2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO HEPTACHLOR AND HEPTACHLOR EPOXIDE IN THE UNITED STATES

Heptachlor is a polychlorinated cyclodiene insecticide that was extensively used prior to 1970 to kill termites, ants, and soil insects in seed grains and on crops. In 1983, the manufacturers voluntarily cancelled its registered uses, with the exception of termite and fire ant control. Currently, the only permitted use of heptachlor is for fire ant control in buried power transformers; however, there are no actively registered pesticides containing heptachlor as an active ingredient. Heptachlor epoxide is the primary degradation product of heptachlor. Heptachlor epoxide is more persistent in the environment than heptachlor and biomagnifies in the terrestrial food chain. Although the use of heptachlor is restricted, exposure to the general population can occur through the ingestion of contaminated food, inhalation of vapors from contaminated soil and water, and dermal contact with contaminated soil and water. The food classes most likely to contain residues are milk and other dairy products, vegetables, meat, fish, and poultry.

2.2 SUMMARY OF HEALTH EFFECTS

There are limited data on the toxicity of heptachlor or heptachlor epoxide following inhalation or dermal exposure. Most of the available information on the toxicity of heptachlor comes from oral exposure studies in laboratory animals, although some human data have been identified. Oral exposure of laboratory animals to heptachlor results in a variety of adverse effects including liver effects, neurological effects, reproductive system dysfunction, and developmental effects. Although there are very few studies involving exposure to heptachlor epoxide, it is likely that the effects resulting from heptachlor exposure are due to its metabolism to heptachlor epoxide. Several human studies have examined the possible relationship between increased serum levels of heptachlor or heptachlor epoxide and adverse health outcomes. Most of these studies involved exposure to a variety of organochlorine pesticides and the observed effects cannot be ascribed to heptachlor. Additionally, a small number of studies have examined populations consuming milk products from cows fed heptachlor-contaminated feed. In these types of studies, there is a greater degree of certainty in attributing the observed effects to heptachlor exposure.

In mature animals, liver and neurological effects appear to have similar thresholds of toxicity. Acute- or intermediate-duration exposure of rats or mice to 5–10 mg/kg/day has resulted in a variety of liver effects

including increases in serum alanine aminotransferase activity, necrosis, hepatocytomegaly, hepatitis, and increased liver weights. These studies suggest that the severity of the hepatic lesions is related to the duration of exposure. No alterations in serum liver enzyme activity levels or hepatocytomegaly incidence were observed in individuals exposed to heptachlor and heptachlor metabolites in contaminated milk products. Neurological alterations indicative of excitability and increased arousal were observed in rats exposed to 7 mg/kg/day for an acute duration and mink exposed to 1.7 or 6.2 mg/kg/day for an intermediate duration. At higher doses (17 mg/kg/day), mice exhibited difficult standing, walking, and righting. Seizures have also been observed in mink prior to death.

The reproductive system may be a more sensitive target of heptachlor toxicity than the liver or nervous system. A decrease in fertility and an increase in resorptions were observed in female rats acutely exposed to 1.8 mg/kg/day. Exposure of males to 0.65 mg/kg/day for 70 days resulted in decreased epididymal sperm count and increased resorptions when the males were mated with untreated females. In contrast, two acute studies involving exposure to 10 mg/kg/day heptachlor, 8 mg/kg/day heptachlor epoxide, or 15 mg/kg heptachlor/ heptachlor epoxide mixture did not find dominant lethal effects. Reduced fertility has also been observed in mice exposed to 8.4 mg/kg/day.

The available data provide suggestive evidence that the developing organism is the most sensitive target of heptachlor toxicity. Increases in pup mortality have been observed at doses of 5.0 mg/kg/day and higher in rats and at 1.7 mg/kg/day in mink; these doses were also associated with serious maternal toxicity. Decreases in pup body weight have also been observed at 4.5 mg/kg/day and higher. Heptachlor does not appear to increase the occurrence of anomalies or malformations. Perinatal and postnatal exposure adversely affected the development of the nervous and immune systems; the lowest-observed-adverse-effect level (LOAEL) for these effects is 0.03 mg/kg/day. No adverse effects were observed in the developing reproductive system. Studies of a population exposed to heptachlor-contaminated milk products found similar effects to those reported in animals. The risk of fetal or neonatal deaths, low birth weight infants, or major congenital malformations was not significantly altered. However, alterations in neurobehavioral performance were found when the children of women exposed to contaminated milk products reached high school. In particular, abstract concept formation, visual perception, and motor planning were adversely affected.

The carcinogenicity of heptachlor and heptachlor epoxide has been evaluated in a number of human studies. In general, these studies have examined possible associations between heptachlor and/or heptachlor epoxide tissue levels or a surrogate of heptachlor exposure and the prevalence of cancer.

