National Toxicology Program
U.S. Department of Health and Human Services



Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP)

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Preface

The National Toxicology Program (NTP) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in 1998. The CERHR is a publicly accessible resource for information about adverse reproductive and/or developmental health effects associated with exposure to environmental and/or occupational chemicals. The CERHR is located at the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health and Dr. Michael Shelby is the director.[1]

The CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. The CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Chemicals are selected for evaluation based upon several factors including the following:

- potential for human exposure from use and occurrence in the environment.
- extent of public concern.
- production volume.
- availability of scientific evidence for reproductive and/or developmental toxicity.

The CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. The CERHR expert panels use explicit guidelines to evaluate the scientific literature and prepare the expert panel reports. Expert panel reports are made public and comments are solicited.

Next, the CERHR prepares the NTP-CERHR monograph. The NTP-CERHR monograph includes the NTP brief on the chemical evaluated, the expert panel report, and all public comments. The goal of the NTP brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's interpretation of the potential for the chemical to adversely affect human reproductive health or children's health. The NTP-CERHR monograph is made publicly available electronically on the CERHR web site and in hard copy or CD-ROM from the CERHR.

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[1]Information about the CERHR is available on the web at http://cerhr.niehs.nih.gov or by contacting the director:

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Introduction

In 1999, the CERHR Core Committee, an advisory committee composed of representatives from NTP member agencies, recommended seven phthalates for expert panel review.

These chemicals were selected because:

(a) there is the potential for human exposure from their widespread use and occurrence within the environment,
(b) they have a high production volume,
(c) there is substantial scientific literature addressing the reproductive and/or developmental toxicities of these chemicals, and
(d) they are of concern to the public.

These seven phthalates are as follows:

- di(2-ethylhexyl)phthalate (DEHP)
- di-isononyl phthalate (DINP)
- di-isodecyl phthalate (DIDP)
- di-n-butyl phthalate (DBP)
- butyl benzyl phthalate (BBP)
- di-n-octyl phthalate (DnOP)
- di-n-hexyl phthalate (DnHP)

Phthalates are a group of similar chemicals widely used to soften and increase the flexibility of plastic consumer products such as shower curtains, medical devices, upholstery, raincoats, and soft squeeze toys. They are not bound to the plastics and can leach into the surrounding environment. The scientific literature on the reproductive and developmental toxicities of several phthalates is extensive. In addition, there is widespread public concern about the safety of phthalates.

As part of the evaluation of phthalates, the CERHR convened a panel of scientific experts (Appendix I) to review, discuss, and evaluate the scientific evidence on the potential reproductive and developmental toxicities of each phthalate. There were three public meetings of this panel (August 17-19 and December 15-17, 1999 and July 12-13, 2000). The CERHR received numerous public comments on the phthalates throughout the evaluation process.

The NTP has prepared an NTP-CERHR monograph for each phthalate. This monograph includes the NTP brief on BBP, a list of the expert panel members (Appendix I), the expert panel's report on BBP (Appendix II), and all public comments received on the expert panel's reports on phthalates (Appendix III). The NTP-CERHR monograph is intended to serve as a single, collective source of information on the potential for BBP to adversely affect human reproduction or development. Those interested in reading this report may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP brief included within this report presents the NTP's interpretation of the potential for exposure to BBP to cause adverse reproductive or developmental effects in people. It is based upon information about BBP provided in the expert panel report, the public comments, and additional scientific information available since the expert panel meetings. The NTP brief is intended to provide clear, balanced, scientifically sound information on the potential for BBP exposures to result in adverse health effects on development and reproduction.

Developmental Toxicity versus Reproductive Toxicity

While there are biological and practical reasons for considering developmental toxicity and reproductive toxicity as 2 separate issues, it is important to keep in mind that life in mammals, including humans, is a cycle. In brief, the cycle includes the production of sperm and eggs, fertilization, prenatal development of the offspring, birth, post-natal development, sexual maturity, and, again, production of sperm and eggs.

