**MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY**

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**INTEROFFICE COMMUNICATION**

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September 20, 1999

To: File for Dibutyl Phthalate (CAS# 84-74-2)

From: Michael Depa, Toxics Unit

Subject: Screening Level Determination

The initial threshold screening level (ITSL) for dibutyl phthalate (DBP) is 50 µg/m³ (8-hour average).

The following references or databases were searched to identify data to determine the ITSL: Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS), the Registry of Toxic Effects of Chemical Substances (RTECS), the American Conference of Governmental and Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs), National Institute of Occupational Safety and Health (NIOSH) Pocket Guide to Hazardous Chemicals, MDEQ's Environmental Protection Bureau Library, International Agency for Research on Cancer (IARC) Monographs, Chemical Abstract Service (CAS) On-line (1967-May 12, 1998), National Library of Medicine, Health Effects Assessment Summary Tables, and National Toxicology Program (NTP) Status Report. Review of these sources found that EPA has not established a Reference Concentration (RfC) for DBP. the RfD for dibutyl phthalate is 0.1 mg/kg/day. Both the American Conference of Governmental and Industrial Hygienist (ACGIH) Threshold Limit Value (TLV) and the National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Level (REL) are 5 mg/m³. The NTP performed extensive feeding studies on dibutyl phthalate (DBP). A document (see summary below) was obtained from the EPA (1990) entitled "Documentation of the Not Verifiable Status for the Inhalation RfC of Di-n-Butylphthalate (CAS # 84-74-2)". The molecular weight of DBP is 278.34 g and the vapor pressure is $1.0x10^{-5}$ - $1.4x10^{-5}$ mmHg at 25 °C (ATSDR, 1990).

**Animal Studies - Inhalation Exposure**

In a report reviewed by the EPA (1990), groups of male Wistar rats were exposed to mists/vapors of dibutyl phthalate (DBP) at 0 (n=11), 0.31 - 0.56 (n=12), or 45-59.2 mg/m³ (n=14) for 6 hours per day for 5 days and for 3 hours on Saturdays for a period of 6 months (Kawano, 1980a). The ATSDR calculated the exposure concentrations to be 0.5 and 50 mg/m³ (ATSDR, 1990). No histopathologic examinations were performed on any of the rats. In the high exposure group there was a marked decrease in weight gain that was apparent after 50 days; at 6 months the average differential between this group (n=7) and the controls (n=5) was 72 g, representing a 12% decrease. At 6 months relative but not absolute brain and lung weights in this group were both significantly increased ($p<0.01$). The testicular weights were not decreased in either group. The hematological parameters measured in this group showed increased levels of GOT (not indicated as significant), BUN values were elevated (23.4 mg/dl, normal range 15-21), and were accompanied by decreased levels of serum electrolytes Na and Cl, all of which indicate possible renal dysfunction. Possible liver dysfunction was indicated by altered levels of triglycerides and cholesterol, elevation of serum

glucose, and elevation of serum enzymes, although the latter alterations were apparently not significant. In the low exposure group, weight loss was apparent only after about 100 days and was limited to a 4% differential compared to controls at 6 months. Hematological parameters measured were all within normal limits. BUN levels were elevated, but not significantly at 6 months. Elevations in ALP and GPT at 3 but not 6 months may indicate a transient effects on liver function. Based in a weight loss of >10%, and indications of kidney dysfunction, the 50 mg/m$^3$ exposure concentration represents a LOAEL in this study; the lack of these indicators in the low exposure group (0.5 mg/m$^3$) would indicate this dose as a NOAEL. The small number of animals (groups of 3 to 7), the lack of statistical limits on data presented, the use of only male rats, the lack of any food consumption data, and the lack of any histopathology all detracted the use of this study in the derivation of an ITSL.

In another report reviewed by the EPA (1990), groups of male albino rats (15/group) were exposed to "round the clock" exposure at either 0, 0.098, 0.256, or 0.98 mg/m$^3$ DBP for 93 consecutive days (Men'shikova, 1971). Because of the continuous exposure conditions, these values also represent the exposures adjusted for duration. Effects were evaluated from behavior, weight dynamics, nerve velocity measurements, serum protein fractions (the gamma-globulins, an unreliable measure of liver function) and hematology. Although clinical observations and weight data were not presented, the report stated that "the animals remained healthy and gained weight uniformly". White blood cell counts fluctuated upward beyond the normal range (6000-17000/mm$^3$) during the highest exposure reaching a peak of about 20,000/mm$^3$. Serum gamma-globulins appeared to be significantly elevated in the highest exposure group at the end of the study. In the EPA (1990) review of this study EPA stated, "The significance of these findings is not apparent or corroborated by any other toxicological data within the study (hepatic histopathology, data on change in liver weight)." No other data was presented in the EPA summary of Men'shikova (1971). The highest level of exposure in this study, 0.98 mg/m$^3$, represents a free-standing NOAEL. Notable shortcomings of this study include the failure to report histopathology and organ weights measurements. This study was found to be sufficiently poor in quality to preclude its direct use in derivation of an ITSL.