Mixed results have been reported across tumor types and within tumor types. Interpretation of the studies is limited by the lack of information on heptachlor exposure, variables that may affect organochlorine levels (including diet and body mass index), and possible concomitant exposure to other chemicals. Increases in the incidence of hepatocellular carcinoma were observed in mice exposed to 2.4 mg/kg/day heptachlor and higher for 2 years, but not in rats exposed to 2.6 mg/kg/day and higher. The EPA has classified heptachlor and heptachlor epoxide in group B2 (probable human carcinogen) and the International Agency for Research on Cancer (IARC) considers heptachlor as possibly carcinogenic to humans (Group 2b). EPA has derived an oral slope factor of 4.5 per (mg/kg)/day for heptachlor and 9.1 per (mg/kg)/day for heptachlor epoxide. These slope factors correspond to drinking water unit risk levels of $1.3x10^{-4}$ and $2.6x10^{-4}$ per (µg/L), respectively.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for heptachlor. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. 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 within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

Data on the toxicity of heptachlor or heptachlor epoxide following inhalation exposure are limited to several mortality studies of pesticide applicators or manufacturers (Blair et al. 1983; MacMahon et al.

1988; Shindell and Associates 1981; Wang and MacMahon 1979a) and a case control study examining the possible association between organochlorine pesticide exposure and aplastic anemia (Wang and Grufferman 1981). No significant associations were found. Interpretation of the results are limited by co-exposure to other organochlorine pesticides and lack of monitoring data. No inhalation exposure animal studies were identified.

The available inhalation data are considered inadequate for the development of MRLs for heptachlor and heptachlor epoxide.

Oral MRLs

Heptachlor.

- An MRL of 0.0006 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to heptachlor.

A number of studies have examined the toxicity of heptachlor following acute-duration oral exposure; many of the toxicity studies are limited by the lack of statistical analysis and poor reporting of the observed effects, including incidence data. Despite the limitations in the studies, the acute database does identify several targets of toxicity including the liver, nervous system, reproductive capacity, and the developing offspring. Although some other adverse health effects have been reported, they have not been replicated in other studies or were observed at lethal doses. The most sensitive effect following acute-duration exposure appears to be a decrease in fertility and an increase in resorptions observed in female rats administered via gavage 1.8 mg/kg/day heptachlor in groundnut oil for 14 days prior to mating (Amita Rani and Krishnakumari 1995). Gestational exposure to 4.5 or 6.8 mg/kg/day resulted in decreases in pup body weight (Narotsky and Kavlock 1995; Narotsky et al. 1995); a decrease in pup righting reflex was also observed at 4.2 mg/kg/day (Purkerson-Parker et al. 2001b). At twice these dose levels, an increase in pup mortality was observed (Narotsky et al. 1995; Purkerson-Parker et al. 2001b). Liver effects were observed at doses similar to those resulting in developmental effects. Increases in serum alanine aminotransferase and aldolase activity levels, hepatocytomegaly, and minimal monocellular necrosis were observed in rats administered 7 mg/kg/day heptachlor in oil for 14 days (Berman et al. 1995; Krampl 1971). Exposure to 7 mg/kg/day also resulted in excitability and increased arousal in rats administered heptachlor in oil via gavage for 1 or 14 days (Moser et al. 1995).

The lowest LOAEL identified in the acute-duration oral database is 1.8 mg/kg/day for reduced fertility and an increase in resorptions in female rats (Amita Rani and Krishnakumari 1995). In this study, groups of 30 female CFT-Wistar rats received gavage doses of heptachlor in groundnut oil for 14 days (presumably 7 days/week). The total administered doses were 25 and 50 mg/kg body weight and the daily doses were 1.8 and 3.6 mg/kg/day. A vehicle control group was also used. After 14 days of exposure, the animals were mated with unexposed male rats. A significant decrease in the number of pregnant females (56.3 and 44.4%) and increase in the number of resorptions (18.90 and 11.40%) were observed in both groups of heptachlor-exposed rats. Significant decreases in estradiol-17beta and progesterone levels were also observed in the 1.8 mg/kg/day group. No alterations in the number of implantations were observed. The investigators noted that focal necrosis was observed in the liver; however, they did not note at which dose level and no incidence data were provided. This LOAEL of 1.8 mg/kg/day was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from rats to humans, and 10 for human variability) and a modifying factor of 3 to account for the use of a serious end point, resulting in an acute-duration oral MRL of 0.0006 mg/kg/day.

- An MRL of 0.0001 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to heptachlor.

