MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

TO: File for Hexachlorobenzene (CAS No. 118-74-1)

FROM: Robert Sills, Toxics Unit Supervisor, Air Quality Division

SUBJECT: ITSL Basis (and Attached: IRSL and SRSL basis)

DATE: October 5, 2017

The Initial Threshold Screening Level (ITSL) for hexachlorobenzene (HCB) is 0.35 ug/m^3 with a 24 hour averaging time (AT). This represents a change from the previous ITSL of 0.035 ug/m^3 (24 hour averaging time; see Attachment 1).

As described in Attachment 1, the IRSL and SRSL for HCB are 0.0022 ug/m^3 and 0.22 ug/m^3, respectively, with annual averaging time.

The current screening level review focused on the ITSL. As with the previous assessment (Attachment 1), this review found a lack of an EPA RfC, ATSDR (2015) inhalation MRLs, or a Texas (TCEQ) Effects Screening Level (ESL). EPA (1988) provides an RfD of 8E-4 mg/kg/day based on chronic exposure and the critical effect of liver toxicity. However, EPA (2010) and ATSDR (2015) have more recently derived short-term benchmarks that are more restrictive than this EPA (1988) chronic RfD, based on the critical effect of reproductive toxicity. EPA (2010) derived a subchronic provisional RfD (PPRTV) for the Superfund program of 1E-5 mg/kg/day, and ATSDR (2015) derived an intermediate duration oral Minimal Risk Level (MRL) of 1E-4 mg/kg/day.

Both of these benchmarks (of EPA (2010) and ATSDR (2015)) are based on a key study by Bourque et al.(1995). In this study, twenty 6- to-13 year old cynomolgous monkeys were randomly assigned to five groups, and received HCB in capsules at doses of 0, 0.01, 0.1, 1.0, and 10 mg/kg b.w. for 13 weeks. Ultrastructural changes of ovarian follicles were then evaluated. Lesions were observed in the follicles of monkeys given all concentrations of HCB, including condensed mitochondria in the developing ova and follicular cells that contained nuclei with deep indentations and abnormal accumulation of cytoplasmic lipid droplets. The most sensitive organelle appeared to be the mitochondria, which were condensed, with abnormal intracristal spaces in the lower-dosage groups and marked degeneration in the 10 mg/kg group. These effects appeared in a dose-related manner. The LOAEL was the lowest dose group, 0.01 mg/kg/day, with mitochondria that appeared condensed with swollen cristae and abnormal intracristae spaces, and, a few follicular cells contained abnormal nuclei. The authors stated that the effects in the ova in the lower-exposure groups were typical of reversible cellular injury; only a small fraction of the mitochondria observed appeared

normal. They also stated that, "…mitochondrial alterations usually represent nonspecific manifestations of cell injury and cannot be directly related to a specific cellular response or class of disease. Nevertheless, mitochondria of the developing follicles are adversely affected after exposure to HCB; that, in turn, is likely to impair fecundity of the animal." "In human beings, it is hypothesized that increased rate of atresia of primordial follicles caused by this pollutant may be a cause of early onset of menopause." (Bourque, 1995). A NOAEL was not established. Previously, Jarrell et al. (1993) reported that low exposures of cynomolgous monkeys to HCB can decrease the total number of primordial follicles; they found a LOAEL of 0.1 mg/kg b.w./day with a dose-related toxic effect in primordial germ cells at this lowest dose despite no evidence of systemic or hepatic effects. They did not find effects on % fertilization at doses up to and including the highest dose of 10 mg/kg/day, although they did find a significantly decreased number of primordial follicles at this high dose. "The increased levels of HCB correlated with a lesion of the primordial germ cell best viewed ultrastructurally. This degeneration was present in all oocytes at the 0.1 mg/kg/day dosage and became progressively more severe with higher dosages. At 10 mg/kg/day there was an actual reduction in the total number of primordial follicles in the ovary….the fact that fertilization rates were unchanged in response to HCB suggest that either all oocytes are not equally sensitive to HCB or are not equally exposed to the administered chemical within the ovary." (Jarrell et al., 1993).

