

OVERVIEW OF PHTHALATES TOXICITY APRIL 12, 2010

Introduction

The summary indicates that “certain developmental effects of phthalates are believed to be additive. In fact, available data do not support the contention that developmental effects are additive at the environmental levels seen for these products. At present additivity has only been seen at exposure levels at relatively high levels – well above the environmental levels that have been measured.

Chemistry and Use

Diisononyl phthalate (DINP) is not used in teething rings as suggested. It was voluntarily removed from these products over 10 years ago.

Toxicokinetics

Although it is correct that the oxidative metabolites for DINP are preferred biomarkers, it is not correct to suggest that studies using the monoester of DINP (MINP) “lack sensitivity.” As further discussed in the comments on DINP review, exposure estimates can be made from monoester biomonitoring data.

Role of PPAR α

“Phthalate syndrome” is a hypothesis that developmental effects seen in laboratory studies with some phthalates share the same etiology. It is not clear that structure-activity relationships suggest that the effects included in the hypothesis are independent of PPAR α .

The discussion of the CERHR conclusions appears to suggest that “the most severe effects” are observed in male infants. While evidence exists that high levels of phthalates can disrupt normal sexual development in laboratory animals, there is no evidence to suggest that similar effects occur in humans. In fact, studies have not observed developmental effects in male primates exposed to relatively high levels of some phthalates. Similarly there is little evidence to suggest that phthalate exposure results in behavioral effects in female offspring.

Table 5 – DIDP has been examined for adverse effects in male sexual development. In a guideline, 2-generation reproduction and developmental toxicity study, there were no effects on anogenital distance, nipple retention, changes in male reproductive organ weights, or fertility. Therefore, “no effects” should be noted in the table for DIDP.

Developmental Effects of Ortho DAPs in Animals

Evidence for additivity of antiandrogenic effects has only been observed at high exposure levels. Studies at typical environmental level do not suggest additivity.

The summary fails to discuss the primate data suggested that male development is not affected by phthalate exposure.

Table 7 – A discussion of the results for DINP in *Gray et al (2000) and Ostby et al (2000, 2001)* and their use in hazard characterization can be found in the response to the CPSC toxicity review for DINP.

Studies in Humans

The discussion of the study by Swan et al fails to mention several of the study's limitations.

Table 8 – The liver acceptable daily intake (ADI) for DIDP can not be calculated from the dog study. A discussion of this study's limitations can be found in the response to the CPSC toxicity review for DIDP.

Table 8 – The doses cited in the Cho et al (2008) are incorrect and have been updated in a recent announcement (Cho et al., 2010). A discussion of this paper can be found in the response to the CPSC toxicity review for DIDP.

TOXICITY REVIEW OF DI-n-BUTYL PHTHALATE
(DIBUYTL PHTHALATE OR DBP), APRIL 7, 2010

1. The review incorrectly states the conclusion of the 2004 publication by Hauser et al. which describes medications as a source of human exposure to phthalates (discussed in the “Exposure” section on Page 6 of the review). The review states that Hauser concluded that “concentrations of levels approaching a NOAEL of 50 mg/kg/day can possibly contribute to the testicular dysfunction reported to be associated with DBP exposure in other studies.” The paper by Hauser et al. does not draw this conclusion. The conclusion of this paper was that they had:

“identified an individual with a urinary MBP level two orders of magnitude higher than the U.S. population 95th percentile and linked this unusually high urinary MBP . . . concentration with the use of a specific medication that contained DBP. However, because this is a case report on a single patient, replication of this finding in other . . . populations is needed to definitively conclude that the medication was the main contributor to the very high urinary concentration of MBP.”

The CPSC DBP review seems to downplay the fact that Hauser’s paper was a description of a case study involving a single patient. In addition, the misstatement of Hauser’s conclusions gives the reader the incorrect impression that typical daily use of medications may lead to exposures close to the NOAEL.

2. On page 9 of the review, in the section on “Systemic Effects”, the author’s incorrectly use the term “hypospadiac.” The correct name of the disorder is hypospadias.
3. On page 12 of the review, in the summary of the section on “Sensitization,” the last statement is very misleading. The statement that “There is not sufficient data to conclude that DBP is a strong sensitizer under the FSHA” implies that there may be a lesser issue of DBP as a sensitizer. It should be noted that DBP is not classified as sensitizer in the US or EU, and is in fact still listed as an acceptable ingredient in personal care products by the Cosmetic Ingredient Review (CIR) Expert Panel (pages 34-32 of: Annual Review of Cosmetic Ingredient Safety Assessments--2002/2003. Int.J.Toxicol. 24 Suppl 1:1-102, 2005.)
4. There are a few statements in the discussion section, which begins on page 23, which are either mischaracterizations or misstatements of the data on DBP.

- On page 24 of the review, we disagree with the statement that “The human data by Swan et al. showed an association between MBP and anogenital distance (AGD) in male infants.” The Swan study utilized a weight-corrected measure, which was referred to as Anogenital Index, or AGI. The clinical significance of AGI in humans is not known, as it is a measure that was defined by this study.

- In this same paragraph, it is misleading to state at the end that “These studies provide sufficient evidence that DBP can be considered developmentally toxic under the FHSA.” This statement implies that Swan’s data can be used to support an FHSA designation of “developmentally toxic,” while the relevance of this study in supporting an FHSA designation is not clear.

- Once again, the discussion section states that there is not enough evidence “to consider DBP a strong sensitizer.” This misleads the reader to believe that there are sensitization issues with DBP, when no such issues exist.

TOXICITY REVIEW OF n-BUTYL BENZYL PHTHALATE (BBP)
APRIL 7, 2010

BBP Hazard Identification

1.0) INTRODUCTION.

Monsanto is no longer the US producer of BBP. Ferro Corporation is the US manufacturer of BBP.

The Introduction should state that “when BBP is added during the manufacture of a vinyl product, it is not bound to the final product”.

2.0) TOXICOKINETICS.

It is not clear from the description of Elsis, et al., what dermal absorption rate CPSC intends to use for BBP. Elsis, et al., have been criticized for not including a detailed description of exposure conditions in their dermal absorption work. At least one group, The Institute for Human Health and Consumer Protection of the European Union, has concluded that the dermal absorption rate of BBP is 5% (EURAR, 2007).

Citing Nativelle, 1999, the CPSC discussion of BBP metabolism appears to suggest that the observed ratio of the two major BBP metabolites in rats, monobutyl phthalate (MBuP) and monobenzyl phthalate (MBzP) is due to something other than different rates of hydrolysis of the ester.

Nativelle, et al., 1999 showed that rats receiving from 150 to 1500 mg/kg BBP orally produced 2.88 to 4.14 times more MBuP than MBzP. In addition to Nativelle, et al., Eigenberg showed that rats preferentially metabolize BBP to MBuP 2.75:1 (Eigenberg, et al.,1986). Monsanto (Monsanto, 1997) showed that a different strain of rat, the Alpk:APfSD, produced 2.1 to 5 times more MBuP than MBzP following oral administration and 3.6 to 4 times as much MBuP as MBzP following subcutaneous administration. Dogs, alternatively, receiving an oral dose of 5000mg/kg BBP excreted 88% and 91% unchanged (male vs female, respectively) and 4% as phthalic acid (Erickson, 1965). This point is notable because important species differences are known to exist in BBP metabolism. Anderson, et al., (2000) reported on human volunteer tests with deuterated BBP. In their study, which employed only 7 volunteers per group, the ratio of MBzP to MBuP ranged from 10 to nearly 100 in low to high-dose groups. Humans, therefore, preferentially hydrolyze BBP in the opposite manner of rats.