In an inhalation study, investigators found that a 2-hour exposure in mice to an aerosol concentration approximating 250 mg/m DBP resulted in severe irritation of the eyes and upper respiratory tract, labored breathing, incoordination, partial paralysis, convulsions, narcosis, and in some animals, death from paralysis of the respiratory system (Voronin,1972; abstract). After chronic dosing of rats with 40 mg/m$^3$ irritating effects, weight loss and blood abnormalities were observed. The "under-threshold value" was reported as 20 mg/m$^3$. It should be noted that the information reported here was from an abstract translated from Russian for the Chemical Abstract Service (CAS-Online) and that no mention of the use of control animals, statistical analysis, exposure conditions or strain of animals was made. The term "irritating effects" was not defined and it is unknown if these effects occurred in the lungs. Additionally, in the Documentation of the TLV for DBP (ACGIH, 1993) the ACGIH stated that the threshold for effects was 4 mg/m$^3$ and not the 40 mg/m$^3$ as stated in the abstract obtained from CAS-Online. All these factors are major deficiencies and undermine the validity of the results.

The EPA (1990) reviewed an inhalation pharmacokinetic study by Kawano (1980b). The results from this study indicate that DBP is absorbed from the lungs and distributed throughout the body by the circulation. Furthermore, DBP was found to accumulate in the liver but not in the lungs during protracted periods (up to 6 months) of inhalation exposure. The ATSDR (1990) summarized the metabolism, distribution and excretion of DBP. Studies using oral and intravenous routes of exposure indicate that DBP is efficiently metabolized to mono-butyl phthalate by esterases present in several organ systems including liver, kidney and gut.

**Animal Studies - Oral Exposure**

The NTP performed several 13-week feed studies in rodents (NTP, 1995) using di-n-butylphthalate (DBP). These studies focused primarily on developmental and reproductive effects.

MAXIMUM PERINATAL EXPOSURE (MPE) DETERMINATION STUDY (F344/N RATS):
A range finding feed study was used to find the maximum perinatal exposure (MPE) concentration. This study consisted of groups of 10 pregnant F344/N rat dams exposed to various concentrations of DBP during gestation and through lactation. Some of their pups were selected and received the perinatal exposure concentrations in feed for 4 weeks. The 10,000 ppm dose was chosen for the MPE+Subchronic Study. RESULTS**:** Decreased weight gains were noted in dams exposed to 20000 ppm during gestation and to dams exposed to 10000 ppm during lactation. The gestation index (number of live pups per breeding female) was significantly lower in the 20000 ppm group than in the control, and pup mortality in this groups was marked (100% by day 1 of lactation); however, survival was 89% or greater in all other treatment groups. Hepatomegaly was observed in males in all exposed groups and in females receiving 2500 ppm or greater. No gross lesions were observed at necropsy. Moderate hypospermia of the epididymis was diagnosed in all male rats in the 7500 and 10000 ppm groups; mild hypospermia of the epididymis was diagnosed in 2 of 10 males in the 5000 ppm group. No degeneration of the germinal epithelium was detected in the testis of these rats.

MPE DETERMINATION STUDY(MICE)
This feed study consisted of C3H male mice and C57BL/6 female mice and their offspring (B6C3F1 mice). The pregnant dams were exposed during gestation and lactation to 0, 1250, 2500, 5000, 7500, and 10000 ppm, and the pups were exposed for 4 weeks post weaning to the same concentrations of as the dams. RESULTS: The gestation period was longer in dams that received 2500 ppm or greater than in the controls, and gestational body weight gain depressions were noted in dams receiving 7500 ppm or greater. Only 5 of 20 females in the 10000 ppm group delivered live pups, and none of the 20 females receiving 2500 ppm delivered live pups. Only one pup in the 10000 ppm groups survived past lactation day 1; the number of live pups per litter in the 7500 ppm groups also remained low throughout lactation. No deaths of either male or female pups occurred after weaning. Initial postweaning and final body weights of male pups receiving 2500 ppm or greater were significantly less than those of the control group. Hepatomegaly in male mice in all exposed groups, and the absolute liver weight of males administered 7500 ppm was greater than that of the controls; although a similar change was apparent in females, no statistical differences between the liver weights of exposed and control female were detected. No treatment-related gross lesions were identified at necropsy, and no histopathologic lesions definitively associated with treatment were observed in male or female mice in the 7500 ppm groups. Developmental toxicity and fetal and pup mortality were suggested at concentrations as low as 7500 ppm. No subchronic toxicity study with prior MPE exposure was conducted with mice, although an MPE concentration of 5000 ppm was suggested by the data.

