Peer Review Charge for

Hexachlorobutadiene Toxicological Profile - Draft for Public Comment

Reviewer #3

This toxicological profile is an update of a previous one published in 1994. This update has focused on Chapter 2, Health Effects, and resulted in the derivation of a new acute-duration oral MRL; a new oral intermediate MRL. The intermediate MRL was derived based on the reclassification of an endpoint from a LOAEL to a NOAEL

Thus, we would like for you to focus on the health effects section (Chapter 2), and the MRL issues presented below.

CHARGE TO REVIEWER:

CHAPTER 1:

Does Chapter 1 adequately summarize the published literature regarding the health effects present in Chapter 2 for this substance?

CHAPTER 2:

First, does Chapter 2 adequately reflect the published literature regarding health effects for this substance? Are you aware of any studies that are not included that may be relevant in the derivation of MRLs for this chemical?

Second, we would like you to focus on the current data assessment which resulted in the changes noted above and presented in detail below.

MRLs:

Oral MRLs

Acute Duration (Revised)

An acute-duration oral MRL of 0.006 mg/kg/day was derived for hexachlorobutadiene based on an increased incidence of renal proximal tubule degeneration in rats exposed to hexachlorobutadiene in the diet for 14 days (Harleman and Seinen 1979). The MRL is based on a LOAEL of 5.9 mg/kg/day and a total uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Note: *An acute-duration oral MRL was not derived in the 1994 toxicological profile because of the small number of animals evaluated in the two available acute studies. Upon re-evaluation, the six male and six female rats per group was considered adequate since the lesions appear to have occurred in all hexachlorobutadiene-exposed rats and the findings are supported by several intermediate-duration studies.*

Three studies have evaluated the acute oral toxicity of hexachlorobutadiene. Two studies involved a single gavage dose of hexachlorobutadiene, which resulted in necrosis in the renal proximal tubules in rats exposed to ≥100 mg/kg (Birner et al. 1995; Jonker et al. 1993a); at 200 mg/kg, there was evidence of impaired renal function (increases in blood urea nitrogen, urine volume, urinary protein, and urinary glucose levels) (Jonker et al. 1983a). The third study was a range-finding study that found renal proximal tubule degeneration in male and female rats exposed to 5.9 or 6.2 mg/kg/day, respectively, hexachlorobutadiene in the diet for 14 days (Harleman and Seinen 1979). Decreases in body weight gain (9.5%) were also observed in the female rats exposed to 6.2 mg/kg/day and the male rats exposed to 19 mg/kg/day (21%). The study did not find any histological alterations in the liver of rats exposed to doses as high as 59 or 62 mg/kg/day.

The available acute oral studies identify the kidney as the most sensitive target of hexachlorobutadiene toxicity. Although the studies examined a limited number of potential endpoints, more extensive intermediate-duration studies confirm that the kidney is the most sensitive target of toxicity (Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977). The observed effects are also supported by several acute-duration parenteral studies (Bouroshaki 2003; Chiusolo et al. 2008; Cristofori et al. 2013; Kirby and Bach 1995; Maguire et al. 2013; Swain et al. 2011; Zanetti et al. 2010).

Since the Harleman and Seinen (1979) study identified the lowest LOAEL for renal effects, it was selected as the principal study for the MRL. In this study, groups of six male and six female Wistar rats were exposed to 0, 50, 150, or 450 ppm hexachlorobutadiene in the diet for 2 weeks. Doses of 0, 5.9, 19, and 59 mg/kg/day for males and 0, 6.2, 20, and 62 mg/kg/day for females were estimated using reported body weights and EPA's allometric equation to calculate food intake. Parameters used to assess toxicity included body weight, food consumption, liver and kidney weights, and histopathological examination of the liver and kidney. Significant decreases in body weight gain were observed in males at 19 and 62 mg/kg/day (21 and 31% of controls, respectively) and in females at 6.2, 20, and 62 mg/kg/day (9.5, 26, and 33% of controls, respectively). Decreases in food intake were also observed; however, decreases in food efficiency (growth/food intake) were only observed at the highest dose. Significant increases in relative kidney weight were observed in the males and females at ≥19 and 20 mg/kg/day; no alterations in relative liver weight were observed. Degeneration of the proximal tubular epithelial cells was observed at all hexachlorobutadiene exposure levels; no alterations were observed in the liver. Although incidence data were not provided, the investigators noted that histological changes were observed in the kidneys of all animals exposed to hexachlorobutadiene.