3.0) IRRITATION/ALLERGIC RESPONSE

This section cites Bornehag, et al. and Kolarik, et al. which allege but do not address, let alone establish, causality between BBP and allergy or asthma. Each study relies on an association between indoor airborne levels of BBP and the incidence of allergic rhinitis in children. The association reported by Kolarik, et al, was not statistically significant. Neither

research team was able to isolate BBP exposures and all groups in the studies had mixed exposures to a number of different environmental agents. Also, the diagnostic approach for allergic rhinitis differed across study cohorts. Work by Butala, et al. (2002, 2004) showed that phthalates including BBP do not act as respiratory sensitizers in a mouse model developed to predict humans respiratory sensitization and asthmagenicity. This work should be included in the CPSC report on BBP.

4.0) ENDOCRINE EFFECTS

This section presents reports of BBP or metabolites of BBP activities in PPAR receptor assays. The section also describes human subject studies. The discussion of Main, et al, 2006 should include information on the size study cohort (130 women) and that a large variation was seen in breast milk levels of phthalate metabolites included MBuP and MBzP. The CPSC summary mentions that no correlation was found between phthalate levels and cryptorchidism and that several serum hormone levels correlated with MBuP but does not mention that correlations were not seen with MBzP.

The discussion on Duty, et al, 2003 and 2005, should point out the number of study subjects (Duty, et al., 2005) was 295 and all were patients at an andrology clinic. Assessment of phthalate levels were based on a single spot urine test. Likewise, Huang, et al., 2007, also used a very small study cohort, 76 subjects and relied on a single serum and spot urine analysis for phthalate assessment. The subjects in Huang, et al., were all patients in an amniocentesis clinic because of abnormal levels of alpha fetal protein or choriogonadotropic hormone or because of age at pregnancy (35 years of age or older). Each of these human subject studies should be reviewed and interpreted with caution because of the small study cohort, limited sampling of phthalate levels and the lack of representativeness of the study subjects to the general population.

The CPSC document presents a discussion of Lampen, et al., but fails to mention a more recent study by Bility, et al (2004). Bility, et al., tested monoesters of various phthalates including BBP with mouse and human PPAR α , β , γ in transactivation assays, target gene expression assays and PPAR γ mediated differential assays. Although active with mouse PPAR β , MBuP and MBzP showed no activity with human PPAR β . With PPAR α and PPAR γ MBuP and MBzP were either not active or 3-10 times less active with human PPAR than mouse PPAR. This information should be included in the CPSC toxicity review for BBP. Moreover, the statement on page 12 of the CPSC review that "BBP can act like hormones" should be modified to account for the data discussed above and for the CPSC statement appearing on page 13 of the CPSC document, "At this time it cannot be concluded that BBP caused detrimental hormone effects" (in humans).

5.0) REPRODUCTIVE EFFECTS

The CPSC cites a 10-week rat study by NTP (1997) and identifies 20 mg/kg/day as the NOEL and relate this to decreased sperm concentration (LOAEL = 200mg/kg/day). However,

the 1997 NTP report that BBP decreased caudal epididymal spermatozoa concentration in a 10-week feeding study could not be replicated by NTP in their 26-week feeding study. In that study, also a 1997 study, a change in sperm concentration was not seen in rats dosed for 26 weeks at 30, 60, 1180 or 550 mg/kg/day and only occurred at dose of 1660 mg/kg/day. The CPSC report does not mention the NTP 26-week study.

The most comprehensive study of BBP reproductive toxicity is the study of Tyl, et al. (2004) which establishes an F0 and F1 parental systemic and F1 reproductive no observable adverse effect level (NOAEL) of 3750 ppm (~250 mg/kg/day). The offspring toxicity NOAEL issuing from that study was 3750 ppm (~250 mg/kg/day), and the offspring toxicity no observable effect level (NOEL) was 750 ppm (~50 mg/kg/day), based on reduced AGD in F1 and F2 males at birth at 3750 ppm, with no effects on reproductive development, structures, or function. From these studies, the reproductive NOEL for BBP should be no lower than 50 mg/kg/day and arguably much higher. The actual NOEL in the Tyl study lies somewhere between 250 and 50 mg/kg/day, based on AGD differences in pups seen at 250 mg/kg/day. Those effects are of questionable health significance, given the absence of effects in those pups on reproductive development, structures or function, indicating that 250 mg/kg/day could be used as a conservative NOAEL.

6.0) DEVELOPMENTAL EFFECTS

When describing the animal studies the CPSC review should state that “the effects most commonly seen were reduced AGD in males, undescended testes, reproductive tracts malformations, male nipple/areolae retention, and reduced fertility” are seen in rats and not other species. Paul Foster’s 2006 article mentioning the “Phthalate Syndrome” and cited in the CPSC review refers to effects in rats. The Saillenfait article, also cited in the CPSC review, discusses effects in mice and rats but the effects reported by Saillenfait, et al., are closure defects and other malformations that do not include the suite of male reproductive tract anomalies associated with high-dose phthalate effects in rats.

7.0) GENOTOXICITY AND CARCINOGENICITY

The CPSC Toxicity Review claims that it presented studies that “provide strong data showing BBP can alter the expression of genes and have carcinogenic activity”. In fact, the CPSC review presented a single study of poor quality (Morel, et al, 2007) and refers to it as “studies” investigating the potential of BBP to alter genes and gene expression. In taking this approach CPSC ignored numerous mutagenesis and genotoxicity studies of BBP. These studies are listed in the table below. The table appears in the 2007 European Union risk assessment for BBP and is reproduced in its entirety. No studies were omitted. All are negative except one that is considered ambiguous or negative and one that showed weak SCE and chromosome aberration activity *in vivo* at several time points. The EU assessment concluded that “Based on the available data, and according to EU criteria, BBP should not be considered a mutagen.”

Morel, et al, 2007 dosed female rats with 500mg/kg/day BBP via oral gavage following parturition on pnd 2-20. Female pups were collected and used for various assays on pnd 21, 35, 50 or 100. Data on body weight, uterine weight and date of vaginal opening were obtained for a subset of these female pups but the time (pnd day) these data were collected is not provided in the report. No bodyweight data are not provided in the report. This omission is important since only relative uterine weight was changed (increased) and vaginal opening co-varies with body weight. The authors' claim that BBP increased uterine weight/body weight ratios at pnd 21 and decreased body weight at the time of vaginal opening are difficult to assess because body weight data (or point when it might have been collected) are not provided and the age and body weight of the animals when vaginal patency occurred is also not provided. From the data presented it can be seen that uterine weight was not affected by BBP treatment. What is not clear is the impact of reduced body weight gain that may have occurred in lactating pups. Food consumption during lactation is not reported but it is known that lactating rats begin to self feed after the first week of nursing as they continue to nurse. Self-feeding would not have contributed to the pup intake of BBP but could have been reduced as a result of lactational transfer of BBP and contributed to weight gain suppression. Pup body weight and the age of the pup when body weight was measured are important co-variables for the parameters the authors claimed were impacted by BBP. In the absence of these data it is difficult to assess growth effects presented by the authors. This is particularly the case with uterine growth since Zacharewski, et al.(1998) reported on a definitive uterine growth assay and showed that BBP at oral doses of 20, 200 or 2000mg/kg did not affect uterine weight in immature ovariectomized rats nor did it affect the degree of vaginal epithelial cornification in mature ovariectomized rats.

With regard to mammary gland effects, BBP has been tested in lifetime carcinogenicity bioassays in rats (and mice) at the maximum tolerated dose and did not produce an increase in incidence of mammary tumors in either species at any dose. In fact,. The only finding in mammary tissue associated with BBP was a dose-related reduction in spontaneous mammary tumor rates seen in rats (NTP, 1982).

Contrary to what the CPSC says, the data carcinogenicity data base for BBP does not provide significant evidence for carcinogenic activity. NTP concluded after its 1982 cancer bioassays that BBP was probably carcinogenic for female rats and was inconclusive for carcinogenicity for male rats and negative for carcinogenicity in male and female mice. Following the 1997 bioassays, NTP concluded that there is some evidence of carcinogenic activity in male rats and equivocal evidence in female rats. In looking at the same data base as cited by CPSC, IARC concluded in 1999 that BBP is not classifiable as to its carcinogenicity to humans (Group 3).

