2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO HEXACHLOROBENZENE IN THE UNITED STATES

Hexachlorobenzene is a fully chlorinated hydrocarbon industrial chemical that is practically insoluble in water, but is very soluble in fat, oils, and organic solvents. Hexachlorobenzene is one of the most persistent environmental pollutants, and bioaccumulates in the environment, in animals, and in humans. It is not currently manufactured as a commercial product in the United States, and virtually all commercial production ended in the late 1970s. However, some hexachlorobenzene is produced as a by-product or impurity in the manufacture of chlorinated solvents and other chlorinated compounds (e.g., carbon tetrachloride, chlorophenols, mirex, tetrachloroethylene, trichlorobenzenes, trichloroethylene, trichlorotoluenes, and vinyl chloride), including several pesticides currently in use (pentachloro-nitrobenzene, chlorothalonil, Dacthal®, picloram, pentachlorophenol, atrazine, simazine, and lindane). It is estimated that 3,500–11,500 kg of hexachlorobenzene were inadvertently produced in the manufacture of chlorinated solvents in 1984. There are no current commercial uses of hexachlorobenzene in the United States, although hexachlorobenzene was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains until 1984, when its registration as a pesticide was voluntarily canceled. Hexa-chlorobenzene had also been used in the production of pyrotechnic and ordinance materials for the military and in the production of synthetic rubber.

The general population is not likely to be exposed to large amounts of hexachlorobenzene, but some exposure is likely, as many studies have detected small amounts in food and air samples. Traces of hexa-chlorobenzene have been found in almost all people tested for hexachlorobenzene or its metabolites. These amounts of hexachlorobenzene are most likely the result of consumption of low levels in food, with a estimated yearly uptake of 68, 22, and 5 μg for adults, toddlers, and infants, respectively. Other sources of exposure may include contact with contaminated soil and air. Hexachlorobenzene has been detected in agricultural soil at levels up to 440 ng/g, and in lake sediment at #15 ng/g. Ambient air samples have usually been reported to range from 0.1 pg/m^3 to 1.5 ng/m^3. Hexachlorobenzene has a very low solubility in water, so exposure by water is not likely to be significant; ambient water samples have usually been below 0.1 parts per trillion (ppt).

Children are expected to be exposed to hexachlorobenzene by the same routes as adults. Additionally, if hexachlorobenzene is present in their mothers, unborn children may be exposed through the placenta and

nursing children may be exposed to hexachlorobenzene present in milk. Human milk samples from the general population found hexachlorobenzene concentrations in the range of 11–70 ng/g fat.

See Chapter 6 for more detailed information regarding concentrations of hexachlorobenzene in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

Hexachlorobenzene is a toxic organochlorine that has been shown to cause death, systemic (e.g., liver, skin, bone, and thyroid), neurological, developmental, endocrine, and immunological toxicity in humans. Animal studies have demonstrated that hexachlorobenzene causes reproductive toxicity and increases the risk for cancer formation. The most sensitive target organs for hexachlorobenzene are the liver, ovary, and central nervous system.

A limited number of occupational studies have associated inhalation of hexachlorobenzene with liver effects (strongly with increased porphyrins and weakly with hepatocellular carcinoma), immunological effects (decreased neutrophil activity, increased immunoglobulins, and susceptibility to infection), and renal effects (microproteinuria) Queiroz et al. 1997, 1998a; Richter et al. 1994; Selden et al. 1999). Most data for the inhalation effects of hexachlorobenzene in humans were presented by studies of workers from an organochlorobenzene factory and the residents of a nearby rural town (Flix, Spain). Exposure to hexachlorobenzene (primarily airborne) pollution has been linked with elevated blood levels of hexachlorobenzene and hepatic effects (increased porphyrins and hepatic enzymes), thyroid effects (decreased thyroxine levels; weakly with hypothyroidism, goiter, and thyroid cancer), and impaired development of locomotor skills in infants.