As indicated above, ATSDR (2015) derived an intermediate duration oral MRL for HCB at 1E-4 mg/kg/d based on the Bourque et al (1995) and Jarrell et al. (1993) studies. The ATSDR (2015) noted that the Bourque et al. (1995) 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. ATSDR (2015) regarded this as a LOAEL and applied a UF_L = 3 to account for the lack of a NOAEL in this study, along with UF_A = 3 for extrapolation from animals (monkeys) to humans, and a UF_H = 10 for human variability; the total UF_T was thus 90. The point of departure for the intermediate duration MRL was thus the LOAEL of 1E-2 mg/kg/day, and with the application of UF_T = 90, the resulting MRL = 1E-4 mg/kg/day (after rounding).

As indicated above, EPA (2010) used the same key study (Bourque et al., 1995) and LOAEL = 0.01 mg/kg/day in derivation of the provisional RfD (PPRTV) = 1E-5 mg/kg/day. They differed from ATSDR's approach by applying 10-fold UFs for each UF_H, UF_A, and UF_L, for a total UF_T = 1000.

It is also noted that ATSDR (2001) derived a TLV-TWA = 2 ug/m^3 for HCB, based on minimizing the potential for increased formation and excretion of porphyrins leading to dermal lesions and ulcerations, neurotoxicity, and possible liver cancer. They did not identify developmental toxicity as a critical effect for the TLV derivation, and they did not cite Bourque et al. (1995) or Jarrell et al. (1993). A potential ITSL based on this TLV-TWA under Rule 232 (1)(c) would be TLV/100 = 0.02 ug/m^3 (8 hour averaging time).

The conclusion of this ITSL review is that reproductive toxicity is a critical short-term effect of HCB, and we concur with the conclusions of ATSDR (2015) and EPA (2010) that the ultrastructural effects seen at the lowest dose of Bourque et al. (1995) constitute a LOAEL of 0.01 mg/kg/day. The reduced UF_A and UF_L applied by ATSDR

(2015) appear appropriate, therefore the short term ITSL is derived from the ATSDR (2015) intermediate duration oral MRL of 1E-4 mg/kg/d under Rule 231(1)(b) with exposure route conversion, as follows:

$$\text{ITSL} = 1\text{E-}4 \text{ mg/kg/d} \times \frac{1000 \text{ ug}}{\text{mg}} \times \frac{70 \text{ kg}}{20 \text{ m}^3\text{/d}} = 0.35 \text{ ug/m}^3 \text{ (24 hour averaging time)}$$

This ITSL is well below the TLV-TWA of 2 ug/m^3 and is considered a more appropriate ITSL derivation in this particular case than a TLV/100. The ATSDR (2015) intermediate duration MRL and the ITSL focus on the critical effect of reproductive effects, while the TLV is stated to be based, in part, on consideration of potential carcinogenicity (ACGIH, 2001). Because the critical effect is reproductive, based on a subchronic duration key study, the appropriate averaging time is 24 hours rather than annual.

References:

ACGIH. 2001. Documentation of the TLVs and BEIs. 7th Edition. Hexachlorobenzene TLV-TWA.

ATSDR. 2015. Toxicological Profile for Hexachlorobenzene. US DHHS. August 2015.

Bourque, A.C., et al. 1995. Ultrastructural changes in ovarian follicles of monkeys administered hexachlorobenzene. Am J Vet Res 56 (12): 1673-1677.

EPA. 2010. Provisional Peer-Reviewed Toxicity Values for Hexachlorobenzene (CASRN 118-74-1). Superfund Health Risk Technical Support Center, NCEA, ORD.

EPA. 1988. IRIS database. Chemical file for hexachlorobenzene. Oral RfD last revised 9/26/1988. Retrieved 9/21/2017.

Jarrell, J.F. et al. 1993. Hexachlorobenzene toxicity in the monkey primordial germ cell without induced porphyria. Reproductive Toxicology 7: 41-47.