There appear to be inconsistent statements regarding mutagenicity and carcinogenicity of BBP in the CPSC summary. The final paragraph of the Genotoxicity and Carcinogenicity section begins with the statement, "(T)hese studies provide strong data showing BBP can alter the expression of genes and have carcinogenic activity". The final statement of that paragraph says, "the data is (sic) considered limited, and is not sufficient to consider BBP carcinogenic under FHSA". These statements are repeated in the Discussion section. Based on the

conclusion, i.e., that data are limited and not sufficient to consider BBP carcinogenic under FHSA. the initial statements appear to overstate the case for BBP genetic and oncogenic effects.

IN VITRO AND IN VIVO GENOTOXICITY STUDIES COMPLETED ON BBP*

Study Design	Results	Reference
In vitro, prokaryotes and lower eukaryotes		
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.1, 1.0, 5.0, 10.0 µl BBP/plate	Negative	Monsanto (1976b)
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.001, 0.01, 0.1, 1.0, 5.0, 10.0 µl BBP/plate	Negative	Monsanto (1976c)
Salmonella typhimurium Strain TA98, TA100, TA1535 and TA1537; S9 +/-; 100, 333, 1,000, 3,333 and 10,000 µgBBP/plate	Negative	NTP (1997)
In vitro, Mammalian cells		
E. Coli; 30 mg/plate; mutation test.	Negative	Omori (1976), original data Kurata (1975)
B. Subtilis; 30 mg/plate; repair test.	Negative	Omori (1976), original data Kurata (1975)
Saccharomyces cerevisiae Strain D4, S9 +/-; 0.1, 1.0, 5.0, 10.0 µl BBP/plate; mutation test.	Negative	Monsanto (1976b)
Mouse lymphoma cells L5178Y TK; S9 +/-; 0.08, 0.16, 0.32, 0.65, 1.25, 2.5 or 5.0 µl BBP/ml, insoluble at 1.25, 2.5 and 5.0 µl/ml. Mutation test	Negative	Monsanto (1977d)
Mouse lymphoma cells L5178Y TK; S9 +/-; 5, 10, 20, 30, 40, 60 nl BBP/ml. Mutation test	Negative	NTP (1997)
Chinese hamster ovary (CHO) cell; S9 +/-; Up to 1,250 µg BBP/ml; CA and SCE assay.	Negative or ambiguous	Galloway (1987)
Syrian hamster embryo cells; 25, 50, 100, 150, 250 µg BBP/ml in the 7 days study, 1,2 5,10 and 20 µg BBP/ml in the 24 hours study, precipitation at conc. ≥ 25 µg BBP/ml. Cell transformation test	Negative in the 24 hours study; positive at 2.5 and 10 µg/ml in the 7 days study	Le Boeuf (1996)
BALB/3T3 cells; 10, 20, 40, 80, 160 nl BBP/ml.	Negative	Monsanto (1985)

Transformation test		
In vivo		
Drosophila melanogaster; 250, 10,000 and 50,000 ppm BBP in feed; sex-linked recessive lethal mutation	Negative	Valencia (1985)
Alpk:APfSD (AP) rats. 182.6 µg/kg/day of BBP during gestation and lactation; 19 rats; micronucleus	No induction of micronucleus	Ashby et al. (1997)
B6C3F1 mice, CD-1 mice; 400-600, 1,280-1,840, 3,200-4,560 mg BBP/kg bw, s.c.; dominant lethal mutations.	No increase in foetal deaths	Bishop (1987)

* Entire table reproduced from European Union Risk Assessment report for BBP (2007), pp 150-151.

Full citation for references cited in table can be found in the EU report.

REFERENCES

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Bility, M. T., J.T. Thompson, R.H. McKee, R.M. David, J.H. Butala, J.P. Vanden Heuvel and J.M. Peters. (2004) Activation of mouse and human peroxisome proliferators-activated receptors (PPARs) by phthalate monoesters. *Toxicol Sci.*, 82:1, 170-182.

Butala, J.H., R.M. David, G. Gans, R.H. McKee, T.L. Guo, V.L. Peachee, K.L. White, Jr. (2004). Phthalate treatment does not influence levels of IgE or TH2 cytokines in B6C3F1 mice. *Toxicology*. 201, 77-85.

Butala, J. H., R.M. David, G. Gans, R.H. McKee and K. White, Jr. (2002) Evaluation of Respiratory Sensitization Potential of DEHP. *Toxicological Sciences Late Breaking Abstracts Supplement*, 66 (S-1) p. 22

Erickson NG (1965). The metabolism of diphenyl phthalate and butylbenzyl phthalate in the beagle dog. *Dis. Abstr.* 26, 3014-3015.

EURAR (2007). European Union Risk Assessment Report on BBP. European Chemical Bureau, Institute for Human Health and Consumer Protection, Volume 76, European Commission Joint Research Center, Ispra, Italy.

NTP National Toxicology Program. NTP-80-25, NIH Publication No. 82-1769. (19820 Technical Report series no. 213. Carcinogenesis bioassay of butyl benzyl phthalate in F344/N rats and B6C3F1 mice (feed study).

Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR and Matthews JB (1998) Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. *Toxicol. Science.* 46, 282-293.

TOXICITY REVIEW
OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP)
APRIL 1, 2010

1. In the executive summary, the statement that “Sufficient animal data existed to support the conclusion that DEHP was a carcinogen and a reproductive and developmental toxicant” should be clarified to reflect that while NTP still makes this conclusion, IARC considers DEHP to be Group 3 – not classifiable as to its carcinogenicity to humans.
2. In the “Introduction” section on page 1 of the review, it is stated that the review is “intended to be utilized as part of an individual and cumulative phthalate risk assessment.” We believe that this statement is somewhat premature, as the panel has not made a decision as to the role of the cumulative risk approach in their review.
3. On page 5, in the “Manufacture, Supply, and Use” section, the statement in the first paragraph that “DEHP can also contain bisphenol A (CAS No. 80-05-7) at concentrations ranging from 0.025 to 0.5%.” is very misleading. This statement is made immediately after a discussion of the impurities that are typically found in DEHP, incorrectly implying that bisphenol A is an impurity of the DEHP manufacturing process or that BPA is part of the DEHP manufacturing process. The cited source (ECB, 2008) actually states that “Some DEHP is, when requested by the user, supplied with ‘Bisphenol A’; 4,4’-isopropylidenediphenol (CAS No. 80-05-7) as an additive in the range of 0.025 to 0.5%.” While we are not aware of the sale of such a product where bisphenol A is intentionally added to DEHP. Regardless, it is both unfair and scientifically unjustified to cloud the review of DEHP with this mention of bisphenol A.
4. On page 6, also in the “Manufacture, Supply, and Use” section, the statement that “DEHP uses can be divided into two categories: 1) use as a polymer, and 2) use as a nonpolymer” is incorrect. DEHP is used *in* polymers and in non-polymer applications. DEHP is not a polymer, nor does it polymerize under normal use conditions. It is slightly troubling to see such a blatant error in the basic description of the uses of DEHP.
5. On page 29, in the “Metabolism: oral exposure” section, it is stated that dimethyl phthalate (DMP) is a “major urinary metabolite” in both mice and hamsters (Albro, et al., *J. Chromatography*, 244: 65-79, 1982). To the best of our knowledge, this is not in

agreement with the currently accepted understanding of the metabolism of DEHP. This reference to DMP as a metabolite of DEHP appears to be unique to this particular journal article, and is not supported by any other studies.

6. On page 38, in the “Hazard Information” section, the discussion of carcinogenicity data does not mention the fact that IARC currently considers DEHP to be in Group 3, “not classifiable as to its carcinogenicity to humans.” While the National Toxicology Program’s Report on Carcinogens supports the review’s conclusion that DEHP is a “possible human carcinogen”, not presenting the IARC designation for DEHP unfairly ignores an entire wealth of research and discussion regarding the potential mechanism of DEHP-induced carcinogenicity in rodents, and the relevance of these mechanisms to humans.

