiment not related to methoxychlor) for parasite control.

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APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical Name: Methoxychlor
CAS Number: 77-43-5
Date: September 2002
Profile Status: Final Post Public
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 33
Species: Mice

Minimal Risk Level: 0.005 [X] mg/kg/day [ ] ppm

Reference: Chapin RE, Harris MW, Davis BJ, et al. 1997. The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. Fundam Appl Toxicol 40:138-157.

Experimental design: Pregnant female rats were administered 0, 5, 50, or 150 mg 95% pure methoxychlor/kg/day from gestation day 14 to postpartum day 7 by gavage in corn oil, then pups were dosed directly in the same manner until postpartum day 42. At birth, pups were weighed, measured for anogenital distance, and checked for external malformations. The first day of vaginal opening or preputial separation was recorded. At about 12 weeks of age, one male and one female from each litter were mated: each treated male to two untreated females and every two treated females to one fertile untreated male. Pups were counted, sexed, and weighed at birth and removed from the dam at postpartum day 11. The dams were mated again and killed at gestation day 19. Uterine contents were analyzed for fetal number, sex, and number of dead implants and resorptions. Blood was drawn from treated males for hematology and clinical chemistry. The gonads and associated reproductive organs were examined for structural malformations, weighed, and fixed for histology. Testicular spermatid head count and epididymide sperm motility and sperm count were performed. Blood was collected from 10 unmated treated females for determination of serum estradiol, progesterone, and follicle stimulating hormone (FSH).

Effects noted in study and corresponding doses: Dams exposed to 150 mg/kg/day methoxychlor had fewer live pups/litter than controls. Precocious vaginal opening was evident (statistically significant) in all methoxychlor-treated groups (postnatal days 37.4, 35.2, 30.8, and 33.4, respectively, for groups 0, 5, 50, and 150 mg/kg/day). Estrus cycles were highly irregular or absent in the 50 and 150 mg/kg/day females. There was a severe reduction in the number of females that conceived in the 50 and 150 mg/kg/day groups (3/15 and 0/15, respectively, compared to 13/15 in the controls). There were no statistically significant effects on litter size or pup mortality or weight gain to postpartum day 10. In the second mating, the 50 mg/kg/day litters had only 32% of the implants of the controls. Empty uterine weight of pregnant females was reduced by 20 and 51% in the 5 and 50 mg/kg/day groups, respectively. Non-pregnant females also showed reduced uterine weight at the high dose. Histologically, uteri from the rats in the 50 mg/kg/day group that did not get pregnant showed mild to severe endometrial squamous metaplasia and endometrial hyperplasia; vaginas of six of the rats were cornified, and one rat had vaginal epithelial hyperplasia. Ovaries in this group were generally polycystic, two females had cystic oviducts, there were few corpora lutea, and mammary tissue was underdeveloped. The effects in the 150 mg/kg/day group were similar.

In the male mating trials, the number of females with vaginal sperm was statistically significantly reduced in the 150 mg/kg/day group. Litter size, postpartum pup death, and pup weight were not affected, and

there was no increase in resorptions or preimplantation losses. Preputial separation was delayed by 8 and 34 days in the 50 and 150 mg/kg/day groups, respectively. Weights of the testes and right epididymis were significantly reduced in the 50 and 150 mg/kg/day group, and weights of the left cauda epididymis, seminal vesicles, and ventral prostate were significantly reduced at 150 mg/kg/day. Sperm motility and epididymal sperm density were also reduced at 150 mg/kg/day. A dose-related inhibition of testes development was observed. Mild epithelial disorganization was seen in the testes of one rat in each of the 50 and 150 mg/kg/day groups and the testes of one rat in the 150 mg/kg/day group were completely atrophic. No treatment-related changes were noted in clinical chemistries and hematology. The serum estrogen:progesterone ratio was significantly elevated in the 50 and 150 mg/kg/day groups, and the FSH levels were significantly suppressed in all methoxychlor-treated rats verified to be in estrous.

Dose and end point used for MRL derivation: 5 mg/kg/day from gestation day 14 to postpartum day 42, accelerated onset of puberty

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose?
If so, explain: None needed.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:
N/A

Other additional studies or pertinent information which lend support to this MRL: The MRL is supported by other observations of reproductive effects associated with intermediate-duration exposure including elevated levels of prolactin in the pituitary of male rats exposed to 50 mg/kg/day (Goldman et al. 1986; Gray et al. 1989), decreased seminal vesicle weight, caudal epididymal weight, and caudal epididymal sperm count (Gray et al. 1989), and increased gonadotropin releasing hormone in the mediobasal hypothalamus in male rats exposed to 50 mg/kg/day (Goldman et al. 1986). At higher exposures levels, intermediate-duration studies show decreased fertility in male rats at doses of 60–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974), and in female rats at doses of 50–150 mg/kg/day (Bal 1984; Chapin et al. 1997). A study in humans described by Stein (1968), Coulston and Serrone (1969), and Wills (1969) that identified a NOAEL of 2 mg/kg/day for effects to the testes and menstrual cycle was not chosen as the basis for an MRL because reproductive function was not evaluated, and such an MRL may not be protective of reproductive and developmental effects in the fetus or child.