Attachment 1

Justification for the current IRSL and SRSL for Hexachlorobenzene, and superseded justification for the previous ITSL

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

To: File for Hexachlorobenzene (CAS # 118-74-1)

From: Doreen Lehner, Air Quality Division, Toxics Unit

Subject: Screening Level Determination for Hexachlorobenzene (CAS# 118-74-1)

Date: October 28, 2015

There was previously no initial threshold screening level (ITSL) for hexachlorobenzene. An ITSL is now being established at 0.035 µg/m^3 based on a 24-hour averaging time. The initial risk screening level (IRSL) is 0.0022 µg/m^3 based on an annual averaging time. The IRSL is based on an increase in hepatocellular carcinoma in female Sprague-Dawley rats exposed via diet for two years (Erturk et al., 1986).

Hexachlorobenzene (CAS # 118-74-1) also known as perchlorobenzene or pentachlorophenyl chloride, is an organochloride with the molecular formula C_6Cl_6 and a molecular mass of 284.40 g/mol. It is a white, crystalline solid that is soluble in halogenated solvents, esters, and hydrocarbons, but is not soluble in water. Hexachlorobenzene is a fungicide formerly used as a seed treatment, especially on wheat, but has been banned globally as it is a persistent organic pollutant. Hexachlorobenzene is an animal carcinogen and has been classified by the International Agency for Research on Cancer (IARC) as a Group 2B Carcinogen (possibly carcinogenic to humans).

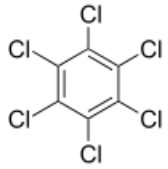

Figure 1. Chemical structure of hexachlorobenzene.

A literature review was conducted to determine the screening level: Chemical Criteria Database (CCD), United States Environmental Protection Agency (USEPA) Integrated Risk Information System (IRIS), National Institute for Occupational Safety and Health (NIOSH), American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values and Biological Exposure Indices (TLV/BEI) 2014 guide, National Toxicology Program (NTP) Study Database, International Agency for Research on Cancer (IARC) Monographs, National Library of Medicine (NLM) online, EPA Aggregated Computational Toxicology Resource (ACToR) Database, EPA Toxic Substance Control Act Test Submission Database (TSCATS), and the Registry for Toxic Effects of Chemical Substances (RTECS).

IRSL Discussion and Derivation

The USEPA has derived an estimate of carcinogenic risk from inhalation exposure to hexachlorobenzene with an inhalation unit risk (IUR) of 4.6 x10^{-4} (µg/m^3)$^{-1}$ based on a study by Erturk et al., (1986) Spague-Dawley rat 2-year oral bioassay. “Groups of 94 Sprague-Dawley rats/sex/dose were fed 0, 75, or 150 ppm hexachlorobenzene (purity >99.5%) in the diet for up to 2 years…. Interim kills four rats/group were performed at weeks 0, 1, 2, 3, 4, 8, 16, 32, 48, 64, and 80. The remaining 50 animals/group were observed until natural death or until sacrifice at two years. Treated animals of both sexes surviving past 12 months showed significant increases in liver and renal tumors. Hemangiohepatomas, hepatocellular carcinomas and bile duct tumors were significantly increased in treated females; males and females in both dose groups had increased incidences of renal cell adenomas and hemangiohepatomas. Females were far more susceptible to hepatocarcinogenicity while males were generally more sensitive to renal carcinogenicity. The time-to-tumor onset in each dose group was generally longer than one year. The increase in hepatocellular carcinomas and bile duct tumors in males was not statistically significant. In this same study hepatomas were reported in Syrian golden hamsters that had been exposed for at least 90 days to 200 or 400 ppm hexachlorobenzene in the diet and killed after varying observation periods” (USEPA, 1996).

The following table is a summary of results for hepatocellular carcinoma in female Sprague-Dawley rats, which was the most significant dose related toxicity endpoint for hexachlorobenzene.

Table 1. Dose-Response Data for Hepatocellular Carcinoma after Oral Administration of Hexachlorobenzene (EPA, 1996)

Administered Dose (ppm)	Human Equivalent Dose (mg/kg)/day	Tumor Incidence
0	0	0/52
75	0.73	36/56
150	1.46	48/55