TOXICITY REVIEW OF DIISONONYL PHTHALATE (DINP)
APRIL 7, 2010

This appendix provides comments on the toxicity review of DINP produced by CPSC staff and posted to the CPSC website (conforming changes also should be made to the CPSC staff Overview of Phthalates Toxicity).¹ Unless otherwise noted, page citations are to that document. If a document cited in our comments is among the references to the toxicity review, we do not repeat that citation here. Additional references are given at the end of the section in which they are cited.

Overall Remarks

The toxicology review is comprehensive with respect to the DINP database. However, it also includes tangential information on other phthalates and even other un-related chemicals. This is inappropriate since the purpose of the document is to address “potential toxicity associated with diisononyl phthalate (DINP)”. The extraneous information adds an additional layer of complexity that can be misinterpreted, leading to inaccurate conclusions on DINP.

Chemistry and Use

Chemistry

The second sentence of the second paragraph of the Chemistry subsection (p. 4) states: “DINP-1 is also known by the trade name Jayflex®.” The Jayflex line includes a variety of plasticizer products and, while a trademark, the name is not registered. The sentence should read: “DINP-1 is also known by the trade name Jayflex™ DINP.”

Acute Toxicity, Skin and Eye Irritation

Sensitization

The second paragraph of the Sensitization subsection (p. 8) states that there was no evidence of dermal irritation in the human repeated insult patch test (HRIPT) for DINP. It should be noted that there also was no evidence of sensitization.

Toxicokinetics

Oral Toxicokinetics

The subsection on human oxidative metabolites states: “In earlier biomonitoring studies, MINP was non-detectable in most individuals, which led to the conclusion that human exposure to

¹ CPSC Health Sciences (2010). Toxicity Review of Diisononyl) Phthalate (DINP). Memo from M. Babich and C. Osterhout to M. Danello, April 11, <http://www.cpsc.gov/about/cpsia/toxicityDINP.pdf>; CPSC Health Sciences (2010). Toxicity Review of Diisononyl) Phthalate (DINP). Memo from M. Babich to M. Danello, April 12, <http://www.cpsc.gov/about/cpsia/phthalover.pdf>.

DINP was low. However, studies based on MINP may underestimate human exposure. OH_MINP and CO₂-MINP are more sensitive and should lead to more accurate estimates of exposure” (pp. 11-12, citations omitted). The logic in these statements is faulty. The fact that oxidative metabolites are more sensitive does not necessarily mean that the MINP studies have underestimated exposure. Because of the addition of oxygen atoms to the metabolic forms, the oxidative metabolites from a given aliquot of DINP will give a higher concentration of oxidative metabolites, in micrograms per liter of urine, than of MINP. If the detection level in ug/L is the same for both types of metabolites, then the oxidative metabolites are more likely to be detected, but it does not follow that the DINP exposures are higher than those indicated by MINP. In fact, conversion of oxidative metabolite concentrations to the associated DINP exposures gives exposure levels very similar to those indicated by MINP concentrations. While the oxidative metabolites have enabled quantification of DINP exposure in a larger percentage of the population, those quantified values are very similar to those obtained using MINP, and they still show that that exposure is very low. See Attachment 10 (Human Exposure to Diisononyl Phthalate (DINP)), Tables 1-3, which provides exposures estimated from both MINP and oxidation metabolite data.

Percutaneous Absorption

Given the data available for DINP, the inclusion of discussion of “Other Phthalates” is not necessary and therefore not appropriate. The Elsis et al. (1989) study demonstrated that dermal absorption decreases with increasing alkyl chain length and that absorption of DIDP is ten-fold less than that of DEHP. DINP was not studied by Elsis et al., but the indication is that dermal absorption of DINP would be between that of DEHP and DIDP. This is born out by the results of the Stoltz and El-hawari studies of DINP discussed in this subsection.

Systemic Health Effects

Overall comment on primate data

The toxicity review includes a mention of the primate studies on DINP in the subsection on liver effects. However, these studies deserve more comprehensive discussion with respect to systemic effects in general. The 14-day oral study in monkeys (Pugh et al., 2000) and 13-week oral study in marmosets (Hall et al., 1999) show that orally administered DINP has no serious adverse systemic effects in primates at concentrations up to 2500 mg/kg/day. In particular, there were no changes in the liver or kidney weights and no treatment-related changes in histopathology. Systemic effects unquestionably would have occurred in rodents at such doses.

Although the primate studies used a small number of animals and were no longer than 13 weeks, they nevertheless provide valuable information about the likelihood that effects observed in rodents would occur in humans exposed to DINP. First, primates are much more closely related to humans than are rats (e.g., Lindblad-Toh, 2004). Thus, the lack of effects in primates is highly probative evidence that humans are refractory to systemic effects from DINP. Second, a 13-week, or even 2-week, study is sufficient to observe systemic effects in rodents. For example, liver and kidney weights were increased in a 28-day study of rats (BIBRA, 1985). Liver weight increases were seen as early as 1 week after the beginning of treatment in the rat

chronic bioassay (Moore, 1998a). Thus, the primate studies were of sufficient length to assess the potential for DINP treatment to influence liver and kidney weights. That such effects were not seen in the primates at doses and durations that would cause such effects in rodents strongly indicates that humans likely would not be affected by DINP in the manner of rodents.

Because the primate data are highly relevant in assessing the potential toxicity of DINP to humans, those data should be more completely and prominently discussed in the toxicity review.

K. Lindblad-Toh (2004). Genome sequencing: Three's company. *Nature* 428, 475-476, Figure 2 (Mammalian evolution and genome sequencing), http://www.nature.com/nature/journal/v428/n6982/fig_tab/428475a_F2.html.

Spongiosis Hepatis

While a good discussion of spongiosis hepatitis is presented, two key pieces of information are overlooked. First, Karbe and Kerlin (2002), and Anthony (2001) provide evidence that spongiosis hepatitis is a spontaneous degenerative change seen in aging rats without a counterpart in human hepatic pathology. Careful review of rodents over the last twenty or more years by the National Toxicology Program has led to only a rare incidence of neoplasms arising from stellate cells in mice (13 cases from more than 90,000 mice), but these lesions differ morphologically from spongiosis hepatitis. There was no evidence of a lesion resembling spongiosis hepatitis in a review of 163 human livers (Su et al., 1997). Indeed, in the chapter on liver neoplasia from a definitive text on human liver disease, *Pathology of the Liver*, edited by MacSween et al., the authors state: "To the best of our knowledge no human counterpart of the spongiotic pericytoma [spongiosis hepatitis] has ever been described" (Anthony, 2001). Reports of lesions with similar characteristics in humans or non-human primates also are not found in the literature. This lesion or lesions with similar appearances are not described in any of a number of standard texts on neoplasia or systemic pathology in domestic animals, and there are no reports of this lesion in dogs. The only other species in which this lesion has been reported is the teleost fish (Couch, 1991). Given the large number of laboratory dogs and primates that have been exposed to a broad variety of chemicals over a considerable number of years, the absence of descriptions of this lesion would support the view that spongiosis hepatitis is primarily confined to male rats and teleost fish.

Attachments A-1 and A-2 are evaluations by two separate experts in liver pathology, Dr. John Cullen and Dr. Dawn Goodman. Drs. Cullen and Goodman provided these evaluations to the ACC Phthalate Esters Panel with respect to a toxicity review of DINP conducted by the US Environmental Protection Agency.² After reviewing the relevant information, both Drs. Cullen and Goodman conclude that spongiosis hepatitis is not a serious liver effect, even in rats. Dr.

² The Cullen and Goodman opinions were included in comments on the EPA's toxicological review of DINP, submitted to EPA in 2005 by the Phthalate Esters Panel, in response to EPA's notice of opportunity for comment (70 Fed. Reg. 34437 (June 14, 2005)). To date, EPA has not issued a revised toxicological review nor responded to the comments received in response to its notice.

Cullen's opinion also addresses liver enlargement and liver enzyme induction in rats treated with DINP.