Intermediate-duration oral exposure studies have identified a number of targets of heptachlor toxicity including the liver, nervous system, reproductive system, and the developing offspring. Other less documented effects have also been observed. In the absence of maternal toxicity, heptachlor is not associated with alterations in pup mortality or body weight gain (Lawson and Luderer 2004; Purkerson-Parker et al. 2001b; Smialowicz et al. 2001). Additionally, gestational exposure does not appear to result in significant alterations in the occurrence of anomalies or abnormalities (Narotsky et al. 1995; Smialowicz et al. 2001) or the development of the reproductive system (Lawson and Luderer 2004; Smialowicz et al. 2001). *In utero* exposure followed by postnatal exposure (until postnatal day 42) did not alter reproductive function (Smialowicz et al. 2001), but did adversely affect neurobehavioral performance (Moser et al. 2001) and immune function (Smialowicz et al. 2001). The neurological effects included impaired spatial memory at 0.03 mg/kg/day and higher, impaired spatial learning at 0.3 or 3 mg/kg/day, and decreased righting reflex (Moser et al. 2001; Purkerson-Parker et al. 2001b) and increased open field activity (Moser et al. 2001) at 3 mg/kg/day. When the exposure was terminated at postnatal day 21, rather than postnatal day 42, spatial memory and learning were not adversely affected (Moser et al. 2001). The difference in results may have been due to higher heptachlor epoxide body burden in rats exposed to postnatal day 42, testing at different ages, or exposure may have occurred during a critical window of vulnerability. The effects observed in rats are consistent with those observed

in humans. Impaired performance on several neurobehavioral tests, including abstract concept formation, visual perception, and motor planning, was observed in high school students presumably prenatally exposed to heptachlor from contaminated milk products (Baker et al. 2004b). Alterations in immune function were also observed in the rats exposed until postnatal day 42. At 0.03 mg/kg/day and higher, suppression of the immune response to sheep red blood cells was observed (Smialowicz et al. 2001). A reduction in the percentage of B lymphocytes was also observed in the spleen of rats exposed to 3 mg/kg/day. Other tests of immune function were not significantly altered.

The liver effects observed in rats or mice exposed to heptachlor in the diet include increased liver weights (Izushi and Ogata 1990; Pelikan 1971), increased serum alanine aminotransferase activity levels (Izushi and Ogata 1990), steatosis (Pelikan 1971), and hepatitis and necrosis (Akay and Alp 1981). The lowest LOAEL values for these effects range from 5 to 8.4 mg/kg/day. Neurological signs such as hyperexcitability, seizures, and difficulty standing, walking, and righting were observed at similar dose levels; LOAELs ranged from 1.7 to 17 mg/kg/day (Akay and Alp 1981; Aulerich et al. 1990; Crum et al. 1993). Decreases in epididymal sperm count were observed in rats administered 0.65 mg/kg/day heptachlor in groundnut oil for 70 days (Amita Rani and Krishnakumari 1995). This dose also resulted in increased resorptions when the exposed males were mated with unexposed females. Reduced fertility was observed in all mice exposed to 8.4 mg/kg/day heptachlor for 10 weeks (Akay and Alp 1981).

The intermediate-duration oral MRL for heptachlor is based on the results of the study reported by Moser et al. (2001) and Smialowicz et al. (2001), which found alterations in development of the nervous and immune systems. In this study, groups of 15–20 pregnant Sprague Dawley rats were administered via gavage 0, 0.03, 0.3, or 3 mg/kg/day heptachlor in corn oil on gestational day 12 through postnatal day 7; pups were also exposed from postnatal day 7 to 21 or 42. The liver, kidneys, adrenals, thymus, spleen, ovaries, uterus/vagina, testes, epididymides, seminal vesicles/coagulating glands, and ventral and dorsolateral prostate were histologically examined in the offspring on postnatal day 46. Neurological (functional observational battery tests, motor activity, passive avoidance tests learning and memory, and Morris water maze test to assess spatial and working memory) and immunological (splenic lymphoproliferative responses to T cell mitogens, and to allogeneic cells in a mixed lymphocyte reaction, primary immunoglobulin M (IgM) antibody response to sheep red blood cells, examination of splenic lymphocytes subpopulations, and delayed-type and contact hypersensitivity) function tests were performed on the offspring exposed until postnatal day 42; neurological function tests were also performed on offspring exposed until postnatal day 21. Reproductive assessment included evaluation of vaginal opening (index of female puberty) and prepuce separation (index of male puberty) beginning at

postnatal days 25 and 35, respectively. The offspring were mated with an untreated mate and the dams were allowed to rear the first litter to postnatal day 10. The results of the neurobehavioral assessment were reported by Moser et al. (2001); the remaining results were reported by Smialowicz et al. (2001).