In the past, toxic effects were often studied in a "life stage specific" manner. Thus, concerns for developmental toxicity were addressed by exposing pregnant mothers and looking for adverse effects in fetuses. Developmental toxicity was detected as death, structural malformations, or reduced weights of the fetuses just prior to birth. Reproductive toxicity was studied by exposing sexually mature adults to the chemical of interest and effects were detected as impaired capacity to reproduce. Over the years, toxicologists realized that exposure during one part of the life cycle could lead to adverse effects that might only be apparent at a different part of the life cycle. For example, exposure of a sexually mature individual to an agent capable of inducing genetic damage in eggs or sperm might have no apparent effect on the exposed individual. However, if a genetically damaged egg or sperm from that individual is involved in fertilization, the induced genetic damage might lead to death or a genetic disorder in the offspring. In this example, chemical-induced damage is detected in the next generation. In contrast, the reproductive system begins developing well before birth and continues until sexual maturity is attained. Thus, exposure of sexually immature animals, either before or following birth, to agents or conditions that adversely affect development of the reproductive system can result in structural or functional reproductive disorders. These effects may only become apparent after the exposed individual reaches the age of puberty or sexual maturity.

Thus, in the case of genetic damage induced in eggs or sperm, what might be considered reproductive toxicity gives rise to developmental disorders. Conversely, in the case of adverse effects on development of the reproductive tract, developmental toxicity results in reproductive disorders. In both these examples it is difficult to make a clear distinction between developmental and reproductive toxicity. This issue is important in considering the phthalate evaluations because evidence of developmental toxicity affecting reproductive capacity in later stages of the life cycle is reported for at least 3 of the phthalates -BBP, DBP, and DEHP.

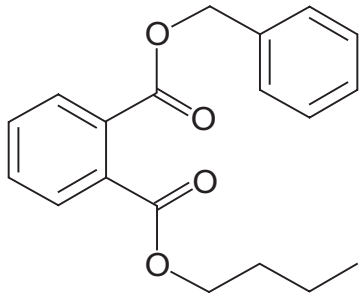
NTP Brief on Butyl Benzyl Phthalate (BBP)

What is BBP?

BBP is a clear, slightly viscous liquid with the chemical formula $C_{19}H_{20}O_4$ and the structure shown in Figure 1. It is one of a group of industrially important chemicals known as phthalates. Phthalates are primarily used as plasticizers to add flexibility to plastics. The largest use of BBP is in the production of vinyl tiles. It is also used in a variety of other products such as food conveyor belts, artificial leather, automotive trim, and traffic cones. There is no evidence that BBP is used in toys or medical devices.

BBP is produced by sequentially reacting butanol and benzyl chloride with phthalic anhydride. U.S. annual production figures for BBP were not available.

Figure 1. *Chemical structure BBP*



Are People Exposed to BBP?*

Yes. There are several ways that people may be exposed to BBP at home or at work. Human exposure can occur during the manufacture of BBP, during the manufacture of BBP-containing products, during the use of such products, or through the presence of BBP in the environment.

Environmental exposures can occur through air, water, or food. Most people are probably exposed to BBP primarily through food. BBP migrates into foods, particularly fatty foods, from BBP-containing materials that are used to process food.

The expert panel estimated that the U.S. general population is exposed to approximately 2 μg/kg bw/day (micrograms per kilogram body weight per day). This reflects a total daily exposure of approximately 140 μg per person per day. By comparison, a small drop of water weighs approximately 30,000 μg and a grain of table salt weighs approximately 60 μg.

A recent study not available to the expert panel determined the amount of BBP metabolites in human urine (Blount *et al.*, 2000). Kohn *et al.* (2000) and David (2000) used the data from that study to estimate daily exposure levels of BBP. Kohn *et al.* estimated that 95% of people exposed to BBP are exposed to 4 μg/kg bw/day or less, very close to the expert panel's estimate.