Striking epidemiological evidence was found in studies of a population orally exposed to hexachlorobenzene in southeast Anatolia, Turkey. In the 1950s, widespread ingestion of bread made from grain that had been treated with hexachlorobenzene as a pesticide caused an epidemic in this region. The ingested dose of hexachlorobenzene was estimated to be in the range of 0.05–0.2 g/day, equivalent to 0.7–2.9 mg/kg/day for an average person. An extremely high (95%) rate of mortality occurred in infants under 2 years of age who had been breast fed by mothers who had ingested the contaminated bread. Poisoned infants displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (annular erythema). The infant deaths were primarily associated with cardiorespiratory failure secondary to this disease. Other clinical symptoms in these infants included weakness and

convulsions. A disease called *kara yara* or "black sore" was observed most frequently in children between the ages of 6 and 15 years, although some younger children and adults were also affected. It appeared after approximately 6 months of exposure; symptoms included photosensitivity, skin fragility (causing ulcers and scarring), hyperpigmenntation, and hirsutism. There was a 10% mortality rate among *kara yara* patients. These skin lesions were diagnosed as porphyria cutanea tarda, a specific type of vesiculobullous porphyria. The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins, which may cause tissue damage, especially in the skin. The human studies and supporting animal studies have clearly demonstrated that hexachlorobenzene causes porphyria.

Other symptoms diagnosed in this hexachlorobenzene-exposed population were: loss of appetite, weakness, arthritis (a swelling and spindling of the fingers, but with little pain), hepatomegaly, enlarged thyroid, and inability to perform simple, everyday activities such as handling eating utensils, rising from a squat, and climbing stairs. Clinical findings persisted in most subjects, including high porphyria, dermal lesions, multiple neurological effects, skeletomuscular effects, enlarged liver, and enlarged thyroid. In adult pregnant women who had been exposed as children, suggestive (but not conclusive) evidence of elevated incidences of miscarriages and stillbirths was found. Similar irreversibility has been seen in animal studies: developmentally exposed rats exhibited a significantly increased response to startling as adults and acutely exposed rats still exhibited porphyria more than 500 days after exposure.

No studies were located regarding health effects in humans or animals following dermal exposure to hexachlorobenzene. However, an acute study in rats suggested that hexachlorobenzene can be absorbed across the skin.

The primary target systems for hexachlorobenzene are hepatic toxicity, reproductive toxicity, developmental toxicity, and carcinogenesis; these are discussed below. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for more detailed information and discussions of additional effects.

Hepatic Effects. The most consistently identified effect following exposure of humans or animals to hexachlorobenzene is porphyria. The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins (see Section 3.5, Mechanisms of Action). The build-up of high levels of porphyrins in the body is known to cause liver (including cirrhosis, siderosis [accumulation of iron], focal necrosis, hyperplasia), and kidney (renal

failure) damage. Additionally, phototoxicity occurs as porphyrins accumulated in the skin are activated by sunlight to generate reactive oxygen species, causing tissue damage. As a result, skin lesions occur most commonly on areas exposed to sunlight, such as the hands and face. Available data also suggest that porphyrins may activate the immune system. Porphyria, diagnosed by the presence of high levels of porphyrins in the blood, feces, or urine, has been detected following exposures to hexachlorobenzene in workers, in the residents of Flix, Spain (primarily inhalation exposures resulting from a nearby organochlorine factory), and in the Turkish epidemic (oral exposure with contaminated grain). Exposed patients from the Turkish epidemic also exhibited hepatomegaly.

Several studies in rats and mice have observed porphyria, but no clear relationship has been established between porphyria and other hepatic effects seen in animals, such as peribiliary lymphocytosis and fibrosis, hepatomegaly and increased liver weight, enzyme induction, and degenerative pathological changes.

Reproductive Effects. Although no reliable evidence of reproductive toxicity has been observed in humans, the reproductive performance of rats has been adversely affected at doses as low as 16 mg/kg/day of hexachlorobenzene (decreased fertility, increased numbers of stillborn pups).

