United States
Consumer Product Safety Commission
Washington DC 20207-0001

MEMORANDUM

Date: AUG 31 1998

To: Michael A. Babich, PhD, Phthalate Esters Project Manager, Division of Health Sciences

Through: Mary Ann Danello, PhD, Associate Executive Director, Directorate for Epidemiology and Health Sciences MAD

Through: Marilyn L. Wind, PhD, Director, Division of Health Sciences MLW

From: Brian C. Lee, PhD DABT, Toxicologist, Division of Health Sciences MLW for BCL

Subject: Health effects update, acceptable daily oral intake, and carcinogenicity of diisononylphthalate (DINP)

INTRODUCTION

Diisononylphthalate (DINP) (CAS# 68515-48-0, 28553-12-0) is a plasticizer used in the production of various plastic products, including those intended for use by children. DINP is not a single chemical entity since it contains a nonspecific mixture of branched and straight chain phthalates with an average of 9 carbons [1]. Due to the potential variability of DINP, the results of toxicological testing may be mixture-specific.

A previous assessment [1] examined the toxicity of DINP and derived an acceptable daily intake of 0.15 mg DINP /kg body weight /day and a cancer risk of 8.1 out of 1 million for a 10 kg child exposed to 1 mg DINP-4 (CAS# 71549-78-5) per kg body weight for one year. This memorandum supplements the previous assessment with information on chronic toxicity that has recently become available.

CARCINOGENIC EFFECTS

Aristech rat study

Reports on a 2-yr (104-wk) rat carcinogenicity study on DINP were submitted to EPA under TSCA section 8(e) by Aristech [2-3]. The CAS# used by Aristech suggests it is DINP-1 [Table 1 in [1]], although DINP-1 formulations are not necessarily equivalent. The DINP purity indicated was >99% diisononyl phthalate isomers. This and the CAS# 68515-48-0 used in the 8(e) submissions suggest equivalence to DINP-1 in the previous CPSC assessment [1].

Fischer 344 (F344) rats were fed nominally 0, 500, 1500, 6000, or 12,000 ppm DINP in the diet, corresponding to 0/0, 29/36, 88/109, 358/442, or 733/885 mg DINP /kg body weight /day for males/females [7]. A "recovery" group received 12,000 ppm for 1.5 yr (78 wk) and then was placed on a diet without added DINP until the 104th week termination. This corresponded to 637/774 mg/kg/day for males/females. No analysis of the diet was reported to confirm the actual levels of DINP. A positive control group received 1000 ppm of Wy-14643, a peroxisome proliferator and carcinogen.

Revised data from this study were presented recently to the CPSC staff by Dr. John Butala [7]. Increased incidences of kidney tumors, mononuclear cell leukemia, and liver tumors were reported, but other neoplastic categories were not. Some authors believed that the lower incidence of liver tumors in the recovery group indicates a reversible process when DINP exposure is removed [6]. However, malignant tumors in humans are not known to be reversible by stopping exposure. Therefore, the "recovery" group data should not be used in the staff's cancer risk prediction process.

Results were not reported for the positive control (Wy-14363) group. The lack of positive control results offers no guide as to whether the bioassay was performing as expected. For example, if the Wy-14363 group had few liver tumors, one could conclude that the experiment was not valid and more tumors might have occurred in the DINP-exposed groups.

Kidney tumors occurring in the 6000 ppm and higher levels in male rats were found to be alpha-2-microglobulin-mediated in a different study [5]. Kidney tumors arising through this mechanism are not considered relevant to humans [18].

F344 rats typically have a high rate of spontaneous mononuclear cell leukemia (36.2-46% control males; 13.3-40% control females in the testing laboratory used by Aristech [3]). The incidence of this leukemia among the test groups (34-49% male, 18-46% female) was not dose-related and was at the upper end of the normal range. For this reason, it was not considered a significant effect.