In another recent study (Anderson *et al.*, 2001), it was shown that people efficiently absorb, metabolize, and excrete BBP. Volunteers given an oral dose of BBP excrete approximately 75% of the dose in urine within 24 hours. Most of the dose is excreted as the mono-benzyl phthalate metabolite, with only a minor fraction excreted as the mono-butyl phthalate.

Workers producing BBP or BBP-containing products can be exposed through skin contact or inhalation. It has been estimated that such exposures might be as high as 286 μg/kg bw/day, but are generally thought to be far below this level.

Can BBP Affect Human Development or

* Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

Reproduction?
Probably. Although, there is no direct evidence that exposure of people to BBP adversely affects reproduction or development, studies reviewed by the expert panel and subsequently published studies with laboratory rodents show that exposure to BBP can adversely affect development, including development of the male reproductive tract (Fig. 2). The NTP believes it is reasonable and prudent to conclude that the results reported in laboratory animals indicate a potential for similar or other adverse effects in human populations if exposures are sufficiently high.

Scientific decisions concerning human health risks are generally based on what is known as "weight-of-the-evidence." Recognizing the lack of human data and the evidence of BBP effects in laboratory animals, the NTP judges the scientific evidence sufficient to support the levels of concern for effects on development and reproduction expressed below (Fig. 3).

Summary of Supporting Evidence
As presented in the expert panel report, studies in rats and mice have shown that prenatal exposure to high levels of BBP can result in a range of effects that include prenatal mortality, reduced growth, and skeletal, visceral, and external malformations. Reproductive toxicity studies in male rats reported that oral exposure to BBP can result in reduced sperm counts, histological changes in the testes, and reduced fertility. Such effects were seen at very high doses, typically greater than 1000 mg/kg bw/day. In BBP-exposed females, reproductive effects may have occurred but this was not clear due to the design of the study.

Following completion of the expert panel report, results of a two-generation reproductive toxicity study of BBP in Sprague-Dawley rats were reported (Nagao *et al.*, 2000). Male and female rats were exposed orally to BBP at doses of 0, 20, 100, or 500 mg/kg bw/day. Reproductive performance was not affected at any dose level. The key findings involved developmental effects in the offspring. These effects included reduced birth weights of both males and females, decreased anogenital distance in males, delayed preputial separation in males and, in postpubertal males, reduced serum testosterone levels, decreased spermatocytes and other histopathological changes in the testes. Females were less susceptible than males to adverse developmental effects on the reproductive tract. Most, but not all, of these effects were observed only in the highest dose group. The authors conclude that no effects

Figure 2. *The weight of evidence that BBP causes adverse developmental or reproductive effects in laboratory animals*