Distribution studies have identified the ovaries as a site of hexachlorobenzene accumulation, and intermediate-duration exposures to doses of hexachlorobenzene as low 0.01 mg/kg/day have been shown to cause ovarian lesions in adult female Cynomolgus monkeys. At higher doses, studies in Cynomolgus monkeys, Rhesus monkeys, and rats have reported changes in organ weight, degenerative changes, and disruptions in steroidogenesis (estrogen and progesterone).

Developmental Effects. Human and animal studies have demonstrated that hexachlorobenzene crosses the placenta to accumulate in fetal tissues and is transferred in breast milk.

The poisoning epidemic in Anatolia, Turkey, demonstrated that hexachlorobenzene is a developmental toxin. Children exposed under 2 years of age were the most susceptible (95% mortality, skin lesions). However, children under 15 were also more susceptible than adults, and exhibited both immediate (10% mortality, skin lesions) and persistent (dermal, neurological, skeletomuscular, hepatic, and thyroid effects) symptoms. Exposure for adults was estimated at 0.05–0.2 g/day of hexachlorobenzene (in bread made from contaminated grain) between 1955 and 1959.

Other human studies investigating developmental toxicity have been limited by small study size and low levels of hexachlorobenzene exposure; they have found suggestive evidence of an increased risk of undescended testis and impaired development of locomotor skills in newborn babies.

Animal studies have verified that hexachlorobenzene impaired neurological development and reduced neonatal viability and growth. Although an increased risk of undescended testis has not been observed in animals, the occurrence of cleft palate, renal agenesis, and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene.

Cancer. Among the general population, case-control studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence of breast or other cancers. Data from men exposed to hexachlorobenzene by inhalation (occupationally or as a result of nearby air pollution from an organochlorine factory in Flix, Spain) provide weak evidence for an association between hexachlorobenzene exposure and cancer of the liver, thyroid, and brain. Because hexachlorobenzene produces porphyria, it is noteworthy that several human studies have associated porphyria with the increased incidence of liver cancer.

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumor formation. The evidence of carcinogenicity is strongest in the liver; hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumors (hepatoma in mice and rats; hemagniohepatoma and bile duct adenoma in rats), and malignant tumors (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas (in rats, mice, and hamsters); lymphosarcomas (in rats, mice, and hamsters); adrenal hyperplasia and pheochromocytoma (in rats); parathyroid adenomas (in rats); and hemangioendothelioma and thyroid tumors (in hamsters). In the Ninth Report on Carcinogens, the NTP classified hexachlorobenzene as *reasonably anticipated to be a human carcinogen*. The EPA classified hexachlorobenzene as a *probable human carcinogen*, Group B2, on the basis that oral administration of hexachlorobenzene has been shown to induce tumors in the liver, thyroid, and kidney in three rodent species. IARC has classified hexachlorobenzene as possibly carcinogenic to humans (Group 2B), based on inadequate evidence in humans and sufficient evidence in experimental animals for carcinogenicity. For more information, see Sections 3.2.1.7 and 3.2.2.7, Cancer.

2.3 MINIMAL RISK LEVELS (MRLs)

Inhalation MRLs

Although limited evidence identified organs (liver, kidneys) and systems (endocrine, neurological and immune) that may be targets of hexachlorobenzene toxicity following inhalation exposures in humans and animals, these data are qualitatively and quantitatively inadequate for use in developing acute, intermediate, or chronic inhalation MRLs for hexachlorobenzene.

Oral MRLs

- An MRL of 0.008 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) for hexachlorobenzene.

