MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

TO: File for Diisoheptyl phthalate (CAS #71888-89-6)

FROM: Anne Kim, Air Quality Division, Toxics Unit

SUBJECT: Screening Level Derivation

DATE: February 23, 2007

The initial threshold screening level (ITSL) for diisoheptyl phthalate is 100 µg/m³ based on a 24-hour averaging time.

The following references or databases were searched to identify data to determine the screening level: U.S. Environmental Protection Agency (EPA) Integrated Risk Information System, Registry for Toxic Effects of Chemical Substances, American Conference of Governmental and Industrial Hygienists Threshold Limit Values, National Institute for Occupational Safety and Health Pocket Guide to Hazardous Chemicals, Environmental Protection Bureau Library, International Agency for Research on Cancer Monographs, Chemical Abstract Service (CAS) - Online (1967 – 2006), National Library of Medicine, Health Effects Assessment Summary Tables, and National Toxicology Program Status Report. The EPA has not established a reference concentration or reference dose for diisoheptyl phthalate. The calculated molecular weight of diisoheptyl phthalate is approximately 362 g.

Background

Diisoheptyl phthalate (DIHP) is found in a large class of compounds called aromatic dicarboxylic acid esters, or simply phthalate esters. Phthalate esters are used as plasticizers to provide flexibility to a number of different plastics. Characteristic of most, they are found in liquid form with high boiling points and extremely low vapor pressures. These compounds have the potential to be endocrine disruptors that may contribute to the development of hormone-dependent diseases and affect the capacity of both animal and human reproduction. (Clayton and Clayton, 1981; Zacharewski et al., 1998)

Animal Toxicity

Scarce toxicity data was found after conducting an extensive literature search.

Groups of 30 female and 30 male Crl:CD(SD)IGS BR rats were orally exposed to diisoheptyl phthalate in the diet at concentrations of 0, 1000, 4500, and 8000 ppm in a two-generation reproductive toxicity study (Stump, 2003). Actual consumption doses for the F0 generation are listed below in Table 1. Exposure was initiated at least 70 days prior to mating for both generations. While the litters were potentially exposed in utero and through nursing during lactation, direct exposure to DIHP was not initiated until after PND 21 (for F1 litter) or at all (for F2 litter). Parental rats and litters were sacrificed after pups were weaned (i.e., PND 21).