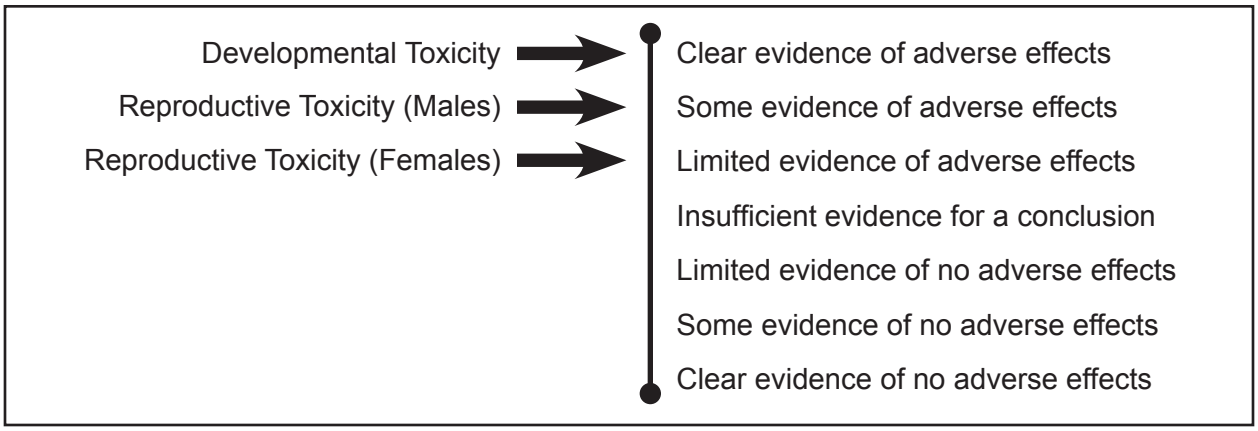
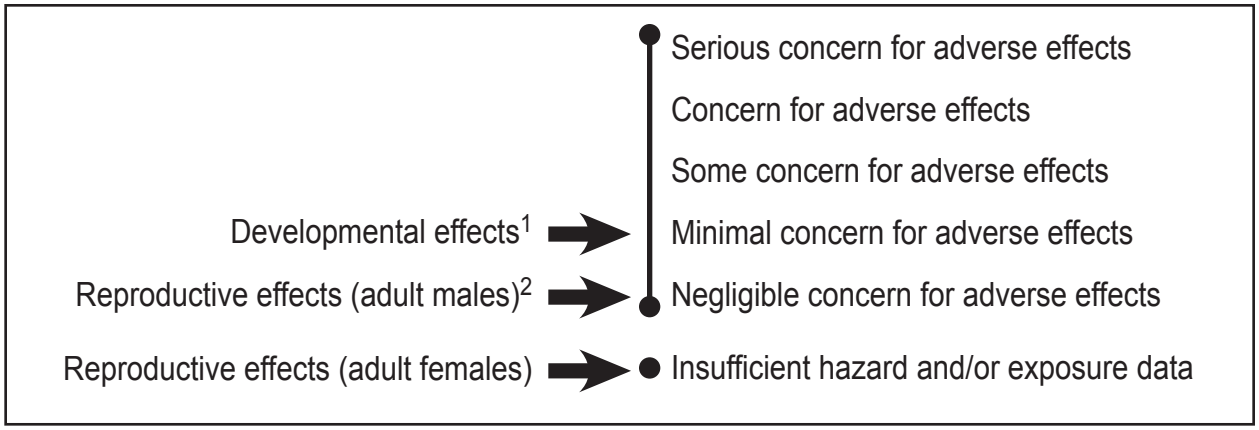


Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to BBP*



[1]Based on Kohn et al. (2000) estimated exposure of women of reproductive age (median, 1.2; 95th percentile, 4.5; maximum, 7.8 μg /kg bw/day)

[2]Based on Kohn et al. (2000) estimated exposures of the U.S. general population (median, 0.88; 95th percentile, 4.0; maximum, 29 μg /kg bw/day).

were observed at 20 mg/kg bw/day. The only effects at 100 mg/kg bw/day were reduced pup weights for both males and females and increased relative kidney weight and decreased relative heart weight in males.

In the rodent developmental toxicity studies available to the expert panel the highest doses at which no effects were observed were 182 mg/kg bw/day in mice and 185 mg/kg bw/day in rats. Noteworthy in the Nagao *et al.* study was that, although reproductive tract changes were observed in pups of both sexes, there were no effects on the capacity of these animals to reproduce when they reached sexual maturity.

In another study (Piersma *et al.*, 2000), two gavage treatment regimens (gestation days 5 through 16 or 5 through 20) were used to study the developmental toxicity of BBP in rats. Study groups included 10 pregnant dams and 10 dose levels that ranged from 0-2100 mg/kg bw/day. Data were submitted to a benchmark approach for calculating Critical Effect Doses (CED) based on the authors' selection of Critical Effect Sizes. The calculated CEDs for the five fetal endpoints considered (frequency of resorptions, fetal weight, extra lumbar rib 13, testicular dislocation, and fetal relative testis weight) were approximately the same as, or higher than, the NOAELs determined by the expert panel.