Incidence data reported by Dr. Butala [7] was based on the 70 or 85 rats initially allocated per dose level per sex. No data on premature deaths were reported and no time-to-death factor was incorporated into the incidence data to adjust for premature deaths. The 12,000 ppm hepatocellular carcinoma incidence jumped to 28% in the males and 8% in the females compared to 2% and 2% for the respective controls. Only the incidence for the males was significantly different from control. The other groups did not experience an increased incidence. The data in the hepatocellular carcinoma+adenoma category were not used since inconsistencies in the data were observed (incidence of carcinoma+adenoma was less than the incidence of carcinoma).

Aristech mouse study

A carcinogenesis bioassay in mice for Aristech also resulted in liver tumors [4]. Dr. John Butala recently presented the results of this bioassay to CPSC staff [7]. B6C3F1 mice were dosed for 2 years with 0, 500, 1500, 4000, or 8000 ppm DINP. The doses were equivalent to an average consumption of 0/0, 90/112, 275/335, 741/910, or 1560/1887 mg/kg/day for males/females.

Data reported by Aristech [7] were based the incidence on the 70 mice that were initially allocated per dose level per sex. Butala indicated a time-to-death factor for prematurely dying mice was used to adjust the incidence. The incidence of hepatocellular carcinoma and combined adenoma plus carcinoma increased in a dose-related manner to a high of 29%/26% (m/f) carcinomas and 44%/46% (m|f) adenomas+carcinomas in the 8000 ppm group compared to controls 14%/1.4% for carcinoma; 23%/4.2% for adenomas+carcinomas.

Body weight depression of 20% in the 4000 ppm group and 40% in the 8000 ppm group was higher than the 10% National Toxicology Program recommendation. The 8000 ppm group's weight depression also exceeds a 20% rule of thumb for maximum tolerated dose. Nevertheless, this does not negate the use of the data for estimating carcinogenic potency [23].

Exxon rat study

Exxon [5,6] fed DINP to F344 rats at 0, 300, 3000, and 6000 ppm for 2 years. This corresponded to average doses of 0, 15, 150, and 300 mg/kg/day but was not reported according to sex. There were initially 80 rats per dose group per sex. Survival was 75-80% in the control groups and 61-70% in the treated groups. Body weights were slightly depressed at 4% and 7% in the 3000 and 6000 ppm male groups, respectively. The body weight depression approaches the 10% National Toxicology Program rule of thumb for dose selection.

Liver tumor categories included "neoplastic nodules" and "hepatocellular cancer." Given the histopathological specificity used in the diagnoses of the other lesions, it is somewhat unusual that these broader terms were used. It will be assumed for this report that these refer respectively to hepatocellular adenoma and hepatocellular carcinoma, which are more specific categories.

The 6000 ppm male rats had (3/81)= 3.7% incidence of hepatocellular cancers with 0% in the controls. This increase was not statistically significant. However, in view of the Aristech results [3], this increase may be near the lowest dose at which tumors may be observed. Female rats in the 6000 ppm group had (1/80)= 1.25% hepatocellular cancer but also had (1/81)= 1.23% in the controls. The data suggests the animals were exclusively categorized to the strongest neoplastic diagnosis. When the hepatocellular cancers and neoplastic nodules are combined, there are no significant differences in liver tumor incidences among the treatment groups.

No differences in the incidence of kidney tumors were found among the groups. Types reported included transitional cell and tubular cell adenomas and carcinomas.

As with the Aristech study [3], mononuclear cell leukemia was within the range of historical control values for this condition, though statistically higher in the 3000 and 6000 ppm groups and was, therefore, not considered a significant effect. The incidence appeared dose-related in the females (22%, 20%, 30%, 43%) but was questionable in the males (33%, 28%, 48%, 51%), respectively, for the 0, 300, 3000, or 6000 ppm groups.