This MRL is based on a critical evaluation of a developmental study (Goldey and Taylor 1992) that observed a lowest-observed-adverse-effect level (LOAEL) of 2.5 mg/kg/day for hyperactivity in offspring rats. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

Human data have shown that the developing central nervous system is a target of hexachlorobenzene toxicity. Many breast-fed infants of mothers who ingested hexachlorobenzene-contaminated bread during an epidemic of hexachlorobenzene poisoning in Turkey between 1955 and 1959 showed symptoms of *pembe yara*, which included weakness, convulsions, and annular erythema prior to death (Cripps et al. 1984; Peters et al. 1982, 1987). In children exposed (at an average age of 7 years) to hexachlorobenzene-contaminated grain, neurological effects persisted into adulthood (weakness, paresthesia, sensory shading, myotonia, and cogwheeling [irregular jerkiness of movement due to increased muscle tone as seen in Parkinson's disease]) (Cripps et al. 1984; Peters et al. 1982). Additionally, a preliminary report of infants from Flix, Spain, found an association between high hexachlorobenzene levels in milk and blood and impaired development of locomotor skills (Sala et al. 1999a).

Toxicology experiments have identified the same targets in developing animals. A developmental study in rats (Goldey and Taylor 1992) provided the most appropriate data from which to derive an acute-duration oral MRL for hexachlorobenzene. Virgin female rats were fed 2.5 or 25 mg/kg/day hexachlorobenzene for 4 days, 2 weeks prior to mating with unexposed males, and the developmental neurotoxicity of hexachlorobenzene was assessed in offspring using a battery of tests (Goldey and Taylor 1992). Pups

treated with either dose reoriented themselves significantly more quickly in a negative geotaxis test (on postnatal days 6 and 8), required less time in an olfactory discrimination test, and demonstrated increased exploratory activity in a motor activity test (on postnatal days 9–11). No significant effects on learning (swim T-maze) or motor activity (measured in older offspring on postnatal days 40 and 50, respectively) were detected. Hexachlorobenzene-exposed offspring at the 25 mg/kg/day dose level exhibited significantly altered acoustic startle responses (decreased at 23 days of age and increased at 90 days of age compared to controls). Thus, the study identified a LOAEL of 2.5 mg/kg/day for hyperactivity in the offspring rats, and this was the most sensitive LOAEL identified for the acute toxicity of hexachlorobenzene.

Other animal studies have demonstrated that exposure to hexachlorobenzene can produce neurological effects. In rats exposed prenatally through adulthood, decreased operant learning ability ("post-reinforcement pause" and "index of curvature") was observed on postnatal day 150 (Lilienthal et al. 1996). Moreover, oral exposure to hexachlorobenzene has also been shown to interfere with the function of the nervous system, inducing mild reduction in conduction velocity of the sciatic nerve and denervation (fibrillations, chronic repetitive discharges) (Sufit et al. 1986), hyperexcitability, tremors, muscle fasciculations, clonic convulsions, ataxia, lethargy, and paralysis in adult rats (Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Koss et al. 1978; Nikolaev et al. 1986; Ockner and Schmid 1961); convulsions, tremors, and progressive weakness in litters of female rats (Cripps 1990); tremor in adult C57B1/6J mice (Hahn et al. 1988); dysrhythmic electroencephalogram in adult Beagle dogs (Sundlof et al. 1981); severe tremors and muscular weakness in adult Rhesus monkeys (Knauf and Hobson 1979); hypoactivity, lethargy, and ataxia in infant Rhesus monkeys (Iatropoulos et al. 1978); and tremors, panting, and unsteady gait in adult SPF pigs (Den Tonkelaar et al. 1978).

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

This MRL is based on a LOAEL of 0.01 mg/kg/day for minimal ovarian effects in monkeys (Babineau et al. 1991). An uncertainty factor of 90 was used (3 for extrapolation from monkeys to humans, 10 for human variability, and 3 for use of a minimal LOAEL). Ultrastructural studies of ovaries collected from monkeys (Babineau et al. 1991; Bourque et al. 1995; Jarrell et al. 1993) provide the most appropriate data for use deriving an intermediate oral MRL for hexachlorobenzene. Female Cynomolgus monkeys were fed doses of 0.01–10 mg/kg/day of hexachlorobenzene in glucose in gelatin capsules for 90 days; the studies focused exclusively on end points relevant to reproductive toxicity (ovarian function and

histopathology). The LOAEL of 0.01 mg/kg/day for reproductive effects is the most sensitive LOAEL for the intermediate toxicity of hexachlorobenzene (Babineau et al. 1991).