BBP was studied to determine if it produced antiandrogenic-like effects on sexual development in male rats exposed from gestation day 14 to postnatal day 3 (Gray *et al.*, 2000). Pregnant dams were exposed by gavage to 750 mg/kg bw/day. Exposure induced shortened anogenital distance in male but not female pups, reduced testis weights, female-like areolas/nipples in some male pups, as well as some malformations in male reproductive tracts. This study demonstrates the antiandrogenic effects of BBP but, the use of a single high dose limits its utility in evaluating the potential for BBP to affect human reproduction or development.

Are Current Exposures to BBP High Enough to Cause Concern?

Probably not. More data are needed to better understand human BBP exposure levels and

how these exposures vary across the population. Although the general U.S. population presently appears to be exposed to BBP at levels that are not of immediate concern for causing adverse reproductive or developmental effects, data are not available to permit conclusions regarding the possibility of effects in various age groups, occupations, or socioeconomic strata. Based on the expert panel report, and more recent data on rodent toxicity and human exposure, the NTP offers the following conclusions.

The NTP concludes that there is minimal concern for developmental effects in fetuses and children.

This is based on the observation of no effects in rats at 20 mg/kg bw/day and the human exposure estimates of Kohn *et al.* (see footnotes to Fig. 3)

The NTP concurs with the CERHR Phthalates Expert Panel that there is negligible concern for adverse reproductive effects in exposed men.

The data are insufficient to reach conclusions for exposed women.

These conclusions are based on the information available at the time this brief was prepared. As new information on toxicity and exposure accumulate, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

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Kohn MC, Parham F, Masten SA, Portier CJ, Shelby MD, Brock JW, Needham LL. Human exposure estimates for phthalates. *Environmental Health Perspectives,* **108**: A440-A442 (2000).

Nagao T, Ohta R, Marumo H, Shindo T, Yoshimura S and Ono H. Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study. *Reproductive Toxicology*, **14**:513-532 (2000).

Piersma, AH, Verhoef, A, te Biesebeek, JD, Pieters, MN, Slob, W. Developmental toxicity of butyl benzyl phthalate in the rat using a multipledose study design. *Reproductive Toxicology,* **14**: 417-425 (2000).

Appendix I. NTP-CERHR Phthalates Expert Panel Report on BBP

A 16-member panel of scientists covering disciplines such as toxicology, epidemiology, and medicine was recommended by the Core Committee and approved by the Associate Director of the National Toxicology Program. Over the course of a 16-month period, the panel critically reviewed more than 500 documents on 7 phthalates and identified key studies and issues for plenary discussions. At three public meetings[1], the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. At the final meeting, the expert panel reached conclusions on whether estimated exposures may result in adverse effects on human reproduction or development. Panel assessments were based on the scientific evidence available at the time of the final meeting. The expert panel reports were made available for public comment on October 10, 2000, and the deadline for public comments was December 11, 2000 (*Federal Register* 65:196 [10 Oct. 2000] p60206). The Phthalates Expert Panel Report on BBP is provided in Appendix II and the public comments received on that report are in Appendix III. Input from the public and interested groups throughout the panel's deliberations was invaluable in helping to assure completeness and accuracy of the reports. The Phthalates Expert Panel Reports are also available on the CERHR website <*http://cerhr.niehs.nih.gov*>.

[1]Phthalate Expert Panel meeting dates were: August 17-19, 1999, in Alexandria, VA; December 15-17, 1999, in Research Triangle Park, NC; and July 12-13, 2000, in Arlington, VA.

Appendix I. NTP-CERHR Phthalates Expert Panel
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National Toxicology Program
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Center For The Evaluation Of Risks To Human Reproduction

Appendix II

NTP-CERHR EXPERT PANEL REPORT

on

Butyl Benzyl Phthalate

October 2000 *NTP-CERHR-DINP-00*

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PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June, 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

The following seven phthalate esters were selected for the initial evaluation by the Center: butyl benzyl phthalate, di(2-ethylhexyl) phthalate, di-isodecyl phthalate, di-isononyl phthalate, di-n-butyl phthalate, di-n-hexyl phthalate, and di-n-octyl phthalate. Phthalate esters are used as plasticizers in a wide range of polyvinyl chloride-based consumer products. These chemicals were selected for the initial evaluation by the CERHR based on their high production volume, extent of human exposures, use in children's products, published evidence of reproductive or developmental toxicity, and public concern.