In vitro studies and peroxisomal proliferation

Dr. Jacqueline Smith recently presented cell culture data on interspecies differences of the hepatocellular response to DINP-1, DINP-2, and MINP-1 (monoester of DINP-1) [8]. Intercellular hepatocyte communication was examined by the amount of lucifer yellow dye diffusion through confluent primary hepatocyte cultures and by the number of gap junction and nexus ultrastructures between the cells. These communication parameters were decreased by 1000 ppm and 12000 ppm *in vivo* dietary exposures in rats and mice, but not in monkey hepatocytes. Peroxisomal β-oxidation and DNA synthesis rates were increased in the hepatocytes of exposed rats and mice, but not in monkeys. Stronger effects on gap junctions, peroxisomal β-oxidation, and DNA synthesis were seen with MINP, suggesting that cleavage of DINP to the monoester is a metabolic activating step.

The *In vitro* hepatocellular responses emphasized by Dr. Smith are features of peroxisome proliferators. The data have been interpreted by others as suggesting that phthalates would not be peroxisome proliferators in humans and therefore, unlikely to be carcinogenic [15,17]. Dr. Raymond David, Chemical Manufacturers Association Phthalate Esters Panel, indicated a potential genetic explanation in support of this hypothesis [9]. He mentioned a study at the Chemical Industry Institute of Technology with a "knock-out" mouse variety [19,20] which lacks the gene for the peroxisomal proliferator-activated alpha receptor. In these mice, a peroxisome proliferator failed to induce liver tumors or peroxisomal proliferation after 1 year of dietary exposure. The respective normal mouse variety had tumors and peroxisomal proliferation.

Peroxisomal proliferator-activated receptors have been identified in hepatocytes from various experimental animals and humans. Activation of the receptor appears to be necessarily associated with peroxisomal proliferation [15]. In interspecies comparisons, 4 receptor types and a wide range of receptor responses to phthalates have been found [15]. Alpha receptors in rats and mice show generally greater response to phthalates than hamsters and guinea pigs which are greater than non-human primates.

However, there is insufficient data to show that the receptors and subsequent peroxisome proliferation are directly involved in initiating phthalate carcinogenesis. Hypothesized mechanisms involve generation of hydrogen peroxide by peroxisomes, and enhanced cell proliferation [15,16]. Certain chemicals, e.g. clofibrate, which cause peroxisomal proliferation,

have not been found to be carcinogenic in the human experience, although the dose compared to experimental animals is low. There are also many carcinogens that do not cause peroxisomal proliferation.

Phthalates are worthy of additional future consideration due to the apparent epigenetic mode of action. However, more studies are needed to regard phthalate hepatocarcinogenicity in rodents as irrelevant to humans. A role for peroxisomal proliferation in a carcinogenic mechanism has not been determined. Interspecies comparative receptor binding studies on phthalates are lacking.

NON-CARCINOGENIC EFFECTS

Aristech rat study

Histopathological examination data were not reported for tissues other than liver and kidney in the Aristech study described under Carcinogenicity [2-3]. Spongiosis hepatis, a perisinusoidal cell degeneration, occurred in the male rats in the 1500 and 6000 ppm (33% of the survivors) and 12,000 ppm (56% of survivors) compared to the controls (2%). However, the occurrence in the control group was not stated. In the Exxon study [5], the spontaneous occurrence of spongiosis hepatis was high (25%) so the relevance of the increase in this bioassay is unclear. Other histopathological effects noted were cytoplasmic eosinophilia and hepatocellular enlargement (hypertrophy). Non-neoplastic kidney lesions in the males of those groups included mineralization of the renal papilla, pigment in renal tubule cells, but percentages were not stated.

Only a few of the non-neoplastic parameters in the revised data presentation were listed despite the purported evaluation of several parameters [7]. Butala indicated that only the significant changes were presented and only for dose levels with those changes. No data showed that the no-observable adverse effect levels (NOAEL) selected by Butala were truly without adverse effects. Although serum enzymes indicative of liver damage were elevated in the 12000 ppm groups, the data presented was too limited to use for estimating an acceptable daily intake.

Aristech mouse study

The few non-neoplastic parameters in the presentation of this study were too limited for estimating an acceptable daily intake despite the purported evaluation of several parameters [7]. Butala indicated that only the significant changes were presented and only for dose levels with those changes. No data was presented to indicate that the NOAELs he suggested were truly without adverse effects. The average dose in the higher levels was somewhat lower than expected due to the body weight depression of 20% in the 4000 ppm group and 40% in the 8000 ppm group. This is higher than the 10% National Toxicology Program recommendation. The 8000 ppm weight depression also exceeds the 20% heuristic for maximum tolerated dose.