Two early studies (Babineau et al. 1991; Jarrell et al. 1993) used doses of 0.1, 1, and 10 mg/kg/day of hexachlorobenzene. In all treated animals, hexachlorobenzene decreased the total number of oocytes and primary follicles, caused oocyte necrosis and follicular degeneration, and induced histopathological changes in the ovarian epithelium (cell stratification; decreased nuclear membrane distinction; increased density and granularity of oocyte nuclei; and increased numbers of aggregated lysosomes, vesicles, and vacuoles). The severity was dose-dependent.

The Bourque et al. (1995) follow-up study extended the observation of ultrastructural effects in the ovary to 0.01 mg/kg/day. At this dose, mitochondria in developing follicles were condensed and deformed. At higher doses (up to 10 mg/kg/day), the mitochondria were progressively more damaged and other changes were noted, such as indentation of nuclear membranes and abnormal accumulation of lipid in the cytoplasm of follicular cells. Because these effects were not associated with changes in oocyte fertility (measured *in vitro*), they were considered minimally adverse. Thus, these studies identify a LOAEL of 0.01 mg/kg/day for minimal reproductive effects in the treated monkeys.

Other monkey studies have also observed evidence of ovarian effects. In female Cynomolgus monkeys given capusles with at least 0.1 mg/kg/day of hexachlorobenzene for 90 days, a dose-related decrease in serum progesterone levels during the luteal (but not follicular and periovulatory) phase of the menstrual cycle, lengthening of the menstrual cycle, ultrastructural changes in surface epithelium of the ovary (indicative of cellular degeneration), and changes in ovary surface epithelial cell shape (length to width ratio) were detected (Foster et al. 1992a; Sims et al. 1991). In female Rhesus monkeys, gavage doses of at least 64 (but not lower doses up to 32) mg/kg/day of hexachlorobenzene for 60 days induced degenerative changes of the ovarian follicle, stroma, and germinal epithelium (Iatropoulos et al. 1976), and suggestive evidence of unusual steroidogenic activity (depressed serum potassium) was seen in Rhesus monkeys given 128 mg/kg/day for at least 60 days (Knauf and Hobson 1979).

Rat studies have confirmed the reproductive toxicity of hexachlorobenzene in the ovary. Increased serum progesterone levels and elevated ovarian weights were observed in superovulated female Sprague-Dawley rats orally administered $1 mg/kg/day hexachlorobenzene by gavage for 21 days (Foster et al. 1992b).

Super-ovulated (but not normal cycling) female Sprague-Dawley rats gavaged with 50 mg/kg/day of hexachlorobenzene for 5 days exhibited significant elevation of serum levels of progesterone (Foster et al. 1993). In a subsequent study with ovariectomized female Sprague-Dawley rats, gavage doses of at least 1 mg/kg/day for 30 days significantly decreased circulating corticosterone and cortisol levels, without affecting levels of circulating aldosterone and progesterone levels or adrenal gland weight (Foster et al. 1995a). The investigators concluded that hexachlorobenzene exposure induces alterations in steroidogenesis of cells of the adrenal cortex inner zone.

Although clear evidence of reproductive toxicity has not been observed in humans, suggestive data indicated that hexachlorobenzene may increase the risk for spontaneous abortion (miscarriages and stillbirths). Therefore, the intermediate-duration oral MRL is considered relevant to human health.

- An MRL of 0.00005 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) for hexachlorobenzene.