MPE+SUBCHRONIC STUDY
Groups of 10 pregnant F344/N rat dams were exposed to 10,000 ppm (the MPE dose) in feed during gestation and through lactation. The pups were exposed to 10,000 ppm DBP in the feed from weeks 4 to 8. A 13 week adult exposure in feed started at 8 weeks of age with concentrations of DBP at 0 (with and without perinatal exposure), 2500, 5000, 10000, 20000 or

40000 ppm. In the male pups the doses are 0, 138, 279, 571, 1262 and 2495 mg/kg/day. In the female pups the doses are 0, 147, 294, 593, 1182 and 2445 mg/kg/day. RESULTS**:** No mortality or toxicity was observed in dams during the perinatal phase of the study; however, before pups were culled at 4 days postpartum, the percentage of live pups per litter was 86% to 93% that of the controls. At the time of weaning, hepatomegaly and markedly increased peroxisomal enzyme activities (approximately 19-fold greater than the control values) were observed in exposed pups. During the 13-week adult exposure phase, the body weight gains of rats treated with DBP decreased with increasing exposure concentration. Hepatomegaly was observed in male rats receiving 5000 ppm or greater and in females receiving 2500 ppm or greater. In males receiving 2000 ppm as adults, testis and epididymal weights were less than in the controls; males in the 40000 ppm groups also had a lower testis weight than controls. Results of hematologic analyses conducted at the end of the 13-week exposure period suggested a mild anemia in male rats administered 10000 ppm or greater as adults and female rats administered 40000 ppm as adults. Histopathologic examination of the testes revealed degeneration of the germinal epithelium, a mild to moderate focal lesion in rats in the 10000 and 20000 ppm groups and a marked, diffuse lesion in all males receiving 40000 ppm; at 40000 ppm, an almost complete loss of the germinal epithelium resulted. Rats administered 20000 ppm had fewer spermatid heads per testis than the unexposed controls, and epididymal spermatozoal concentration was less than that in the MPE+0 ppm group.

SUBCHRONIC STUDIES (RATS and MICE)
F344/N rats were exposed to 0, 2500, 5000, 10000, 20000 or 40000 ppm DBP in the feed continuously for 13 weeks. In the male rats the doses are 0, 176, 359, 720, 1540 and 2964 mg/kg. In the female rats the doses are 0, 177, 356, 712, 1413 and 2943 mg/kg. B6C3F1 mice were exposed to 0, 1250, 2500, 5000, 10000, or 20000 ppm DBP in the feed for 13 weeks. In male mice the doses are 0, 163, 353, 812, 1601, and 3689 mg/kg. In female mice the doses are 0, 238, 486, 971, 2137, and 4278 mg/kg. RAT RESULTS: Markedly reduced mean body weights were observed in males and females in the 40000 ppm groups (45% and 73% or control body weights, respectively); final mean body weights of males receiving 10000 ppm or greater and females receiving 20000 ppm or greater were lower than those of the controls. Hepatomegaly was observed in males that received 5000 ppm or greater and in females that received 10000 ppm or greater. Testis and epididymal weights of males in the 20000 and 40000 ppm groups were lower than those of the controls. A minimal anemia was detected in male rats receiving 5000 ppm or greater. Histopathologic examination of the testes revealed degeneration of the germinal epithelium, a mild to marked focal lesion in the 10000 and 20000 ppm groups and a marked, diffuse lesion in all males in the 40000 ppm group; at 40000 ppm, an almost complete loss of the germinal epithelium resulted. At 20000 ppm, spermatid heads per testis and per gram testis, epididymal spermatozoal motility, and the number of epididymal spermatozoa per gram epididymis were lower than in the controls. A LOAEL of 5000 ppm (359 mg/kg/day) was identified based on “minimal anemia” reported by the authors. A NOAEL of 2500 ppm (176 mg/kg/day) was also identified. MICE RESULTS**:** No deaths occurred during this study. Mean body weights and weight gains of male and female mice decreased with increasing exposure concentration and the decreased were significant for males and female that received 5000 ppm or greater. Relative liver weights were greater in males and female receiving 5000 ppm or greater than in controls. A minimal anemia was suggested in female mice in the 20000 ppm group. Although no gross lesions were observed at necropsy, microscopic examination revealed hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in male mice receiving 10000 or 20000 ppm and female mice receiving 20000 ppm. A LOAEL of 10000 ppm (1601 mg/kg/day) was identified based on cytoplasmic alterations and glycogen depletion in the male mouse. A NOAEL of 5000 ppm (812 mg/kg/day) was identified.