Exxon rat study

The male controls had a high spontaneous rate of spongiosis hepatis (24/81)= 30%, although a dose-related increase was observed (51/80 in 3000 ppm)= 64%, (62/80 in 6000 ppm)= 78% in treated male groups. This may be somewhat associated with the mononuclear cell leukemia observed in the hepatic sinusoids and slight increases in serum enzyme activities related to liver damage including AST, ALT, alkaline phosphatase [5]. The 6000 ppm males also experienced minor depressions in basic hematological parameters- red blood cell count, hemoglobin, packed cell volume ratio.

Estrogenic activity

DINP and other phthalate esters showed very weak estrogenic activity using recombinant yeast and human breast cancer mitogenesis screening systems [10]. The DINP was supplied by either Exxon or Monsanto and thus may correspond to DINP-1 or DINP-5. The estrogenic potency was estimated at one fifty-millionth that of 17 β-estradiol, a strong, endogenous form of estrogen. DINP was also a weak stimulator of breast cancer cell division.

Reproduction and Fetal development

Reproductive and developmental effects of DINP were noted in an earlier staff assessment [1]. Additional data are reviewed here. Four groups of Sprague-Dawley rats were given diets of 0, 0.2, 0.4, or 0.8% DINP in a study by Exxon [6,21]. No differences were observed in reproductive organ weights, male mating, fertility, fecundity, or gestational indices in P_1 or P_2 generations. Body weights in the F1 and F2 generation were lower, but within the historical control range of the laboratory. The 0.8% diet resulted in an estimated intake of 668 mg/kg/day.

Gavage administration of DINP to female Sprague Dawley rats on days 6-15 of gestation had no effect on live fetuses per litter, resorption frequency, fetal crown-rump length, or fetal weight, according to a summary of an Exxon study [6]. The doses were 0, 100, 500, or 1000 mg/kg/day. The 1000 mg/kg/day group experienced a drop in body weight and inhibited body weight gain. This group also produced an increased incidence of fetuses with rib variations, though none would be physiologically limiting.

Alcohol components which might be related to those released during metabolic cleavage of the ester arms of DINP molecules were tested by gavage administration of isononanol type 1 at 0, 144, 720, 1080, or 1300 mg/kg/day and isononanol type 2 at 0, 130, 650, 975, 1080, or 1440 mg/kg/day on days 6-15 of gestation in Wistar rats [6,22]. Each group had 8-10 animals. Isononanol type 1 consisted of approximately equivalent amounts of 3,4-, 4,6-, 3,6-, 3,5-, 4,5-, and 5,6-dimethylheptanol-1. Isononanol type 2 consisted of 23% 4,5-dimethylheptanol-1, 29%

4-methyloctanol-1, 3% 3-ethylhepatanol-1, 15% 6-methyloctanol-1, and 1% 3-ethyl-4-methylhexanol-1.

Both isononanol types resulted in maternal toxicity, as evidenced by death (10/10 type 1 at 1300 mg/kg/day; 3/10 type 2 at 1440 mg/kg/day), depressed body weight gain at 1080 mg/kg/day (301 vs. 391 g type 1; 345 vs. 391 g type 2), increased resorptions at 1080 mg/kg/day (6.1 vs. 0.4 per dam type 1 only), corpora lutea with 1080 mg/kg/day (14.4 vs 15.8 per dam type 2 only), and/or reduced uterine weight with 1080 mg/kg/day (42.8 vs 81.5 g type 1 only) [6]. Maternal toxic effects did not occur at 720 mg/kg/day with type 1 or at 975 mg/kg/day with type 2. At these levels, incomplete or absent rib ossifications were increased, which increased the percentage of fetuses with developmental retardations (39.4% vs. 21.1% for type 1; 44.0% vs. 21.1% for type 2) [6].