The lowest LOAEL value of 5.9 mg/kg/day identified for renal effects in males was selected as the point of departure for the MRL; the study did not identify a NOAEL value. The lack of incidence data precluded using benchmark dose modeling to calculate a point of departure. This LOAEL was divided by a total uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) resulting in an MRL of 0.006 mg/kg/day.

Intermediate Duration (Revised)

An intermediate-duration oral MRL of 0.002 mg/kg/day was derived for hexachlorobutadiene based on an increased incidence of renal proximal tubule regeneration in mice exposed to hexachlorobutadiene in the diet for 13 weeks (NTP 1991). The MRL is based on a NOAEL of 0.2 mg/kg/day and a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Note: *The MRL of 0.0002 mg/kg/day in the 1994 profile was based on a LAOEL of 0.2 mg/kg/day identified in a mouse study by NTP (1991) and an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). Upon review of the principal study (NTP 1991), it was determined that the 0.2 mg/kg/day dose was a NOAEL since kidney lesions were only observed in 1/10 female rats (incidences in female controls and males exposed to 0.2 mg/kg/day were 0/10 and 0/10); the LOAEL was 0.5 mg/kg/day (kidney lesions observed in 9/10 females).*

A number of studies have evaluated the toxicity of hexachlorobutadiene toxicity following intermediate-duration oral exposure. These studies have identified several targets of toxicity including body weight, liver, kidney, nervous system, hematological system, reproductive system, and developing organism. A summary of the lowest LOAEL values for these endpoints is presented in Table 2.

Table 2. Lowest LOAELs Identified in Intermediate-Duration Oral Studies of Hexachlorobutadiene

Endpoint	Effect	NOAEL[a] (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Body weight	9.9% decrease in body weight gain in mice exposed for 13 weeks	1.5	4.9	NTP 1991
Hematological	Increased hemoglobin concentration in rats exposed for 30 days	3	10	Kociba et al. 1971
Hepatic	Increased cytoplasmic basophilia in rats exposed for 13 weeks	6.3	15.6	Harleman and Seinen 1979
Renal	Proximal tubular epithelial regeneration in mice exposed for 13 weeks	0.2	0.5	NTP 1991
Neurological	Lethargy, hunched posture, incoordination in mice exposed to 40 mg/kg/day for 15 days	12	40	NTP 1991
Reproductive	Infertility in female rats exposed for 15 weeks	15	150	Harleman and Seinen 1979
Developmental	16–19% decrease in pup body weight (rat dams exposed for 18 weeks)		15	Harleman and Seinen 1979

[a]NOAEL identified in the same study as the lowest LOAEL.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

A comparison of the LOAEL values across endpoints supports the identification of the kidney as the most sensitive target of toxicity. A summary of the NOAEL and LOAEL values for renal effects is presented in Table 3.

Table 3. Summary of Renal Effects Observed in Intermediate-Duration Oral Studies of Hexachlorobutadiene

Species	Exposure	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Mouse	13 weeks (F)	0.2 F 1.5 M	0.5 F 4.9 M	Tubular epithelial regeneration	NTP 1991
Rat	147 days (F)	0.2 F 2 M	2 F 20 M	Tubular dilation and hypertrophy with foci of degeneration and regeneration	Schwetz et al. 1977
Rat	13 weeks (GO)	1 F 2.5 M	2.5 F 6.3 M	Enlarged hyperchromatic nuclei in the proximal tubules decreased urine osmolarity in females	Harleman and Seinen 1979
Rat	4 weeks (F)		2.5 F	Decreased BUN in females	Jonker et al. 1993b
Rat	32 days (GO)	1 F	4 F	Focal tubular vacuolization and increased relative kidney weight	Jonker et al. 1996
Rat	18 weeks (F)		15 F	Proximal tubular degeneration and necrosis	Harleman and Seinen 1979
Rat	30 days (F)	10 F	30 F	Tubular degeneration, necrosis, and regeneration	Kociba et al. 1971
Rat	3 weeks (F)	37 M	190 M	Proximal tubules lined with basophilic epithelium	Nakagawa et al. 1998
Rat	30 weeks (F)	94 M			Nakagawa et al. 1998