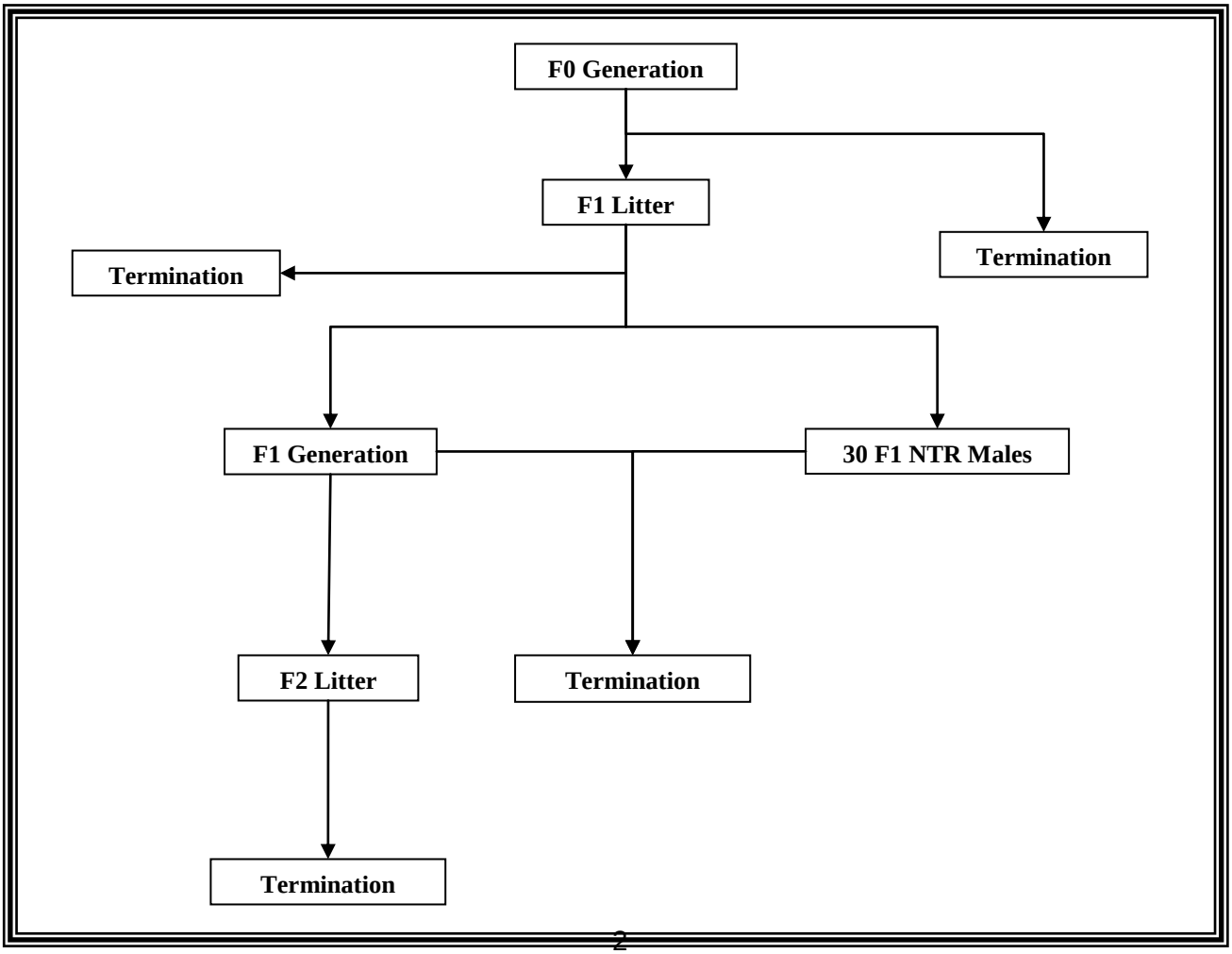
Table 1. F0 generation mean compound consumption in mg/kg/day

	Males		Females		
Target Dose ppm	Prior to Breeding	After Breeding	Prior to Breeding	Gestation	Lactation
0	0	0	0	0	0
1000	81	50	89	64	162
4500	343	222	406	304	716
8000	623	404	726	532	1289

Table 2. F1 generation mean compound consumption in mg/kg/day

	Males		Females		
Target Dose ppm	Prior to Breeding	After Breeding	Prior to Breeding	Gestation	Lactation
0	0	0	0	0	0
1000	91	50	100	64	168
4500	416	227	462	309	750
8000	764	419	833	543	1360

Figure 1. Generalized scheme of study design (Stump, 2003)



Clinical observations were made twice daily during the length of the study. Body weights were recorded weekly throughout the study, with the exception of females during gestation and postnatal periods; female body weights were recorded on gestational days (GDs) 0, 4, 7, 11, 14, 17, and 20 and postnatal days (PNDs) 1, 4, 7, 14, and 21. Food intake was recorded weekly except during the mating period. Female food intake was specifically monitored on the same GDs and PNDs that body weight was observed.

On PND 0, the numbers of stillborn and live pups were recorded. The litters were examined twice daily for survival and for developmental variations and malformations. On PND 4, all litters were reduced to a standardized size of 8 pups per litter to reduce variability. Observations for survival and clinical appearance were made daily, and body weights were recorded and physical examinations conducted on PNDs 1, 4, 7, 14, 21, and weekly thereafter. Other endpoints that were observed in the pups included anogenital distance, thoracic nipple retention, balanopreputial separation (males), and vaginal perforation (females).

Thirty male and 30 female F1 pups were randomly selected to make up the F1 generation, and an additional 30 male rats from the 8000 ppm group were selected to make up the non-treatment recovery (NTR) group due to the significantly decreased anogenital distance seen in the 8000 ppm group males. These selected rats were exposed to the diet containing DIHP starting on PND 22, excluding the NTR group.