As discussed on page 21 of the CPSC toxicity review, the studies by Lington et al.(1997) and Moore (1998a), contain methodological difference such that the Lington et al.(1997) had an inherently higher probability of finding spongiosis hepatitis in a pathological assessment; thus making the comparison of the studies difficult. As per Babich and Green (2000), the studies can be modeled in a manner that normalizes the methodological differences. As demonstrated in Table 5-5 of the toxicity review (p. 25), when the Lington and Moore studies are scaled to a commonality of 4 slides per liver, it is clear that the NOAEL is at least 88 mg/kg/day, such that use of 15 mg/kg/day as a NOAEL is very conservative.

J Couch (1991). Spongiosis Hepatitis: Chemical Induction, Pathogenesis, and Possible Neoplastic Fate in a Teleost Fish Model. *Toxicol Pathol* 19(3):237-250.

P Anthony (2001). Tumours and tumour-like lesions of the liver and biliary tract. In: R MacSween, A Burt, B Portman, K Ishak, P Scheuer, P Anthony (editors). *Pathology of the Liver*. 4th edition, Churchill Livingstone, London.

Q Su, A Benner, W Hofmann, G Otto, R Pichlmayr, P Bannasch (1997). Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia. *Virchows Arch* 431:391-406

Primates

As noted above, the limitations of the primate studies do not negate the valuable information the primate studies provide. The toxicity review notes that "histopathological effects were reported to occur in rats treated for 13-weeks at doses of at least 584 mg/kg/day (Myers, 1991)" but does not discuss the implication of that statement. The lack of liver effects in the primates even at 2500 mg/kg/day for that same length of time indicates that humans are unlikely to experience liver effects even at DINP exposures far in excess of likely exposures.

Endocrine Effects – Animals

The statement on page 30 that "reduced testicular weights, in the absence of histopathological effects, were reported in B6C3F1 mice (Bankston, 1992) and in Fischer 344 rats (Myers, 1991) given \geq 1.0 percent DINP in feed for 13 weeks" is incorrect. In the aforementioned rat study, there was no decrease in testis weight. Further, an *increase* in absolute testis weight in the absence of histopathological findings has been more commonly reported for rats (Lington et al., 1997).

As discussed in Attachment 3 (ECPI Toy Reassessment), the Lee and Koo (2007) study data overall indicate that DINP does not meet the OECD criteria for an androgen antagonist.

Summary

The second paragraph (p. 35) concludes that the NOAEL for systemic effects is 15 mg/kg/day, based the Lington study. However, as discussed above, the combination of the Lington and Moore studies clearly shows that 88 mg/kg/day is a NOAEL.

Reproductive and Developmental Effects

Developmental Effects

On pages 40-43, there is a lengthy discussion on *ortho*-dialkyl phthalates. This information is better suited to the introduction document on phthalates and should be removed since it does not provide information specific to DINP, the purpose of this document.

Gray et al.(2000) and Ostby et al.(2000, 2001)

Gray et al. (2000) conducted a study on the effects of fetal exposure during the late gestational period to DINP and several other phthalates. Timed-pregnant rats were gavaged daily with a single dose of 750 mg/kg/d in corn oil as vehicle from gestational day 14 through postnatal day 3. Data for DINP indicated that at 13 days of age, male pups with retained areolas were observed at an incidence of 22% compared with controls. However, in this study the control incidence for areola retention was reported to be zero, whereas in a subsequent study, control values are reported as 14% (Ostby et al., 2001).

Some of the adult males exposed perinatally to DINP (4/52 pups) had malformations of testis, epididymis, accessory reproductive organs and external genitalia. The low incidence of reported effects was without any dose response and with effects of unclear significance using a small number of rats.). No single endpoint (nipple retention, epididymal agenesis, fluid filled testes, and testes weight) on its own was significantly different from control values. Only by pooling of these different effects, giving a 7.7% incidence, was statistical significance demonstrated. This type of data manipulation is not routinely performed in toxicological safety evaluations, nor is it considered good statistical practice. It should also be noted that Gray et al. (2000) did not see any effects on anogenital distance or on reduction of testosterone levels in the blood with DINP treated animals. Based on the above points, the significance of the reported findings is questionable.

Hass et al. (2003)

This should not be included in the review of DINP since it is only an abstract given at a scientific meeting. The data were never published or made available for review. Furthermore, the abstract states that when birth weight was included as a covariate, AGD was significantly decreased only at the extremely high dose of 900 mg/kg/day.

Borch et al. (2003, 2004)

In a study designed to test effects on testosterone synthesis, 32 pregnant female rats were exposed to either 300 mg/kg-bw DEHP or 750 mg/kg-bw DINP, alone or in combination, from gestation day 7 to gestation day 21 (Borch et al., 2004). The dams were sacrificed on gestation day 21 and the pups were harvested for analysis of testicular testosterone production, testicular testosterone content, plasma testosterone levels, and plasma luteinizing hormone

(LH) levels. The results indicated that testicular testosterone production and testicular testosterone content were significantly decreased in the DINP-exposed pups while plasma testosterone and plasma LH levels were unaltered. However, no mechanism of toxicity can be determined from this paper since it is limited by several confounding factors. First, there were no adverse phenotypic effects reported in the study. Second, the authors sampled testosterone levels on gestation day 21, a time point after the developmental surge of testosterone that occurs during gestation day 16-18 in the rat. After gestation day 18, plasma testosterone levels are naturally declining in the fetal rat. Therefore it is unclear if the decrease in testosterone content is in fact a toxicologically significant response. Compare the results of Adamsson et al. (2009) (no significant increase in testosterone levels with dosage on days 13.5 through 17.5).

Swan et al. (2005)

It is inappropriate to include this study since it does not deal with DINP. Furthermore, this report has been heavily criticized by scientists and statisticians (e.g. McEwen and Renner, 2006). In evaluating Swan et al. (2005) in a recent review of DEHP, an expert panel of the NTP CERHR considered the AGI measurement developed by Dr. Swan to be a “novel index” whose relevance in humans “has not been established” (CERHR, 2005). To date, Dr. Swan’s results have not been repeated by other researchers and Dr. Swan has declined requests by other scientists to review her data.

From a toxicological perspective, there is a difficulty in that the strongest association was with diethyl phthalate – a substance which when tested in rats had no effects on the development of the male reproductive system. From a clinical perspective, the attempt to convert AGD into a kind of index for adverse human health effects is not recognized in human biology. Further there are questions regarding the legitimacy of the AGD measurements in this study, particularly whether there was adequate compensation for the wide variations in age and weight of the measured infants (assuming that the infants could be accurately measured in the first place). As McEwen and Renner (2006) note: “Because little is known about AGD in human infants and its variation, no conclusion can be drawn whether the reported values are normal or abnormal. The range of AGD values seen among study subjects likely represents typical biologic variation that would be expected to occur among normal study subjects.”

Recently, in an updated study, Swan used a revised mathematical analysis for measuring changes in AGD and applied this new methodology to a further population of infant boys. Interestingly, the new data set resulted in contradictory results to some of the previous findings, adding further doubt to the validity of the studies (Swan, 2008).

CERHR (2005). NTP CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) Phthalate, NTP-CERHR-DEHP-05, November 2005, p. 97,
<http://cerhr.niehs.nih.gov/evaluations/chemicals/phthalates/dehp/dehp.html>.

G McEwen, G Renner (2006). Validity of Anogenital Distance as a Marker of in Utero Phthalate Exposure. *Environ Health Perspect* 114:A19-A20.

S Swan (2008). Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environ Res* 108(2):177-84.

Zhang et al.(2009)

It is inappropriate to include this study since it does not deal with DINP and there is no basis on which to extrapolate the findings to DINP.