The USEPA used a linearized, multistage model, with extra risk to derive the IUR of 4.6 x10^{-4} (µg/m^3)$^{-1}$. Rule 231(1) is used to develop an IRSL for hexachlorobenzene. The equation is below:

$$IRSL = \frac{1 \times 10^{-6}}{Unit\ Risk}$$

“Hexachlorobenzene is mutagenic for *Saccharomyces cerevisiae* (Guerzoni et al., 1976), but did not induce dominant lethal mutations in rats exposed by gavage (Simon et al., 1979); nor did it revert histidine auxotrophs of *Salmonella typhimurium* (Lawlor et al., 1979)” (USEPA, 1996). This gives some doubt as to whether hexachlorobenzene is carcinogenic by a mutagenic mode of action and therefore, age-dependent adjustment factors (ADAFs) will not be used at this time.

$$IRSL = \frac{1 \times 10^{-6}}{4.6 \times 10^{-4}} = 0.002173913\ {}^{\mu g}/_{m^3} = 0.0022\ {}^{\mu g}/_{m^3}$$

Rule 231(4) states that the averaging time for IRSLs and SRSLs is an annual averaging time. The initial risk screening level (IRSL) for hexachlorobenzene (CAS# 118-74-1) is 0.0022 µg/m^3 and the SRSL is 0.022 µg/m^3 based on an annual averaging time.

ITSL Discussion and Derivation

The USEPA was unable to derive a reference concentration (RfC) for hexachlorobenzene, but did derive a chronic oral exposure reference dose (RfD) of 0.0008 mg/kg/day in 1991 using the Arnold et al. (1985) study. ATSDR (2015) reviewed all relevant data on hexachlorobenzene and derived a chronic oral minimal risk level (MRL) of 0.00007 mg/kg/day based on the same study by Arnold et al., (1985).

ATSDR used a study by Arnold et al. (1985) where "groups of Sprague-Dawley rats (50 per sex) of the F_1 generation were exposed to dietary hexachlorobenzene at 0, 0.32, 1.6, 8 or 40 ppm (approximate doses of 0, 0.022, 0.11, 0.55, and 2.8 mg/kg/day, respectively, for the F_1 males and 0, 0.026, 0.13, 0.64, and 3.2 mg/kg/day, respectively for the F_1 females) from weaning for life (130 weeks). The groups of F_1 rats had also been exposed via their mothers during gestation and lactation. The F_1 rats in this study were sacrificed after the animals had been on test for 130 weeks. A total of 35 tissues and organs (including brain, heart, liver, extrahepatic bile duct, lungs, spleen, pancreas, small intestine, adrenals, kidneys, bladder, ovaries, uterus, skin, pituitary, thyroid, parathyroid, thymus, prostate, testes, and bone) were histopathologically examined" (ATSDR, 2015).

"Significant dose-response trends were observed in both sexes for hepatic basophilic chromogenesis at >0.55 mg/kg/day, and in males for peribiliary lymphocytosis and fibrosis at or greater than the lowest dose tested (0.022 mg/kg/day). Chronic nephrosis, severe in males, and reduced pup viability were observed at the high dose (2.8 mg/kg/day for males and 3.2 mg/kg/day for females). Tumors were also increased at the high dose, including neoplastic liver nodules in females, parathyroid adenoma in males, and adrenal pheochromocytoma in both males and females. No treatment related effects in the rat offspring were observed with respect to feed consumption or body weight" (ATSDR, 2015).

ATSDR used the increased incidences of peribiliary lymphocytosis and fibrosis in treated males as the 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 0.022 and 2.8 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 in the control, 0.022, 0.11, 0.55, and 2.8 mg/kg/day groups, respectively). For peribiliary lymphocytosis, the incidence was increased in all treated groups (statistically significant in the 0.022, 0.11, and 2.8 mg/kg/day groups), and while the trend with dose was not very impressive, it was statistically significant (16/48, 27/48, 26/48, 21/49, and 32/49, respectively). Incidences of these lesions in the control and treated females were similar to the control males (ranging from 6/49 to 14/49), suggesting that the incidence levels in control males were not 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" (ATSDR, 2015).