CONTINUOUS BREEDING STUDY (RATS)
Groups of 20 breeding pairs of Sprague-Dawley rats received 0, 1000, 5000, or 10000 ppm DBP in feed for 119 days total. Males and females were exposed for 7 day separately and 112 days while paired. RESULTS: The mean pup weight at birth in the 10000 ppm groups was significantly lower than the control pup weight. The average number of live pups per litter in all exposed groups was lower than in the controls. Crossover mating trials in the $F_0$ generation revealed no effects on the fertility of male or female rats receiving 10000 ppm. In contrast to the $F_0$ rats, mating, pregnancy, and fertility indices of the $F_1$ rats were lower in the 10000 ppm group. Interstitial cell hyperplasia was noted in 7 of 10 males in the 10000 ppm group. The authors stated that these effects document the male and female reproductive toxicity of DBP in $F_1$ generation rats receiving 10000 ppm and do not exclude the possibility of developmental toxicity to the $F_2$ offspring.

CONTINUOUS BREEDING STUDY (MICE)
Groups of 20 breeding pairs of CD-1 mice received 0, 300, 3000, or 10000 ppm DBP in feed for 105 days total. Males and females were exposed for 7 days separately and 98 days while paired. The fertility index, average number of litters per breeding pair, and average number of live pups per litter in the 10000 ppm group were lower than in the controls. Crossover mating trials of mice receiving 10000 ppm revealed effects on dams in the $F_0$ generation, with a lower fertility index, number of live pups per litter, and pup weight than in the controls. Liver weights were greater in males and females, and the uterine weight was less in exposed dams than in controls. No other changes were observed at necropsy or on histopathologic examination. These data document the female reproductive toxicity of DBP in $F_0$ mice.

Groups of 6 male Sprague-Dawley rats were administered DBP via gavage at a dose level of 2000 mg/kg/day for 4 days (Cater et al., 1977). DBP was found to produce testicular injury as observed by loss in organ weight, and diminution of both spermatocytes and spermatogonia. Additionally, it was found that DBP treatment adversely affected zinc metabolism. The urinary excretion of zinc was increased and the zinc content in the testes was markedly decreased. Moreover, the turnover rate of this element in testicular tissue was substantially enhanced. DBP treatment did not significantly alter the zinc concentrations in the kidney and liver. The authors stated that coadministration of zinc was found to afford a substantial measure of protection against testicular damage. The authors stated that monobutyl phthalate, a major metabolite of DBP produced the same testicular damage as DBP.

In a metabolic study, groups of 3 male Wistar rats were administered $C^{14}$-labeled DBP orally at a dose of 60 mg/kg (Tanaka et al., 1978). Unlabeled DBP (60 mg/kg) was also given to 3 rats in order to determine urinary metabolites. The $C^{14}$-labeled DBP was excreted mainly as monobutyl phthalate in the urine, with a small portion in the feces. The authors stated that urinary excretion appeared to be much higher than excretion in the feces. Monobutyl phthalate and unchanged DBP (ratio1:1) were found to be the main metabolites in the bile. The authors stated that DBP was rapidly absorbed, metabolized and completely excreted. In distribution studies, specific organ affinity was not markedly observed in rats after a single dosing of $C^{14}$-labeled DBP. The authors stated that since phthalic acid was found in the urine and not the bile that the formation of phthalic acid may occur at sites other than the liver.

**Discussion and Development of Screening Level**

The inhalation toxicity studies available on DBP were inadequate to derive a reference concentration (RfC). Kawano (1980a) found no adverse effects in male rats exposed for 6

months (6 days/week) to DBP at an average concentration of 0.5 mg/m³, but at 50 mg/m³ observed weight loss, kidney dysfunction and increased relative brain and lung weight. And, from the abstract published by Voronin (1972), it was reported that chronic exposure to 40 mg/m³ caused "irritating effects", weight loss and blood abnormalities, yet exposure to 20 mg/m³ was called an "under-threshold value."

Rule 232 hierarchy prescribes that a RfD be used when a reference concentration (RfC) is not available. However, using the RfD to develop an ITSL may be inappropriate when the critical effect of the toxicant occurs in the lung and when this effect is specific to the inhalation route of exposure. Another reason not using the RfD to develop the ITSL would be when the effects observed orally would not be expected to occur via the inhalation route of exposure. An example of this scenario is when the critical effect of oral exposure is toxicity to an organ or organ system and the toxicant is not absorbed into the body via the lung. Keeping this in mind the RfD was examined.