Main et al.(2006)

This study evaluated exposure to DINP in an attempt to associate phthalate monoester levels with reproductive hormone levels and cryptorchidism in male infants, although no such association was observed. Pooled milk samples were obtained from each of 130 women when their children were 1-3 months old. Milk was analyzed using HPLC-MS for the monoesters of di-(2-ethylhexyl) phthalate, di-methyl phthalate, di-n-butyl phthalate, butylbenzyl phthalate and DINP. There were no significant differences in milk phthalate concentrations between the 62 mothers of sons with cryptorchidism and the 68 controls. The children had venous blood sampled at 3 months of age for determination of sex hormone-binding globulin, total and free testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and inhibin B. Individual hormone levels were used to calculate LH/testosterone, LH/free testosterone, and FSH/inhibin B ratios. MINP was found in all milk samples.

Of the parameters tested, MINP was significantly associated with increased serum LH levels. The authors implied that testosterone levels were likely decreased relieving the negative feedback to the pituitary and thereby increasing LH levels. However, no alteration in free or total testosterone was observed (in fact an increase in free testosterone was observed). Further, this association could simply have been a statistical consequence of multiple comparisons to a common control. Overall, the authors concluded that there were “subtle, but significant, dose-dependent associations between neonatal exposure to phthalate monoesters in breast milk and levels of reproductive hormones in boys at three months of age.”

In 2005, the NTP CERHR evaluated this study and indicated a number of weaknesses including confounding and possible contamination of breast milk samples (CERHR, 2005). According to Calafat et al. (2004b), a special treatment of the milk is required upon sample collection to denature milk enzymes and avoid overestimating the concentrations of phthalate metabolites in milk caused by contamination from the ubiquitous phthalate contaminants that may have been incorporated in the milk during the collection, storage, and measurement process. These considerations limited the usefulness of this study in the NTP CERHR evaluation process.

CERHR (2005). NTP CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) Phthalate, NTP-CERHR-DEHP-05, November 2005, pp. 7, 55,

<http://cerhr.niehs.nih.gov/evaluations/chemicals/phthalates/dehp/dehp.html>.

Toxic Effects of DINP

Liver

The toxicity review states, “DINP is considered to be ‘probably toxic in humans’” on the basis of liver effects observed in the rodent studies (p. 118). However, the weight of the evidence indicates that the liver effects in rodents are not relevant to humans. As discussed above and in the opinions of Drs. Cullen and Goodman (Attachments A-1 and A-2), spongiosis hepatitis is not a serious liver effect, even in rats, and has been reported only in rats and fish, never in other mammals, including humans. As noted in the toxicity review (p. 118), hepatomegaly and increased cell number and size are likely due to peroxisome proliferation. As discussed on pages 61-81 of the toxicity review, peroxisome proliferation is not readily induced in humans, if at all, and therefore it is unlikely that the liver and kidney effects observed in animal tests are relevant to humans. Liver enlargement and increase in peroxisomal enzyme levels are classic signs of peroxisome proliferation (Klaunig et al., 2003; Cattley et al., 1998). In primates, no statistically significant liver effects were observed even at doses of 2500 mg/kg/day for 13 weeks – a dose that unquestionably would have caused liver effects in rodents. Therefore, the liver weight changes observed in rodent studies of DINP are likely not relevant to humans.

Kidney

The toxicity review (p. 119) concludes that DINP is “probably toxic” in humans with respect to chronic kidney toxicity. However, no adverse effects have been seen in the highly relevant primate studies at doses up to 2500 mg/kg/day (Hall et al. 1999; Pugh et al., 2000). The effects observed in rats and mice can be attributed to species-specific effects in rodents – peroxisome proliferation and alpha-2u-globulin, and therefore are not likely relevant to humans. Attachment A-3 is a statement by Dr. Gordon C. Hard, an internationally recognized expert in kidney carcinogenesis, renal toxicology, and toxicologic renal pathology.³ Dr. Hard concludes: “In my expert judgment, the kidney-related findings discussed above are not a consequence of toxicity of DINP to the kidney, except for the association between alpha-2u-globulin and pigmentation and linear papillary mineralization. Therefore, in my view there is not sufficient evidence to establish that DINP can reasonably be anticipated to cause serious or irreversible renal effects in humans.”

DINP is an inducer of peroxisome proliferation. Increased kidney weights are a consequence of peroxisomal proliferation and, like increased liver weights, a common observation in studies of peroxisomal proliferation. Woodward (1990) summarized the observations of a number of investigators relating to renal changes induced in rodents by peroxisomal proliferating agents including phthalates. The objective of his summary was to investigate whether cystic lesions in dialysis patients could be the consequence of exposure to DEHP. Woodward found no evidence to support such a link, concluding that “the limited data available seem to suggest that humans are not susceptible to the cystogenic effects of phthalate. . . .”

³ As for the statements of Drs. Cullen and Goodman, Dr. Hard’s statement was made for the ACC Phthalate Esters Panel in conjunction with comments on the 2005 EPA toxicological review of DINP. See note 2, page A-1.

Huber et al. (1996) extended this analysis, concluding that “DEHP and several other [peroxisomal proliferators] led to clear peroxisomal proliferation in rat and mouse kidneys.” The authors noted that the expression of peroxisomal proliferation, including increased weight and increased levels of peroxisomal proliferation-enzymes, in rodent kidneys was less than that observed in rodent livers. The basis for this was demonstrated by Ward et al. (1998). Using mice deficient in the peroxisomal proliferator-activated receptor α (PPAR α knock out mice), they showed that PPAR α “mediates the subacute-chronic toxicity of DEHP in liver, kidney and testis.” Thus, the kidney weight effects of DINP in rat kidneys can be explained through a PPAR α mechanism.

Ward et al. (1998) noted that there were also PPAR α -independent mechanisms of kidney toxicity. Dr. Hard discusses in his statement (Attachment A-3) two such mechanisms that could explain the increased relative kidney weights. One is that infiltration of MNCL cells into the kidney would increase the kidney weight. As discussed on p. 82 of the toxicity review, MNCL is a lesion that occurs spontaneously and almost exclusively in the F344 rat (the species used in both Lington et al. and Moore et al.), and which is not relevant to humans.

For the male rat, alpha-2u-globulin nephropathy also likely contributed to the increase in relative kidney weights. As discussed on pages 82-83 of the toxicity review, the male rat kidneys showed evidence of alpha-2u-globulin nephropathy, a mechanism not relevant to humans. As well as Dr. Hard, Phillips and Cockerell (1984) and Phillips and Egan (1984) also found that increased kidney weights in male rats are associated with induction of alpha-2u-globulin.

W Huber, B Grasl-Kraupp, R Schulte-Hermann (1996). Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. *Crit Rev Toxicol* 26:365-481.

R Phillips, B Cockerell (1984). Effect of certain light hydrocarbons on kidney function and structure in male rats. *Adv Modern Environ Toxicol* 7. M. Mehlman, C. Hemstreet, J. Thorpe, N. Weaver, eds. Princeton Scientific Publishers, Inc. pp. 89-105.

R Phillips, G Egan (1984). Effect of C10-C11 isoparaffinic solvent on kidney function in Fischer 344 rats during eight weeks of inhalation. *Toxicol Appl Pharmacol* 73:500-510.

TOXICITY REVIEW OF DI(ISODECYL) PHTHALATE
APRIL 7, 2010

This appendix provides comments on the toxicity review of DIDP produced by CPSC staff and posted to the CPSC website (conforming changes also should be made to the CPSC staff Overview of Phthalates Toxicity).¹ Unless otherwise noted, page citations are to that document. If a document cited in our comments is among the references to the toxicity review, we do not repeat that citation here. Additional references are given at the end of the section in which they are cited.