ATSDR derived their chronic oral MRL using 0.022 mg/kg/day as the LOAEL for peribiliary lymphocytosis and fibrosis of the liver in the F_1 male rats. An uncertainty factor of 300 was used (3 for LOAEL to NOAEL; 10 for extrapolation from animals to humans; and 10 for human variability) resulting in a chronic oral MRL of 0.00007 mg/kg/day. EPA derived their oral RfD using the NOAEL of 1.6 ppm (diet) [0.08 mg/kg/day] in the Arnold et al. (1985) for liver effects as the point of departure. EPA used an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability) resulting in an oral RfD of 0.0008 mg/kg/day. EPA's conversion from ppm to mg/kg/day were slightly different than ATSDRs conversion factors. EPA "doses were based on actual food consumption and body weights provided by Arnold at 30 weeks of

exposure (EPA, 1985, 1988)" (EPA, 1996). ATSDR used the "EPA (1988) chronic reference body weight (male: 0.523 kg; female: 0.338 kg) and food consumption (male: 0.036 kg/day; female 0.027 kg/day) for Sprague-Dawley rats were used to calculate hexachlorobenzene dose from concentration in food. Sample calculation for males: (0.32 mg hexachlorobenzene/kg food [0.32 ppm] x 0.036 kg food consumed/day)/0.523 kg body weight = 0.022 mg hexachloro-benzene/kg/day" (ATSDR, 2015). Even though EPA and ATSDR used the same study by Arnold et al. (1985), the difference in EPA's final RfD value and ATSDR's chronic oral MRL comes from the difference in: the food consumption conversion (EPA used both 1985 and 1988 guidance for conversions while ATSDR used only EPA 1988 guidance); the point of departure, EPA used a NOAEL of 1.6 ppm and ATSDR used a LOAEL of 0.32 ppm; and in the uncertainty factors used (EPA used an uncertainty factor of 100 while ATSDR used an uncertainty factor of 300). As ATSDR provided a more detailed explanation of their derivation and the specific liver effect for the point of departure (ATSDR used increased incidences of peribiliary lymphocytosis and fibrosis in treated males), the ATSDR chronic oral MRL of 0.00007 mg/kg/day value was deemed more appropriate for deriving a candidate ITSL.

According to Rule 232(1)(b) an ITSL can be derived from an oral reference dose (such as an RfD or MRL) using the following equation:

$$ITSL = Oral\ Reference\ dose\ \times \frac{70\ kg}{20\ m^3}$$

Where 70 kg is the default body weight for an average human and 20 m^3 is used to define the minute volume (default ventilation rate) for an average human. Taking the chronic oral MRL of 0.00007 mg/kg/day and using the above equation gives:

$$Candidate\ ITSL = 0.00007\ {}^{mg}/_{kg/day}\ \times \frac{70\ kg}{20 m^3} = 0.000245\ {}^{mg}/_{m^3} = 0.25\ {}^{\mu g}/_{m^3}$$

Therefore the candidate chronic ITSL is 0.25 µg/m^3. According to Rule 232(2)(b) a 24-hour averaging time period should be used, but as this ITSL is based on a full lifetime rat study (exposure occurred during gestation and lactation and from weaning for life [130 weeks]), it is appropriate to utilize a longer averaging time, which would be an annual averaging time. The candidate chronic ITSL for hexachlorobenzene is 0.25 µg/m^3 based on an annual averaging time. This candidate ITSL is protective of chronic liver effects from hexachlorobenzene, but is not protective for reproductive effects of short-term exposure to hexachlorobenzene discussed below. Therefore, this candidate ITSL will not be used.

Derivation of an acute ITSL

PPRTV subchronic oral exposure (SRfD)

Several subchronic studies were used by other regulatory agencies to derive risk assessment values for hexachlorobenzene. U.S. EPA (2010) developed a provisional peer reviewed toxicity value (PPRTV) for their superfund program: a reference dose for subchronic oral exposure (SRfD) of 0.00001 mg/kg/day based on a study by Bourque et al., (1995) where degenerative changes in primary ovarian follicles were detected in monkeys dosed with hexachlorobenzene orally via capsule for 13 weeks. "Female Cynomolgus monkeys (4/dose, 6-13 years of age) were administered hexachlorobenzene (purity not stated) mixed with glucose in gelatin capsules, daily, at doses of 0, 0.01, 0.1, 1.0 or 10 mg/kg-day for 13 weeks. Controls received only glucose. After the period of treatment, monkeys were given FSH and LH during Days 2 through 7 of the following menstrual period. On the eighth day of the cycle, HCG was given; an

ovary from each monkey was subsequently removed 35-38 hours later. Ovaries were sectioned, and primordial, primary, and growing follicles from controls and each hexachlorobenzene treatment group were examined by TEM" (EPA, 2010).