At necropsy, for both the F0 and F1 generation animals, sperm motility, sperm morphology, and sperm production rates were assessed. Select organs were weighed (gonadal, liver, and kidney), and histopathological observations were made in select tissues.

Deaths that occurred before scheduled necropsy and were attributed to DIHP exposure were found in the 4500, 8000, and NTR groups: 1 male in the F1 generation, 2 males each in the F0 and F1 generations, and 2 males in the F1 generation, respectively.

F0 Generation Results:
No significant differences from control were found in clinical observations, fertility indices, mean lengths of estrous cycles, pairing, or coitus, body weight or body weight gain, food consumption or food efficiency, or spermatogenic endpoints.

Statistically significant increases in liver and kidney weights were seen in the 4500 and 8000 ppm groups. These weight increases were correlated with histopathological findings in the liver and kidney of the 4500 and 8000 ppm group males and the liver of the 8000 ppm group females; centrilobular hepatocellular hypertrophy, hepatocellular vacuolization, and chronic progressive nephropathy were observed.

F1 Litter Results:
No significant differences from control were observed in the number of live pups born or in postnatal survival. Mean offspring body weight gain was reduced in the 8000 ppm group during PNDs 7-14 and 14-21 (statistically significant for the PND 7-14 period), and mean individual body weights were reduced in the 8000 ppm group on PNDs 14 and 21 (statistically significant on PND 21). Spleen weights were statistically significantly reduced, both absolute and relative, in the females of the 4500 ppm group and in both sexes of the 8000 ppm group.

Statistically significant reductions in mean anogenital distance were observed in the 8000 ppm group males on PND 1, 21, and at necropsy. The NTR* group males also showed statistically significant reductions in anogenital distance on PND 21 and at necropsy. The males in the 8000 ppm group also showed statistically significant increases in mean thoracic nipple retention. Greater delay for balanopreputial separation was observed in males of the 4500 ppm, 8000 ppm, and NTR groups. These increases were statistically significant compared to control in the 8000 ppm and NTR groups.

***** The 30 F1 litter selected to comprise the NTR group come PND 21.

F1 Generation Results:

In the 2 males of the 8000 ppm group, 1 male of the 4500 ppm group, and 2 males of the NTR group that died before the end of the study (scheduled necropsy), reproductive effects attributable to test diet consumption were observed (e.g., an undescended right testis was observed in an 8000 ppm group male that died week 21).

Clinical observations revealed occurrences of hypospadias in 5 rats, swelling of the prepuce in 5 rats, and undescended testes in 2 rats from the 8000 ppm group. Mating and fertility indices were found to be statistically significantly decreased in the 8000 ppm group animals compared to control. The 4500 ppm group animals also showed non-statistically significant decreases in the indices, supporting a DIHP-related dose response.

While there was no effect on mean body weight or body weight gain due to DIHP exposure, mean body weight gain during the gestation period was statistically significantly reduced compared to control. The reductions seen in mean body weight in the 8000 ppm group during lactation was, then, attributed to the reduced body weights from the gestation period.

Sperm production rate and testicular sperm concentrations were statistically significantly reduced in all DIHP-exposed males, with epididymal sperm concentrations significantly reduced only in the 8000 ppm group males. Incomplete closure of the penis and portions of the male reproductive tract were found to be missing in the 8000 ppm-dosed group and the NTR group. Degeneration of the seminiferous tubules was observed at all dose levels, showing a dose response with statistically significant values only in the 8000 ppm group males.

Statistically significant decreases in mean absolute and relative gonadal weights were seen in both male and female animals in the 8000 ppm group. Mean absolute and relative pituitary weights were also found to be statistically significantly decreased in the 8000 ppm dosed males. Liver and kidney weights were increased in the 4500 ppm and 8000 ppm groups (statistically significant at 8000 ppm only). Liver weight increases were associated with the findings of hepatocellular centrilobular hypertrophy in the 4500 ppm and 8000 ppm males and 8000 ppm females. Hepatocellular vacuolation was also observed in the 4500 and 8000 ppm males (both statistically significant). Dilated renal pelves in all groups, including control, showed a dose-related effect that was attributed to DIHP exposure. Microscopic changes in the kidney differed between the males and females. Males showed hydronephrosis in the 4500 ppm and 8000 ppm groups, with the 8000 ppm group incidences being statistically significant compared to control. The females showed increased incidences of chronic progressive nephropathy that was not statistically significant but, rather, dose-responsive.