BUN = blood urea nitrogen; (F) = feed; F = female(s); (GO) = gavage in oil; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level

In all studies involving exposure of male and female rats or mice, the lower LOAEL values were identified in the females. The lowest LOAEL for renal effects was 0.5 mg/kg/day identified in female mice exposed to hexachlorobutadiene in the diet for 13 weeks (NTP 1991); no effects were observed at 0.2 mg/kg/day. The Schwetz et al. (1977) reproductive/developmental toxicity study also identified a NOAEL of 0.2 mg/kg/day; the LOAEL in this study was 2 mg/kg/day. Because the LOAEL identified in the NTP (1991) was lower than that identified in the Schwetz et al. (1977) study, NTP (1991) was selected as the principal study.

In the NTP (1991) study, groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 1, 10, 30, or 100 ppm hexachlorobutadiene in the diet for 13 weeks; the investigators estimated the doses to be 0, 0.1, 0.4, 1.5, 4.9, and 16.8 mg/kg/day in males and 0, 0.2, 0.5, 1.8, 4.5, and 19.2 mg/kg/day in the females. The following parameters were used to assess toxicity: body weight, food intake (measured weekly), organ weight (brain, heart, kidney, liver, spleen testis), gross necropsy, histopathological examination of major tissues and organs in the controls and high dose animals and all animals dying early; histopathology of kidneys in all groups; sperm morphology; and vaginal cytology.

One male in the 0.1 mg/kg/day group died early. No overt clinical signs were observed in exposed mice. Decreases in body weight gain were observed in males at 4.9 and 16.8 mg/kg/day (9.9 and 15.8%, respectively) and in females at 19.2 mg/kg/day (15%); no alterations in feed intake were noted. Significant decreases in absolute and relative kidney weights were observed at ≥4.9 mg/kg/day in males; relative kidney weight was also decreased in males at 1.5 mg/kg/day. In females, the only significant alteration in kidney weight was a decrease

in absolute weight at 19.2 mg/kg/day. The investigators noted that a decrease in absolute heart weight in males at 16.8 mg/kg/day may be clinically relevant; however, no histological alterations were observed in the heart. Renal tubular epithelial regeneration, prominent in the outer stripe of the outer medullary rays (pars recta) was observed in 0/10, 0/10, 0/10, 0/9, 10/10, and 10/10 males at 0, 0.1, 0.4, 1.5, 4.9, and 16.8 mg/kg/day, respectively, and in 0/10, 1/10, 9/10, 10/10, 10/10, and 10/10 females at 0, 0.2, 0.5, 1.8, 4.5, and 19.2 mg/kg/day, respectively. A significant decrease in sperm motility was observed at 1.5, 4.9, and 16.8 mg/kg/day, but the magnitude of the decrease was not dose-related. No significant alterations in sperm count, incidence of abnormal sperm, estrual cyclicity, or average estrous cycle length were observed.

The NOAEL of 0.2 mg/kg/day identified in female mice was selected as the point of departure for the MRL. The incidence data for renal tubular regeneration was not considered suitable for benchmark dose modeling due to the lack of dose-response data between the extremes in the incidence in the control and lowest dose groups and the incidences in higher dose groups. The MRL of 0.002 mg/kg/day was calculated by dividing the NOAEL of 0.2 mg/kg/day by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

CHAPTER 7:

We would like to know your thoughts on the regulations and guidelines that are presented and any that should be added or removed. Are you aware of any additional regulations or guidelines that we should add? Please provide citations. Are there any that should be removed? Explain.

APPENDIX A:

Please address the MRL worksheets based upon the questions provided above about the MRLs.

APPENDIX B:

Please provide comments about the process utilized in this section.