This evaluation is the result of three public Expert Panel meetings and 15 months of deliberations by a 16-member panel of experts made up of government and non-government scientists. This report has been reviewed by the CERHR Core Committee made up of representatives of NTP-participating agencies, by CERHR staff scientists, and by members of the Phthalates Expert Panel. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that a given exposure or exposure circumstance may pose a hazard to reproduction and the health and welfare of children; (2) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/development health effects are associated with exposure to specific chemicals or classes of chemicals, including descriptions of any uncertainties that would diminish confidence in assessment of risks; and (3) identify knowledge gaps to help establish research and testing priorities.

The Expert Panel Reports on phthalates will be a central part of the subsequent NTP report that will also include public comments on the Panel Reports and any relevant information that has become available since completion of the Expert Panel Reports. The NTP report will be transmitted to the appropriate Federal and State Agencies, the public, and the scientific community.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website <*http://cerhr.niehs.nih.gov/*> or from:

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A Report of the CERHR Phthalates Expert Panel:

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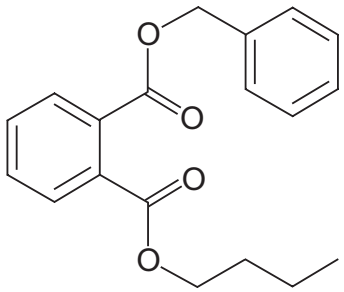
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1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of Butyl Benzyl Phthalate



Butyl benzyl phthalate (BBP) (CAS 85-68-7) is produced by sequentially reacting butanol and benzyl chloride with phthalic anhydride (1).

Table 1: Physicochemical Properties of BBP

Property	***Value***
Chemical Formula	$C_{19}H_{20}O_4$
Molecular Weight	312.35
Vapor Pressure	6 x 10^{-7} mmHg at 25 ^oC
Melting Point	-40.5 ^oC
Boiling Point	370 ^oC
Specific Gravity	1.12
Solubility in Water	slight – 2.7 mg/L
Log K_{ow}	4.59

(1)

1.2 Exposure and Usage

According to the American Chemistry Council (ACC, formerly CMA) (1), the largest use of BBP is in vinyl tile. BBP is also a plasticizer in PVC used to manufacture food conveyor belts, carpet tile, artificial leather, tarps, automotive trim, weather stripping, traffic cones, and is used to a limited extent in vinyl gloves. BBP is also used in some adhesives. BBP may be released to the environment during its production and also during incorporation into plastics or adhesives. Because BBP is not bound to the final product, it can be released during the use or disposal of the product. Phthalates that are released to the environment can be deposited on or taken up by crops that are intended for human or livestock consumption, and thus, can enter the food supply.

General Population Exposure

General population exposure to BBP through food has been estimated by at least two authoritative sources: the International Program on Chemical Safety (IPCS) (2) and the UK Ministry of Agricul-

ture, Fisheries, and Food (MAFF) (3-5).

BBP may enter food by environmental uptake during crop cultivation or by migration from processing equipment or packaging materials. IPCS (2) concluded that BBP exposure to the general population is based almost entirely on food intake; these food exposure estimates were based on a survey of 100 food items that were purchased in four Ontario, Canada supermarkets between 1985 and 1988. BBP was only found in yogurt (0.6 μg/g), cheddar cheese (1.6 μg/g), butter (0.64 μg/g), and crackers (0.48 μg/g). Assumptions used to estimate exposure included a 70 kg body weight, and a daily consumption of 13.61 g butter, 3.81 g cheddar cheese, 1.54 g yogurt, 22.73 g pork, and 3.45 g crackers. Adult BBP intake was estimated at 2 μg/kg bw/day and it was stated that exposure to infants and children could be up to three-fold higher.