"Ultrastructural changes were noted in the ovarian follicles of all hexachlorobenzene-exposed monkeys (Bourque et al., 1995). Incidences of the observed effects were not given; a narrative description of the increasing severity of effects follows. In control monkeys, the developing ova had normal mitochondria that were typically distributed, and follicular cells surrounding the ova were also described as normal in shape and content of their nuclei. In ova of monkeys treated with hexachlorobenzene at a dose of 0.01 mg/kg-day, the majority of mitochondria were condensed with swollen cristae; follicular cells were generally unaffected, but "a few cells" contained abnormal nuclei. In ova of monkeys treated with hexachlorobenzene at a dose of 0.1 mg/kg-day, the mitochondria contained coarsely granular matrices and/or exhibited irregular shapes; many of the follicular cells contained abnormal nuclei (infolding of the nuclear membrane). In ova of monkeys treated with hexachlorobenzene at a dose of 1 mg/kg-day, mitochondria were condensed and swollen; in addition, herniation of the ooplasm (suggesting rupture of the zona pellucida) was noted, along with abnormal nuclei in follicular cells and abnormal spaces between follicular cells. In ova of monkeys treated with hexachlorobenzene at a dose of 10 mg/kg-day, the mitochondrial changes were more severe (many had electron-lucent matrices); in follicular cells, the nuclear membrane was highly folded with deep indentations, and there was an abnormal amount of lipid in the cells. Further, the cells of the theca folliculi of the stroma were affected (deformed nuclei) only in monkeys treated with 10 mg/kg-day. The study authors concluded that a NOAEL was not defined (Bourque et al., 1995). The LOAEL for this study is 0.01 mg/kg-day based on degenerative changes in primary and growing ovarian follicles" (USEPA, 2010). A total uncertainty factor of 1,000 was applied: a factor of 10 for animal-to-human extrapolation to account for toxicokinetic and dynamic differences between monkeys and humans; a factor of 10 to account for extrapolation to a potentially susceptible human subpopulation because data for evaluating a susceptible human response are insufficient; and a factor of 10 for extrapolation from a LOAEL to a NOAEL. The resulting subchronic RfD (PPRTV) is 1E-5 mg/kg/day (USEPA, 2010).

ACGIH TLV-TWA

After reviewing multiple studies, ACGIH recommended a threshold limit value time weighted average (TLV-TWA) of 0.002 mg/m^3 based two studies, one in monkeys and one in pigs. The first study considered was performed by Rozman et al. (1978) where "rhesus monkeys (3 male and 3 female) were dosed with 110 µg of HCB for 18 months. This dose (equivalent to 0.033 mg/kg/day) did not affect any of the monitored endpoints which included serum hormone levels, urinary copro- and uroporphyrin levels, and hematology (hemoglobin, hematocrit, white blood count, red blood count), thus representing a no-observed-effect level (NOEL). The second study ACGIH considered was performed by den Tonkelaar et al. (1978) where pigs received 0.05, 0.5, 5, or 50 mg of hexachlorobenzene (HCB)/kg/day for 90 days. "Animals at the highest dosage displayed clinical signs of porphyria cutanea tarda and died during the experiment. The clinical signs were not observed at lower dosages. Increased excretion of coproporphyrin was detected starting with the 0.5 mg/kg/day group. This increase was paralleled by induction of microsomal liver enzymes and increased liver weight in the 5 mg/kg/day group. Characteristic histopatho-logical changes were also noted in the liver. Concentration of HCB in blood and tissues was dose-dependently elevated. Under these experimental conditions a NOEL of 0.05 mg/kg/day was established" (ACGIH, 2001). ACGIH (2001) recommended the TLV-TWA of 2 ug/m^3 to minimize the potential for increased formation and excretion of porphyrins (porphyrogenicity) leading to dermal lesions and ulcerations, neurotoxicity, and possible liver cancer reported only in animals. They noted that, "A large database existed on reproductive/developmental toxicity of

HCB, including a four-generation study with no indication for reproductive toxicity or teratogenicity" (ACGIH, 2001). However, the study citations did not include Bourque et al. (1995), which was the key study utilized by the USEPA (2010) in deriving the SRfD PPRTV based on reproductive effects on monkey ovaries.