As mentioned above the RfD for DBP is 0.1 mg/kg/day. The RfD derived ITSL would be 350 µg/m³ [see Rule 232(1)(b)]. In the Kawano (1980a) study DBP caused kidney effects, indicating that it can be absorbed into the body through the lungs, and cause systemic effects. Kawano (1980b) also showed that once DBP is absorbed by the lungs it is distributed throughout the body. However, NIOSH (1992) indicated that the principal health effects of DBP are irritation to the eyes and upper respiratory tract. However, ACGIH (1993) stated that, "In animals, the primary target for DBP appears to be the reproductive system." In any case, oral studies might not demonstrate the irritant potential of DBP aerosol inhalation. Furthermore, DBP is expected to be an aerosol particulate at ambient temperatures. The RfD derived ITSL (i.e., 350 µg/m³, 24-hr) would be above the National Ambient Air Quality Standard for $PM_{10}$ (i.e., 150 µg/m³, 24-hr).

Unfortunately, there are no reports, either in animals or humans, that describe a threshold dose for the irritation effects of DBP. Presumably the occupational exposure limits (OELs) are protective of these effects, although this is not always the case. To this effect the ACGIH (1993) stated, "There are no reports that the recommended TLV will cause either irritation or systemic effects in man." However, histopathology of the lung was not performed in any of the animal toxicity studies and there are no occupational dose-response reports. There is no data indicating the TLV would not be protective of irritation or systemic effects (e.g., developmental, reproductive, etc.). Additionally, the TLV-based ITSL should provide protection against reproductive/developmental effects as it is below any screening level that would be derived using the animal developmental toxicology studies. Therefore, the TLV was deemed adequate to derive the ITSL. [see Rule 232(1)(c)]

ITSL = OEL ÷ 100

ITSL = 5 mg/m³ ÷ 100

ITSL = 0.05 mg/m³

ITSL = 50 µg/m³ (based on an 8-hour averaging time)

## References

ACGIH (1993) Documentation of threshold limit values and biological exposure indices. Cincinnati, OH. pages 400-402.

ATSDR. (1990) Toxicological profile for dibutyl phthalate. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division of Toxicology/Toxicology Information Branch. 1600 Clifton Rd NE, E-29, Atlanta, Georgia 30333. TP-90-10

Cater BR, Cook MW, Gangolli SD, Grasso P. (1977) Studies on dibutyl phthalate-induced testicular atrophy in the rat: effect of zinc metabolism. Toxicology and Applied Pharmacology. 41: 609-618.

EPA. 1990. Enclosure for di-n-butylphthalate. Documentation of the not verifiable status for the inhalation RfC of di-n-butylphthalate (CAS # 84-74-2). Integrated Risk Information System (IRIS). U. S. EPA. Revised 10/1/90.

Kawano, M. 1980a. Toxicological studies on phthalate esters. I. Inhalation effects of dibutyl phthalate (DBP) on rats. Nippon Eiseigaki Zassho 35 (4), 684-692. (translated and summarized by EPA, 1990 - see EPA reference above)

Kawano, M. 1980b. Toxicological studies on phthalate esters. II. Metabolism, accumulation, and excretion of phthalate esters in rats. Nippon Eiseigaki Zasshi 35 (4), 693-701. (translated and summarized by EPA, 1990 - see EPA reference above)

Men'shikova, T.A. 1971. Hygienic evaluation of dibutyl phthalate in relation to the use of polymer finishes in shipboard living quarters. Gig I Sanit 36 (1-3), 349-352 (translated from Russian and summarized by EPA, 1990 - see EPA reference above).

NIOSH. 1992. NIOSH recommendations for occupational safety and health. Compendium of policy documents and statements. US Dept of Health and Human Services (DHHS), Public Health Service, Centers for Disease Control, National Institute for Occupation Safety and Health (NIOSH), Division of Standards Development and Technology Transfer, Cincinnati, OH. DHHS (NIOSH) Publication No. 92-100.

Tanaka A, Matsumoto A, Yamaha T. 1978. Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. Toxicology. 9: 109-123.

Voronin, A. P. (1972) Toxicological and sanitary characteristics of a dibutyl phthalate plasticizer. Toksikol. Gig. Prod. Neftekhim. Proizvod., 2nd Publication, pp. 83-88. Abstract in Chemical Abstracts 80: 56220r (1974).

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