MAFF (5) estimated adult BBP exposure through dietary intake based on a 1993 survey of fatty foods in the United Kingdom. BBP was detected in carcass meat (0.09 μg/g), poultry (0.03 μg/g), eggs (0.09 μg/g), and milk (0.002 μg/g). In calculating dietary food exposures, MAFF assumed that these types of food likely account for 85% of dietary phthalate intake. Food intake levels were obtained from the Dietary and Nutritional Study of British Adults, but the values were not reported by MAFF. Mean and high-level BBP intakes were estimated at 8 μg/person/day and 20 μg/person/day, respectively. Specific details describing the calculations and assumptions used were not provided. Using the IPCS-assumed adult body weight of 70 kg (2), the exposure values were converted to 0.11-0.29 μg/kg bw/day.

MAFF also addressed BBP exposure in infants resulting from the consumption of infant formula. A survey published in 1996 reported BBP levels of <0.0044-0.24 μg/g in infant formulas purchased in the UK, while a later survey reported BBP levels of <0.003-0.015 μg/g (3, 4). It is speculated that the drop in BBP concentration occurred because infant formula manufacturers were urged to reduce phthalate levels after the MAFF published the results of the 1996 survey (3). Based on the results from the 1998 survey and using an assumed body weight of 2.5-3.5 kg at birth and 7.5 kg at 6 months of age, exposure levels were estimated for infants. Formula intake rates were determined from manufacturer instructions. Exposure levels for infants were estimated at 0.2 μg/kg bw/day at birth and 0.1 μg/kg bw/day at 6 months of age. Infants in the United States are likely exposed to lower levels of BBP through formula. In a survey of infant formulas conducted in 1996, BBP levels were below the detection limit of 0.005 μg/g (6).

BBP was only detected in one sample (2.8 μg/L) collected in 1991 in a survey of 300 drinking water sites in two Canadian provinces from 1985 to 1994. IPCS (2) considered exposure to BBP through drinking water negligible; exposure through soil intake was also considered negligible.

Mouthing of toys and other BBP-containing objects is a potential source of oral phthalate exposure in children. However, BBP is stated not to be used in toys (7). In an analysis of 17 plastic toys, BBP was only detected in a PVC doll's head at 0.02% by weight (8), a level that suggests contamination rather than planned use.

Off-gassing from building materials has been reported as a potential source of BBP exposure-through inhalation; however, exposure has been postulated to be minimal because of BBP's low

vapor pressure. The available data, though minimal, support this view. IPCS (2) reported that median air levels of 0.034-0.035 ng/m^3 were measured in a survey of 125 California homes. BBP levels in outdoor air were also measured for 65 of these homes and the median BBP level was below the detection limit of 0.051 ng/m^3. The 90th percentile levels of BBP in outdoor air ranged from 5.3 to 6.7 ng/m^3 for daytime to evening. IPCS (2) considered BBP exposure through inhalation to be negligible. Pfordt and Bruns-Weller (9) measured BBP levels in 3 flooring samples and found BBP in each sample at levels ranging from 10-250 μg/g.

Dermal contact with products containing BBP is possible, but absorption through skin is most likely minimal. Studies in rats have demonstrated that absorption of BBP through skin is fairly slow (approximately 27% in 7 days) (10). An *in vitro* study conducted with rat and human skin has demonstrated that permeability of human skin to other phthalates (DBP and DEHP) is much lower than that of rat skin (11).

Interpretation of exposure levels for the general population requires caution. The exposure estimates by IPCS and MAFF differed by approximately one order of magnitude. The basis for discrepancies in dietary exposure estimates is difficult to determine for several reasons, including: use of different food types in calculations (e.g., fatty foods vs. a variety of foods); use of different assumptions in calculations; varying BBP levels in foods from different countries; and changing BBP levels in food over time. Dietary intake can vary widely depending on the types of foods eaten and the types of materials in which the foods are packaged. It is noted that the food levels reported by MAFF were collected 12-15 years ago and may not reflect current exposure levels.