No significant alterations in maternal body weight, number of dams delivering litters, litter size, or pup survival were observed. Additionally, no alterations in pup growth rates, age at eye opening, anogenital distance, or age at vaginal opening or preputial separation were observed. A significant decrease in pup body weight at postnatal day 1 was observed at 3 mg/kg/day; this effect was not observed at postnatal days 7, 14, or 21. No consistent, statistically significant alterations in offspring body weights were observed at post natal days 21, 28, 35, or 42. Significant alterations in absolute and relative liver weights were observed in males and females exposed to 3 mg/kg/day; increases in absolute and relative ovary weights were also observed at 3 mg/kg/day. No histological alterations were observed in the examined tissues. No alterations in fertility were observed in the adult males and females mated to untreated partners, and no effects on soft tissue or gross body structure of the offspring (F_2 generation) were observed. No alterations in sperm count or sperm motility were observed.

A dose-related, statistically significant suppression of primary IgM antibody response to sheep red blood cells (sRBC) was found in male offspring, but not females. The primary IgM response to sRBCs was reduced in 21-week-old males exposed to 0.3 mg/kg/day. A second immunization with sRBCs administered 4 weeks later resulted in a significant reduction in IgG antibody response in males administered 0.03, 0.3, or 3 mg/kg/day heptachlor; no response was seen in females. A decrease in the $OX12^{+}OX19^{-}$ (i.e., B/plasma cells) population was also found in the spleen of males exposed to 3 mg/kg/day. No alterations in the following immunological parameters assessed at 8 weeks of age were found: lymphoid organ weights, splenic NK cell activity, splenic cellularity or cell viability, and lymphoproliferative responses of splenic lymphocytes to T-cell mitogens ConA and phytohemagglutinin (PHA) or to allogenic cells in the mixed lymphocyte reaction. The results of this portion of the study suggest that exposure to heptachlor adversely affects the development of the immune system.

Righting was significantly delayed in the female offspring of rats exposed to 3 mg/kg/day heptachlor; no significant alterations were observed in the male offspring. The investigators suggested that this was due to a delay in the ontogeny of righting rather than an inability to perform the task. The following significant alterations in the functional observation battery (FOB) and motor activity tests were found in the offspring dosed until postnatal day 21: increased open field activity in 3 mg/kg/day males, non-dose-related increased activity in figure-eight chambers in females (significant only in 0.03 mg/kg/day group),

and faster decline in habituation of activity in 3 mg/kg/day males. Alterations in the offspring dosed until postnatal day 42 included: increased levels of urination in males in the 0.03 and 0.3 mg/kg/day groups, increased landing foot splay in males in the 0.03 mg/kg/day group, and removal reactivity in males and females in the 0.03 mg/kg/day group. No alterations in the passive avoidance test were observed in the offspring exposed until postnatal day 21; in those exposed until postnatal day 42, an increase in the number of nose pokes was observed in all groups of females. No significant alterations in performance on the water maze test were found in the offspring exposed until postnatal day 21. In those exposed until postnatal day 42, increases in latency to find the platform were observed in males and females exposed to 3 mg/kg/day and increases in the time spent in the outer zone were found in males exposed to 0.3 or 3 mg/kg/day. In the water maze memory trial, no differences in performance were found between controls and animals exposed until postnatal day 21. Alterations in significant quadrant bias were observed in 0.03, 0.3, and 3 mg/kg/day males during the first probe test and in 0.3 and 3 mg/kg/day males and 3 mg/kg/day females in the second probe test. The study investigators noted that the heptachlor-exposed rats did not develop an efficient search strategy for locating the platform; they spent more time circling the outer zone of the tank. By the second week of the test, control rats had learned to venture into the zone where the platform was located.

The Smialowicz et al. (2001) and Moser et al. (2001) study identified a LOAEL of 0.03 mg/kg/day for developmental immunological and neurological effects. These alterations were considered to be minimally adverse and suggestive of immunotoxicity and neurotoxicity. An intermediate-duration oral MRL was calculated by dividing the minimal LOAEL of 0.03 mg/kg/day by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The resulting MRL is 0.0001 mg/kg/day.

There is a limited publicly available database on the chronic oral toxicity of heptachlor. In a multigeneration study conducted by Mestitzova (1967), decreases in litter size, increased postnatal mortality, and increased occurrence of lens cataracts (observed in F_0, F_1, and F_2 generations) were observed at a heptachlor dose of 6 mg/kg/day. Because the only reliable chronic-duration study identified a serious LOAEL at the lowest dose tested, a chronic-duration MRL was not derived for heptachlor.

Heptachlor Epoxide. Publicly available data on the toxicity of heptachlor epoxide are limited to an LD_{50} study (Podowski et al. 1979), and a dominant lethal study (Epstein et al. 1972). Neither of these studies is suitable for derivation of MRLs for heptachlor epoxide.