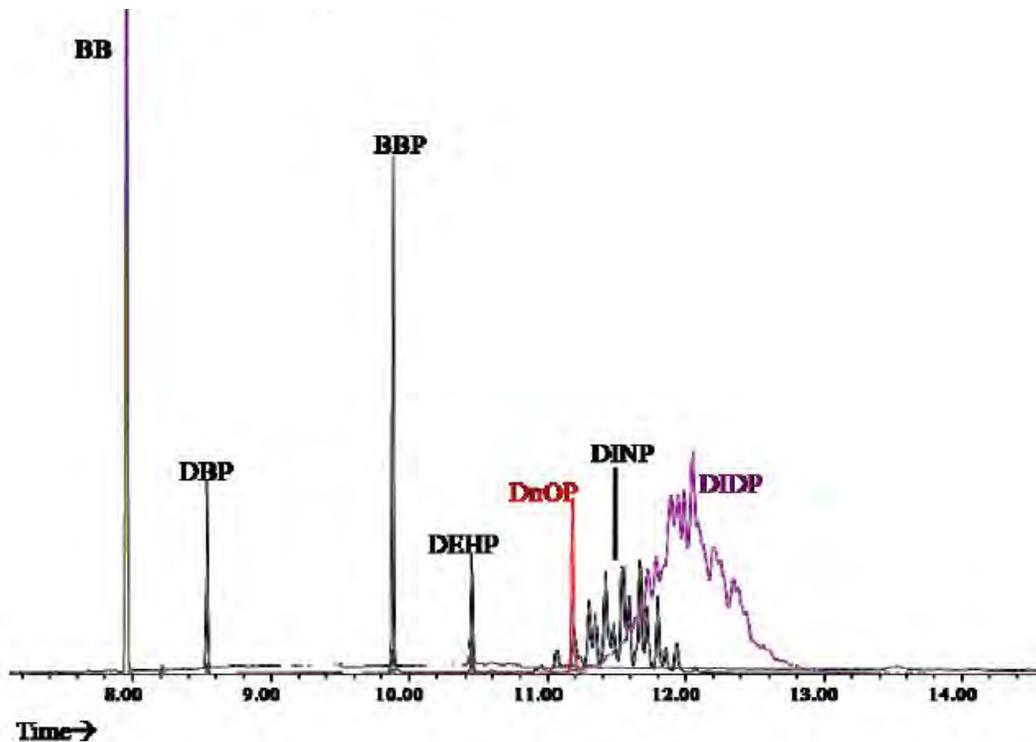
Overall Comment on DIDP Composition

As noted under Physiochemical Properties, commercial DIDP is a mixture of branched C9-11 isomers, but consists primarily of C10 isomers. Likewise, commercial DINP is a mixture of C8-10 isomers, consisting primarily of C9 isomers. Thus, analysis of an item made with commercial DINP will show peaks on the readout corresponding to C10. See Figure A-3, which is taken from the CPSC protocol for testing of phthalates,² and which shows the isomer distributions for DINP and DIDP and their overlap, as they appear on a chromatogram. Verification that commercial DIDP was used requires detection of C11. *Note that it is the commercial products on which toxicity testing has been conducted.*

Figure A-3. Chromatogram of Various Phthalates

¹ CPSC Health Sciences (2010). Toxicity Review of Di(isodecyl) Phthalate. Memo from C. Osterhout to M. Babich, April 7, <http://www.cpsc.gov/about/cpsia/toxicityDIDP.pdf>

² Test Method: CPSC-CH-C1001-09.3 – Standard Operating Procedure for Determination of Phthalates, April, 1, 2010, <http://www.cpsc.gov/about/cpsia/CPSC-CH-C1001-09.3.pdf>.



Toxicokinetics

The first paragraph of this section of the toxicity review (p. 3) correctly indicates that DIDP is absorbed at a very low level through the skin. However, the citations are to studies of other phthalates. DIDP dermal absorption was studied by Elsisi et al. (1989). In 7 days, only 2% of dermally applied ¹⁴C-DIDP was recovered in other tissues or excreta. In that study, dermal absorption of phthalates decreased with increasing side chain length beyond four carbons (Elsisi et al., 1989).

A Elsisi, D Carter, I Sipes. (1989). Dermal absorption of phthalate diesters in rats. *Fund Appl Toxicol* 12:70-77.

The last paragraph of the section (p. 4) hypothesizes that DIDP was present in the DINP formulation used in Silva et al. (2006). As noted above in the Overall Comment, page B-1 above, this could be due to C10 in the DINP formulation.

Exposure

The statement straddling pages 4 and 5 states that the “manufacturer’s exposure limit for DIDP is five mg/m³ based on a value recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).” Note that ACGIH established that value for DEHP; it is conservatively used also for DIDP.

In the Silva et al. (2007) paper discussed in the Exposure section (p. 5), detection of C10 metabolites may have been to C10 isomers that were part of the DINP used in the study. See Overall Comment, page B-1 above.

Systemic Effects

Page 8 of the CPSC toxicity review includes a synopsis of a 13-week diet study in Beagle dogs (Hazelton, 1968b); this study later is used as the key study for derivation of an acceptable daily intake (ADI). As described in the study report, gross necropsy examinations did not reveal any consistent compound-related alterations. Only minor microscopic changes were noted, and there was a lack of significant dose-response in severity and number of animals manifesting these effects. More significantly, this study was not conducted to a standardized protocol and was not conducted according to GLP, and the results were not subjected to statistical analysis due to the small study size. Due to these limitations, this study is inappropriate for risk characterization, such as development of an ADI.

A more appropriate study for risk characterization of systemic effects is the rat study conducted by Hazelton Laboratories (1968), discussed on page 8 of the toxicity review. It involved four groups of 10 male and 10 female rats exposed to DIDP at dietary levels of 0.05%, 0.3% and 1% (approximately 25, 150 and 500 mg/kg/d, respectively) for 13 weeks. No compound-related effects were observed at any dietary level with regard to physical appearance, behavior or survival. Growth of the test rats was not significantly affected. Body weight gains for the two highest levels in males were lower than controls (but not significantly different) and the two test groups were comparable through the ninth week. Overall, weight gains at 13 weeks for the male test groups showed a dose-related, although slight, decrease. Body weight gains for the high dose females were only slightly lower than the controls – not a statistically significant difference. Food consumption values were comparable to the controls. The clinical laboratory values for the test groups showed no significant compound-related differences from control values.

Observations at necropsy revealed the livers of the high dose group animals, particularly the males, to be markedly larger than those of the control rats. Statistical analysis showed the liver weights and liver/body weight ratios for the high dose group males and females to be significantly higher than those for the corresponding controls. No other consistent gross changes were noted in the liver. Histologically, the liver showed no compound-induced alterations. The kidney/body weight ratios but not the absolute weights in the high and intermediate dose group males were significantly higher than those for the corresponding controls. Histologically, the kidneys showed no compound-induced alterations.

A minimal increase in thyroid activity, possibly a compensatory response related to an increased rate of thyroid hormone metabolism due to the overall increased metabolic

capacity in the liver, was observed at the highest dose. It can be concluded from this study that the NOAEL is 0.3% (approximately 150 mg/kg/day)³ based on the observation that the highest dose leads to minor liver and thyroid effects.

The Systemic Effects section concludes that DIDP is a probable toxicant based on increased liver weight, increased peroxisomal enzyme levels and histological changes, and increased kidney weight. It is likely that all these effects are the result of peroxisome proliferation, induced via the PPAR α mechanism. As discussed in the toxicity review for DINP (pp. 61-81), peroxisome proliferation is not readily induced in humans, if at all, and therefore it is unlikely that the liver and kidney effects observed in animal tests are relevant to humans.

As discussed in the Genotoxicity/Carcinogenicity section of the DIDP toxicity review, DIDP is a limited peroxisome proliferator. This can account for the liver effects observed in rodent studies of DIDP. Liver enlargement and increase in peroxisomal enzyme levels are classic signs of peroxisome proliferation (Klaunig et al., 2003; Cattley et al., 1998). Therefore, the liver weight changes observed in rodents studies of DIDP are likely not relevant to humans. The hepatocyte swelling and vacuolation were observed only in the dog study; as discussed above, there was not a dose-response for these effects and there were other limitations that indicate that study should not be used for risk assessment.

Similarly, increased kidney weights are a consequence of peroxisomal proliferation and, like increased liver weights, a common observation in studies of peroxisomal proliferation. Woodward (1990) summarized the observations of a number of investigators relating to renal changes induced in rodents by peroxisomal proliferating agents including phthalates. The objective of his summary was to investigate whether cystic lesions in dialysis patients could be the consequence of exposure to DEHP. Woodward found no evidence to support such a link, concluding that “the limited data available seem to suggest that humans are not susceptible to the cystogenic effects of phthalate. . . .”