ATSDR acute oral MRL

ATSDR reviewed hexachlorobenzene and determined an acute oral minimal risk level (MRL) of 0.008 mg/kg/day based on a study by Goldey et al., (1992) where "groups of 30 virgin female Sprague-Dawley rats were dosed by gastric intubation for 4 days with 0, 2.5, or 25 mg/kg/day hexachlorobenzene to achieve a total dose of 0, 10, or 100 mg/kg/day for the 4-day period. Dosing was completed 2 weeks before breeding. The developmental neurotoxicity of hexachlorobenzene was assessed using a battery of behavioral tests. Negative geotaxic response was assessed in two male and two female pups from each litter on postnatal day (PND) 6, 8, and 10. Olfactory discrimination/homing was assessed in two male and two female pups from each litter on PND 9, 10, and 11. This test simultaneously measures sensory discrimination, motivation, and locomotor ability. The development of exploration and locomotion was assessed in whole litters between PND 15 and 20. Acoustic startle response (ASR) was assessed on PND 23 and 90. Visual discrimination learning, as measured in the water-filled T-maze, was assessed in offspring on PND 40. Motor activity in mature offspring (PND 60) was measured in an open area. These adult animals were again tested for exploratory activity on PND 100" (ATSDR, 2015).

"Hexachlorobenzene affected multiple pathways throughout the developing nervous system, manifested as slight hyperactivity, at a LOAEL of 2.5 mg/kg/day. The offspring rats showed faster response times in negative geotaxis and olfactory discrimination/homing tests at the 2.5 or 25 mg/kg/day maternal dose level. Offspring exposed to maternal doses of 2.5 or 25 mg/kg/day showed either significantly increased exploratory behavior, slight hyperactivity, or both during the early life (19-20 days of age). Hexachlorobenzene-exposed offspring at the 25 mg/kg/day dose level exhibited significantly decreased ASR (23-day-old pups). When rats were tested later as adults (90 days old), response amplitude was significantly elevated in males in both groups exposed *in utero* to 2.5 and 25 mg/kg/day, compared to controls. Maternal exposure of rats to hexachlorobenzene did not result in any significant changes in learning ability, locomotor activity (60-day-old offspring), or exploratory activity (100-day-old offspring)" (ATSDR, 2015).

There are three acute (or subchronic) benchmarks for hexachlorobenzene which are summarized in the following table.

Table 2. Hexachlorobenzene acute toxicity benchmarks and candidate acute ITSLs

Available Benchmark Type	Value	Candidate Acute ITSL (µg/m³)	Candidate ITSL Averaging Time	Candidate ITSL Derivation
PPRTV subchronic oral exposure (SRfD)	0.00001 mg/kg/day	0.035	24-hour	$Oral\ PPRTV \times \frac{70\ kg}{20\ m^3}$
ACGIH TLV-TWA	0.002 mg/m³	0.02	8-hour	$\frac{TLV}{100}$
ATSDR acute oral MRL	0.008 mg/kg/day	28	24-hour	$Oral\ MRL \times \frac{70\ kg}{20\ m^3}$

The ATSDR acute oral MRL of 0.008 mg/kg/day is the most recently derived acute value and used a key study (Goldey et al., 1992) which focused on the effects of early life stage exposure to hexachlorobenzene. ATSDR acute MRLs are applicable to a time period of 1-14 days, therefore an appropriate averaging time for a candidate ITSL based on an acute MRL is 24 hours. However, the EPA (2010) SRfD PPRTV derivation focused on a more sensitive reproductive effect. The candidate ITSL based on the ACGIH TLV is a more restrictive value (0.02 ug/m³) with a shorter (8-hour AT), however, the justification indicates that carcinogenicity was one of the factors accounted for in the TLV derivation. The ITSL is being established at 0.035 µg/m³ based on a 24-hour averaging time to ensure protection from the reproductive effects of HCB, consistent with the EPA (2010) SRfC PPRTV.

References:

ACGIH. 2001. Hexachlorobenzene. TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. ACGIH Worldwide Publications.

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