This MRL was based on a critical evaluation of a multigenerational study (Arnold et al. 1985), which observed a LOAEL of 0.016 mg/kg/day for hepatic effects in F_1 male rats. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

Although studies designed to detect liver histopathology have not been conducted in humans, epidemiological studies have detected hepatic effects as indicated by increased porphyrin and enzyme levels in people exposed for chronic durations to hexachlorobenzene (Herrero et al. 1999; Sala et al. 1999b; Seldon et al. 1999). Analysis of the available human and animal chronic oral toxicity data for hexachlorobenzene indicated that a 130-week study in rats reported by Arnold et al. (1985) provided the most appropriate data for use in the development of an oral chronic MRL for hexachlorobenzene. Briefly, male and female weanling Sprague-Dawley rats were fed 0, 0.016, 0.08, 0.4, or 2 mg/kg/day of hexachlorobenzene for 3 months prior to mating and through weaning of F_1 offspring, when they were sacrificed. The F_1 offspring were continued on their parents' diet from weaning throughout the remainder of their lives (130 weeks). Statistically significant increases in the incidences of peribiliary lymphocytosis and fibrosis were observed in the livers of male rats at $0.016 mg/kg/day in the F_1 generation lifetime study. Significant dose-dependent trends were also found for hepatic basophilic chromogenesis at $0.4 mg/kg/day in both genders of rats. The 2 mg/kg/day dose group rats also exhibited increased pup mortality (in both genders) and severe chronic nephrosis (in males only).

The increased incidences of peribiliary lymphocytosis and fibrosis in treated males were considered to represent a minimal effect. These are common spontaneous lesions in aging rats and occurred in approximately 30% of controls in this study. For peribiliary fibrosis, incidence was increased in all treated groups (statistically significant in the 0.016 and 2 mg/kg/day groups), but there was no clear evidence of a dose-response (13/48, 23/48, 21/48, 21/49, and 23/49, respectively, in the control, 0.016, 0.08, 0.4, and 2 mg/kg/day groups). For peribiliary lymphocytosis, the incidence was increased in all treated groups (statistically significant in the 0.016, 0.08, and 2 mg/kg/day groups), and the trend was also statistically significant (16/48, 27/48, 26/48, 21/49, and 32/49, respectively, in the control, 0.016, 0.08, 0.4, and 2 mg/kg/day groups). Because incidences of these lesions in the control and treated females were similar to the control males (ranging from 6/49 to 14/49), the incidence levels in control males do not appear unusually low. Overall, these findings suggest that hexachlorobenzene produced a minimal hepatic effect in male rats at the lowest doses administered by increasing the incidence of age-related hepatic lesions. The LOAEL of 0.016 mg/kg/day reported in this study for hepatic effects is the most sensitive LOAEL for the characteristic chronic toxicity of hexachlorobenzene, liver toxicity. However, the study did not identify a no-observed-adverse-effect level (NOAEL).

Numerous animal studies have clearly demonstrated that the liver is a major target organ of hexachlorobenzene exposure. Short-term experiments in rats have observed increased liver weight, increased hepatic porphyrins, liver histopathology (cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes), and increased serum cholesterol (Krishnan et al. 1991; Lecavalier et al. 1994; Rajamanickam and Padmanaban 1974; Richter et al. 1981). Intermediate- and chronic-duration experiments in rats have observed these and other signs, including additional liver histopathology (degeneration, hypertrophic hepatocytes with eosinophilic cytoplasm with thready basophilic structures, as well as inflammatory cell infiltrates), decreased liver retinoid levels, and elevated liver enzymes (Andrews et al. 1989, 1990; Den Besten et al. 1993; Elder and Urquhart 1986; Kennedy and Wigfield 1990; Koss et al. 1978, 1983; Kuiper-Goodman et al. 1977; Ockner and Schmid 1961; Smith and Cabral 1980; van Raaij et al. 1993b). Similar effects have been observed in intermediate-duration experiments in Rhesus monkeys (Iatropoulos et al. 1976; Knauf and Hobson 1979), beagle dogs (Sundlof et al. 1981), and pigs (Den Tonkelaar et al. 1978).