Comparison with acute study on which EPA RfD was based.
An RfD of 0.005 mg/kg/day has been derived by EPA (IRIS 2002; RfD derived 1991) based on a NOEL of 5.01 mg/kg/day administered on gestation days 7–19 in New Zealand White rabbits for maternal toxicity observed as excessive loss of litters (Kincaid Enterprises 1986) and an uncertainty factor of 1,000 (10 for interspecies differences; 10 for intraspecies differences; and 10 for the poor quality of the critical study and for the incompleteness of the database on chronic toxicity).

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APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (3-1, 3-2, and 3-3) and figures (3-1 and 3-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 3-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 3-1).

(5) Species The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in Chapter 9 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 3-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 6

Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation

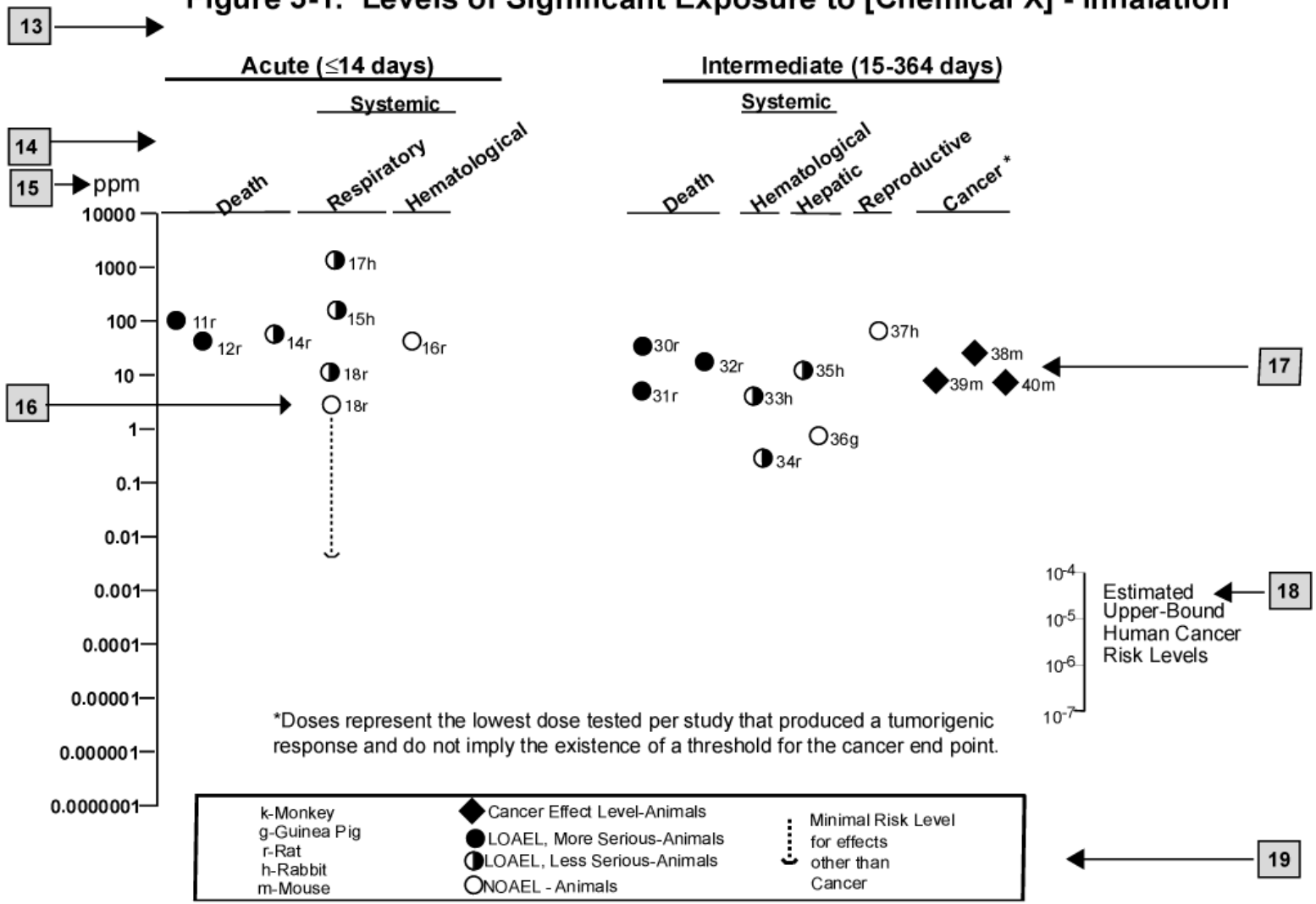
Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 6 INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 6 Systemic	9	9	9	9	9		9
4 6 18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					9		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 3-1.

12 6 [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotranferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

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DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/International Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	*Federal Register*
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LDH	lactic dehydrogenase
LH	luteinizing hormone
LT_{50}	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie

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MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OW	Office of Water

OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than

$	greater than or equal to
=	equal to
<	less than
#	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D

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