F2 Litter Results:
No significant differences from control were observed in the number of live pups born or postnatal survival. Mean offspring body weight gain was reduced in the 4500 and 8000 ppm groups during PNDs 7-14 and 14-21 (statistically significant for the PND 7-14 period in the 8000 ppm group and for the females only in the 4500 ppm group), and mean individual body weights were reduced throughout the postnatal period. Statistically significant reductions in mean anogenital distance were observed in the 4500 and 8000 ppm group males on PND 1.

Spleen weights were statistically significantly reduced, both absolute and relative, in both sexes of the 4500 and 8000 ppm groups. The statistically significant increase in mean relative brain weights in the 4500 and 8000 ppm groups seemed more likely to be a result of the significant reductions seen in mean body weights.

A study conducted by Zacharewski et al. (1998) evaluated the estrogenic activity of DIHP in vitro and in vivo in Sprague-Dawley rats. The estrogenic activity in rats was measured in vitro with an estrogen receptor competitive ligand-binding assay and mammalian- and yeast-based gene expression assays. The effects of in vivo exposure to DIHP in rats were evaluated by measuring changes in uterine wet weight and vaginal cell cornification. Each of the tests was repeated. In the in vitro test, DIHP did not effectively compete with 17beta-estradiol for binding to the uterine estrogen receptor. DIHP did not show positive results in either reporter gene activity assays (mammalian or yeast). Body weight changes were seen after DIHP oral exposure only in the first experiment; the second experiment showed no body weight changes. The in vivo test measuring uterine wet weight showed significant increases after exposure to DIHP; however, the second experiment showed significant *decreases* in uterine wet weight. No vaginal cornification was observed after DIHP exposure.

A study conducted by Smith et al. (2000) reported the effects of DIHP exposure in male rats and male mice. Fischer 344 rats were exposed to 0, 1000, or 12000 mg/kg DIHP in the diet for 2 weeks or 4 weeks. B6C3F1 mice were exposed to 0, 500, or 6000 mg/kg DIHP in the diet for 2 weeks or 4 weeks. At the end of each respective exposure period, all animals were sacrificed by asphyxiation, weighed, and necropsied. The liver was weighed and separated into three sections to test for gap junctional intercellular communication (GJIC), replicative DNA synthesis, and peroxisomal beta-oxidation (PBOX) activity. Three to 5 animals were evaluated for each test.

Rat results: After 2 weeks of DIHP exposure, rats in both the low-dose and high-dose groups showed statistically significant increases in relative liver weight. The relative liver weight continued to be statistically significantly increased after 4 weeks of exposure for the high-dose group only. GJIC was not reduced or inhibited at either dose after 2 or 4 weeks of DIHP exposure. The elevations of periportal DNA synthesis were statistically significant in both doses for the 2-week and 4-week periods. No changes in PBOX activity was found in the low-dose animals of the 2-week or 4-week groups, but a statistically significant increase in PBOX activity was observed after 2 weeks and after 4 weeks of exposure to 12000 mg/kg DIHP.

Mouse results: The liver weights of mice were unaffected by DIHP treatment at either exposure period. Just as no effects on GJIC were seen in exposed rats, no reduction of GJIC was observed in all mice exposed to DIHP. After 2 weeks of exposure, both the low-dose and high-dose groups showed statistically significant increases in periportal DNA synthesis. Only the high-dose group showed significant elevations of DNA synthesis after 4 weeks of DIHP exposure. PBOX activity was not affected after 2

weeks or 4 weeks of exposure to the low dose; however, it was statistically significantly increased in mice exposed to the high dose at the end of both the 2-week and 4-week observations.

Discussion

In the study conducted by Stump (2003), a NOAEL of 1000 ppm was established for the F0 and F1 generations based on the systemic endpoint of increased liver and spleen weights. The same concentration of 1000 ppm also produced reproductive effects in the F1 generation; therefore a reproductive LOAEL was also established at 1000 ppm for the F1 generation animals. A NOAEL of 1000 ppm was established for the F1 and F2 litters based on reproductive toxicity effects observed.