The authors did not judge whether the skeletal retardations would exist at birth or would be physiologically limiting after birth. However, the delayed development suggests a potentially adverse effect of the isononanol components of DINP. No increase in malformations above control levels were observed at 144 mg/kg/day with type 1 and type 2 isononanols. Since the alcohols are about two-thirds the weight of a DINP molecule, this would be equivalent to about (144+ 144/3)= 192 mg/kg/day DINP, assuming 100% ester hydrolysis of the DINP.

Absorption

Longer and more branched alkyl side chains on the phthalate nucleus reduced dermal absorption in male F344 rats [11]. Isodecyl and isooctyl diesters were tested in a series that included ethylhexyl, isobutyl, n-butyl, ethyl, and methyl phthalate diesters. However, no data were located regarding oral absorption of DINP compared to DEHP. In his presentation with the Chemical Manufacturers Association Phthalates Panel to CPSC staff, Dr. David stated that he was not aware of any data regarding comparative oral absorption studies of phthalate diesters [9].

CONCLUSIONS

The acceptable daily intake for DINP is estimated as 0.15 mg/kg/day, based on histopathological changes in male rat livers [5]. The general exposure of humans to DINP has not been estimated. If the usage of DINP was the same as for other phthalates currently in use, oral exposure would be expected from leaching from plastic containers into food, consumption of aquatic animals living in contaminated waters, transfusion of blood or other fluids through medical tubing, and ingestion of contaminated water. No quantitative biomarkers of exposure to DINP or other phthalates are established for humans. It is also not known if the effects of exposure to other phthalates are additive. Therefore, the acceptable daily intake level for DINP cannot be adjusted for exposure from sources other than children's products or other phthalate esters.

The lesions in the rat livers related to DINP are nonspecific but the severity was not described. Without further information, it is assumed that the severity of these lesions was not

slight. The increased incidence of degenerative changes such as focal necrosis and spongiosis indicate some toxic effect is occurring at the higher dose levels. A NOAEL of 300 ppm based on the liver lesions in the Exxon study male rats is selected. This is the same NOAEL identified by Lington [5] and the previous CPSC assessment [1]. The 300 ppm dose level for the male rats was equivalent to 15 mg/kg/day [5]. According to the CPSC chronic health hazard guidelines [12], the acceptable daily intake would then be (15 mg/kg/day /100)= 0.15 mg/kg/day. A LOAEL of 3000 ppm could also be selected based on the liver lesions, but the acceptable daily intake would still be nearly the same (152 mg/kg/day /1000)= 0.15 mg/kg/day. Under the CPSC guidelines [12], the LOAEL would ordinarily be used only in the absence of a NOAEL.

The Aristech information did not provide sufficient information to support the NOAELs identified by Butala [7]. The 8(e) EPA submissions [2,3] have somewhat different data compared to the presentation with the more detailed summary. This was due to re-evaluations by the company. Partial data on several parameters reduced the CPSC staff's ability to consider Butala's NOAELs.

The *in vitro* data and newer studies with knockout gene mice suggests there may be significant interspecies differences in the ability of DINP to cause peroxisomal proliferation and liver cancer [9,19,20]. Some scientists believe that the hepatocellular carcinoma seen in the bioassays is specific to rodents and that the *in vitro* data predicts that humans would be resistant to phthalate-induced neoplasia. However, there is currently insufficient data to conclude that tumors induced by DINP and other phthalates are not relevant to humans. This view is also shared by EPA technical staff [13,14].

REFERENCES

[1] CPSC (1998)- "Preliminary hazard assessment of diisononyl phthalate (DINP) in children's products". Memorandum from MA Babich to RL Medford. 10 March.

[2] Aristech Chemical Corporation (1995). EPA/TSCA 8(e) submission 8EHQ 0794-13083. 13 January.

[3] Aristech (1995). EPA/TSCA 8(e) submission 8EHQ 0794-13083. 20 March.

[4] Aristech (1995). EPA TSCA 8(e) submission 8EHQ 0794-13083. 12 April.