Huber et al. (1996) extended this analysis, concluding that “DEHP and several other [peroxisomal proliferators] led to clear peroxisomal proliferation in rat and mouse kidneys.” The authors noted that the expression of peroxisomal proliferation, including increased weight and increased levels of peroxisomal proliferation-enzymes, in rodent kidneys was less than that observed in rodent livers. The basis for this was demonstrated by Ward et al. (1998). Using mice deficient in the peroxisomal proliferator-activated receptor α (PPAR α knock out mice), they showed that PPAR α “mediates the subacute-chronic toxicity of DEHP in liver, kidney and testis.” Thus, the kidney weight effects of DIDP in rat kidneys can be explained through a PPAR α mechanism, indicating that the are not relevant to humans.

³ Table 4 of the CPSC toxicity review gives the dose value as 170 mg/kg/day.

Cattley, R.C., DeLuca, J., Elcombe, C. et al. (1998). Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul Toxicol Pharmacol* 26:47-60.

Huber, W.W., Grasl-Kraupp, B., and Schulte-Hermann, R. (1996). Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. *Critical Reviews in Toxicology* 26:365-481.

Klaunig, J., Babich, M., Baetcke, K., Cook, J., Corton, J., David, R., DeLuca, J., Lai, D., McKee, R., Peters, J., Roberts, R., and Fenner-Crisp, P. (2003). PPAR α agonist-induced rodent tumors: Modes of action and human relevance. *Critical Reviews in Toxicology*, 33(6):655–780.

Ward, J.M., Peters, J.M., Perella, C.M., and Gonzalez, F.J. (1998). Receptor and non-receptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferators-activated receptor α -null mice. *Toxicologic Pathology* 26:240-246.

Woodward, K. (1990). Phthalate esters, cystic kidney disease in animals and possible effects on human health: A review. *Human and Experimental Toxicology* 9:397-401.

Developmental Effects

This section concludes that DIDP is a probable toxicant based on developmental effects, including increased incidences of minor skeletal variations (pp. 17-18). The skeletal variations were supernumerary cervical and rudimentary lumbar ribs, for which the increase was statistically significant only on a per litter basis at the high dose. These skeletal variations are developmental variants commonly found in developmental toxicity studies that are usually reversed later in life and are generally regarded as not having toxicological significance. Rudimentary ribs in particular are a common finding in rat fetuses and may be related only to transient maternal stress. In addition, the statistical methods used in developmental studies typically do not account for the fact that multiple comparisons are made, leading to the possibility that these apparently statistically significant differences are in fact due to chance.

Table 5 of the CPSC staff Overview of Phthalates Toxicity indicates that male sexual development for DIDP has not been determined. DIDP has been examined for adverse effects in male sexual development. In a guideline, 2-generation reproduction and developmental toxicity study, there were no effects on anogenital distance, nipple retention, changes in male reproductive organ weights, or fertility (Hushka et al., 2001). Therefore, “no effects” should be noted in the table for DIDP

Genotoxicity/Carcinogenicity

We agree with the conclusion that DIDP is not carcinogenic. However, corrected data have been published, resulting in different NOAELs. In the two-year toxicity/carcinogenicity study by Cho et al.(2008), Fischer 344 rats were exposed to 0, 400, 2000, and 8000 ppm DIDP. As published in 2008, the daily mg/kg intakes were 0.85, 4.13, 17.37 for males and 0.53, 3.03, and 13.36 for female. However, these values were deemed to be calculated incorrectly in the original submission. An updated table has been published (Cho et al., 2010a); the corrected values are shown in Table A-1.

Table A-1. Corrected Exposures for Cho et al. (2008)

Daily average food intake (g)	DIDP %	mean daily DIDP intake (mg)	mean bw (g)	mean daily DIDP intake (mg/kg/day)
21.07 - male	0	0.00	382.31	0
21.33 - male	0.04	8.54	390.46	21.9
21.53 - male	0.2	43.06	390.58	110.3
21.70 - male	0.8	173.66	362.39	479.2
14.37 - female	0	0.00	229.25	0
13.36 - female	0.04	5.34	233.19	22.9
15.15 - female	0.2	30.30	236.40	128.2
16.69 - female	0.8	133.59	215.60	619.6

Based on these corrected values, the NOAEL is 479 mg/kg/day for males and 619 mg/kg/day for females since no treatment related neoplastic lesions were observed in internal organs.

Since the writing of the CPSC staff toxicity review, Cho et al. have published a 6-month carcinogenicity study in CB6F1-rasH2 transgenic mice in which increased tumor formation was observed in the high dose males (Cho et al. (2010b). However, the utility of the rasH2-hemizygous transgenic mouse for assessing carcinogenic potential of non-genotoxic compounds (e.g., DIDP) is limited. In addition, transgenic mouse models are screens used when a 2-year bioassay is not available. In this case, the bioassay has been published and serves as the definitive test of the carcinogenic potential of DIDP; it found that DIDP is not carcinogenic (Cho et al., 2008).

W-S Cho, B Seok Hana, B Ahnb, K Taek Nama, M Choia, S Yeon Oha, S Hee Kima, J Jeonga, D Deuk Janga (2010). Corrigendum to “Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer 344 rats” [Toxicol. Lett. 178 (2008) 110–116]. Toxicology Letters 197(2):156.

W-S Cho, J Jeong, M Choi, S Nie Park, B Seok Han, W-C Son (2010b). 26-Week carcinogenicity study of di-isodecyl phthalate by dietary administration to CB6F1-rasH2 transgenic mice. Archives of Toxicology, DOI 10.1007/s00204-010-0536-6.

Discussion

In the discussion of the hazard of DIDP, a series of Acceptable Daily Intakes (ADIs) were calculated.

For subchronic effects, "An ADI based on liver effects calculated from the lowest NOAEL (15 mg/kg/day) divided by a safety factor of 100 [10 (animal to human) x 10 (sensitive populations)] is 0.15 mg/kg DIDP". In this instance, the NOAEL is from a 13-week diet study in Beagle dogs. As discussed previously, this study is limited and not suitable for risk characterization. The most appropriate NOAEL for subchronic risk characterization is 150 mg/kg/day. Applying the same uncertainty factors of 10 and 10, an ADI of 1.5 mg/kg/day is derived.

The ADI based on the two-year chronic toxicity/carcinogenicity study is currently based on incorrect daily intake data and must be updated to reflect the NOAEL of 479 mg/kg/day. See the comments on the Genotoxicity/Carcinogenicity section, above. With this point of departure divided by uncertainty factors of 10 and 10, an ADI of 4.79 mg/kg/day is derived.

The reproductive/developmental toxicity ADI is calculated incorrectly. The key study selected was a one-generation screening study in which rats were exposed to 40, 200, or 1000 mg/kg DIDP. The NOAEL of 40 mg/kg/day was identified on the basis of fetal variations at 200 mg/kg; although the biological significance of fetal variations such as cervical supernumerary ribs remains uncertain. A second one-generation study is also available in which rats were exposed to 0, 100, 500, or 1000 mg/kg/day DIDP in which fetal variations were also noted. The NOAEL of this study was 100 mg/kg/day. When both studies are considered together, it is clear that the true NOAEL is somewhere between 100 mg/kg/day and 200 mg/kg/day, which is consistent with the benchmark doses identified by the update to the Waterman et al., (1999) study report. Thus, for risk characterization purposes, a NOAEL of 100 mg/kg/day is more appropriate than 40 mg/kg/day; adjusted with the total uncertainty factor of 100, an ADI of 1 mg/kg/day is derived.

Based on these data and their correct interpretation, the lowest ADI calculated is 1 mg/kg/day. In conjunction with exposure data, a substantial margin of safety exists between current exposures and the lowest ADI calculated, indicating the continued safe use of DIDP.