Parental toxic effects included increased liver and kidney weights with corresponding hepatocellular hypertrophy, hepatocellular vacuolization, and chronic progressive nephropathy in the F0 generation.

Toxic effects seen in the F1 generation animals included increased liver and kidney weights with the same corresponding histopathology as F0 animals, in addition to reproductive effects: decreased mating and fertility indices, decreased mean body weight during the gestational and, hence, lactation period, decreased testicular sperm concentrations and epididymal sperm concentrations, occurrence of hypospadias, undescended testes, swelling of the prepuce, incomplete closure of the penis, and degeneration, or complete absence, of parts of the male reproductive tract.

The neonatal toxic effects noted in the F1 and F2 litters included decreased birth and postnatal weights, decreased anogenital distance, increased thoracic nipple retention, and a delay, or lack, of balanopreputial separation. Spleen weights were also found to be significantly reduced in both litters. The NOAEL of 1000 ppm from the F1 and F2 litters was based on these reproductive endpoints.

No reproductive effects were observed in the F0 generation, but reproductive effects were found in the F1 generation animals. Although a dose-responsive relationship was not observed, all dose groups showed statistically significant decreases in sperm concentration and sperm production rate. Thus, the study investigators did not propose a NOAEL based on the reproductive findings; 1000 ppm is the LOAEL based on reproductive effects observed in the F1 generation males. A NOAEL of 1000 ppm, however, still was established based on the systemic toxicity endpoint of increased liver and spleen weights seen in the F0 and F1 generations.

The effects seen in the F0 and F1 generation males resulted from a 90-day (70 days prior to breeding plus 21 days after) exposure period to DIHP in the diet. In addition, the F1 generation was also exposed preconceptually, during gestation, and through lactation up to weaning. This study duration is considered to be subchronic, which allows the study to be used to develop an RfD-based initial threshold screening level (ITSL). While the fetal effects seen in this study are critical, since the NOAEL for this effect is the same as the LOAEL for reproductive effects, developing an ITSL based on the F1 generation male spermatogenic effects will also be protective of fetal developmental and/or reproductive effects.

Although the use of F0 generation dose would result in a lower exposure dose (see Table 1 and Table 2), the male F1 generation exposure concentrations were used to calculate the ITSL because the effects were observed in the F1 generation animals.

Use of the F1 exposure concentration is consistent with EPA procedure, where the dose to determine the RfD/RfC is taken from the generation in which the effect is seen (Green, personal communication).

Note: 1000 ppm was determined to be the LOAEL from Stump (2003)
--> 91 mg/kg/day before breeding and 50 mg/kg/day after breeding

Derivations of Screening Level

ITSL = RfD_{RT} x (70 kg)/(20 m^3)

>where RfD_{RT} = reproductive reference dose

Calculation of Dose:

LOAEL = 91 mg/kg/day (70 days/91 days) + 50 mg/kg/day (21 days/91 days)

LOAEL = 81.5 mg/kg/day = 82 mg/kg/day

Calculation of RfD:

$RfD_{RT} = \frac{LOAEL}{UF}$

>where RfD_{RT} = defined above

UF = uncertainty factor

UFs that apply: 1) variation in sensitivity among members of the human population = 10
2) extrapolation from animal data to humans = 10
3) LOAEL to NOAEL = 10
4) sub-chronic to chronic = 3

*3 is used instead of 10 because the effects observed were in the F1 generation of a two-generation reproductive study where exposure occurred preconceptually, in utero, and through lactation, as well as directly for 91 days beginning on PND 21.

$RfD_{RT} = \frac{82 \text{ mg/kg}}{10 \times 10 \times 10 \times 3}$

RfD_{RT} = 0.0273 mg/kg = 0.03 mg/kg

ITSL = 0.03 mg/kg x (70 kg)/(20 m^3)

ITSL = 0.105 mg/m^3

ITSL = 105 ug/m^3 = **100 ug/m^3** - based on a 24-hour averaging time

References

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