[5] AW Lington, MG Bird MG, RT Plutnick, WA Stubblefield, RA Scala (1997)- "Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats." Fundamental Applied Toxicology 36 (1) 79-89.

[6] Exxon Corporation (1997)- "Compilation and summary of toxicological data for diisononyl phthalate." From the European Union Existing Substances Risk Assessment prepared by the European Chemical Industry Council. Prepared on behalf of the Chemical Manufacturers Association Phthalate Ester Panel and Toxicology Research Task Group. October. From laura.keller@exxon.sprint.com, 732-873-6219.

[7] J Butala (1998)- Presentation of Aristech bioassay data on DINP. CPSC HQ, 8 May. DINP chronic rodent toxicology summary, 19 May.

[8] J Smith (1998)- Presentation of Exxon Biomedical Sciences in vitro data on interspecies differences of DINP and MINP. CPSC HQ, 8 May.

[9] R David (1998)- Presentation of Chemical Manufacturers Association Phthalate Task Group information on estrogenic and other effects of phthalates. CPSC HQ, 8 May.

[10] CA Harris, P Henttu, MG Parker, JP Sumpter (1997)- "The estrogenic activity of phthalate esters in vitro." Environ Health Perspect 105 (8) 802-811. August.

[11] AE Elisi, DE Carter, IG Sipes (1989)- "Dermal absorption of phthalate diesters in rats." Fundam Applied Toxicol 12 70-77.

[12] CPSC (1992)- "Labeling requirements for art materials presenting chronic hazards; guidelines for determining chronic toxicity of products subject to the FHSA; supplementary definition of 'toxic' under the Federal Hazardous Substances Act; final rules." Federal Register 57 (197) 46626-46674. 9 October.

[13] EPA/HERD (1996)- "Mechanisms and EPA regulatory position of peroxisome proliferators." Memorandum from D Lai to P Wagner. 26 January.

[14] EPA/OPPT (1998)- "Comments on the expert panel report on review of DEHP carcinogenicity classification submitted by Bergeson & Campbell, 1/20/98." Memorandum from DY Lai to VT Vu. 21 April.

[15] RC Cattley, J DeLuca, C Elcombe, P Fenner-Crisp, BG Lake, DS Marsman, TA Pastoor, JA Popp, DE Robinson, B Schwetz, J Tugwood, W Wahli (1998)- "Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans?" Regulatory Toxicol Pharmacol, in press.

[16] RC Cattley & RJ Preston (1995)- "Does DNA damage play a role in rodent liver cancer induced by peroxisome proliferators?" CIIT Activities 15 (11). http://www.ciit.org/ACT95/ACTIVITIESNOV95/nov95.html .

[17] GM Williams & C Perrone (1996)- "Mechanism-based risk assessment of peroxisome proliferating rodent hepatocarcinogens." Ann NY Acad Sciences 804 554-572.

[18] CPSC/HSHE (1991)- "Reassessment of the relevance of the male rat kidney tumors induced by p-dichlorobenzene." Memorandum by VH Schaeffer.

[19] S S-T Lee, T Pineau, J Drago, EJ Lee, JW Owens, DL Kroetz, PM Fernandez-Salguero, H Westphal, FJ Gonzalez (1995)- "Targeted disruption of the *a* isoform of the peroxisome proliferator-activated receptor gene in mice results in the abolishment of the pleiotropic effects of peroxisome proliferators." Molec Cell Biol 15 (6) 3012-3022.

[20] FJ Gonzalez (1997)- "Recent update on the PPAR*a*-null mouse." Biochimie 79 139-144.

[21] AI Nikiforov, LH Keller, SB Harris (1995)- "Two generation reproduction study with di-isononyl phthalate (DINP)." Tox Lett Suppl.1/78 61. Eurotox'95 abstract.

[22] J Hellwig & R Jackh (1997)- "Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats." Food Chem Toxicol 35 (5) 489-500.

[23] JK Haseman, JE Huff, GN Rao, SL Eustis (1989)- "Sources of variability in rodent carcinogenicity studies. " Fundam Appl Toxicol 12: 793-804.