Medical Exposure
BBP is not approved by the U.S. Food and Drug Administration for use in medical devices.

Occupational Exposure
Exposure in occupational settings can occur through skin contact and by inhalation of vapors and dusts.

Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tank cars (1). Higher exposures to phthalates can occur during the incorporation of the phthalate into the final product if the process is run at a higher temperature than is used in the manufacturing process. The ACC has estimated exposure to BBP in the workplace based upon an assumed level of 1 mg/m^3 during the production of phthalates and 2 mg/m^3 during the manufacture of flexible PVC. An exposure level was estimated by using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 μg/kg bw/workday and 286 μg/kg bw/workday for workers employed in phthalate manufacturing and flexible PVC production operations, respectively. As stated in the General Exposure section, absorption of BBP through skin is expected to be minimal.

The summary for Section 1 is located in Section 5.1.1.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

BBP was not observed to be a primary irritant or sensitizer in skin patch tests with volunteers (2). There are no human data on the general toxicity of BBP alone. Occupational exposures to phthalate mixtures containing BBP have been associated in single studies with respiratory/neurological effects and cancer (2). In a large, population-based case-control study (12), a significant increase in the risk of multiple myeloma has been found among workers employed for 5 or more years in PVC production. In the general population, a significant increase in the risk of bronchial obstruction during the first 2 years of life has been related to presence of PVC flooring (adjusted O.R≥1.89) in a case control study of 251 children and an equal number of matched controls (13). The consequences of exposure to children have not been studied.

2.1.2 Experimental Animal Data

Multiple studies in mice and rats are available describing the acute, sub-chronic, and chronic toxicity of BBP. These studies assess oral as well as inhalation routes of exposure. There is a 90-day dietary toxicity study in dogs that includes effects that are possibly related to decreased food consumption.

Acute Studies

Acute toxicity of BBP is low; an oral LD_{50} value for BBP in rats is reported as 2-20 g/kg (2). Rabbit dermal and ocular studies revealed no significant concern for BBP-induced sensitization or irritation (14).

Sub-chronic Studies

Agarwal et al. (15) published a study that explored previous NTP results indicating effects on male fertility and the hematopoietic system (Table 7-1). Adult male F344 rats, 10 per group, were fed diets containing 0, 0.625, 1.25, 2.5, or 5.0% BBP for 14 days. Using actual pre-treatment body weights (200 g) and reported food intake during the 14-day dosing period, equivalent doses of 0, 447, 890, and 1,338 mg/kg bw/day were calculated for the 3 lower dose groups. Since the high-dose group actually lost weight during the study, average weight during the study was used to calculate a dose of 1,542 mg/kg bw/day. All treated rats showed a dose-related increase in relative liver and kidney weights. No histopathology or hematology changes were observed at the 447 or 890 mg/kg bw/day dose levels. However, at doses of 1,338 and 1,542 mg/kg bw/day, relative decreases in testes, seminal vesicle, and thymus weight were noted; relative epididymis weight was reduced at the high dose. Dose-related histopathological changes in seminal vesicles, testes, and prostate were observed, as was a decrease in bone marrow cellularity at the two highest doses. Mild multifocal hepatitis and cortical lymphocytolysis in the thymus were also observed at the high dose. Increases in luteinizing hormone (LH) were observed at the lowest dose and two highest doses tested. An increase in follicle stimulating hormone (FSH) was observed in the two highest doses, and a decrease in testosterone was observed at the high dose. The decreased body weight seen at the two highest doses may be due to unpalatability of food; decreased food intake was documented. The severity of

the reduced food intake and attendant weight loss precludes associating effects with BBP, or BBP and inanition, at the high dose. The systemic LOAEL determined from these studies is 447 mg/kg bw/day based on increases in organ weight (liver, kidney) and increased LH levels.

Three-month feeding studies were conducted in 4-6 week-old Wistar and Sprague-Dawley (SD) rats fed diets with 2,500-12,000 or 2,500-20,000 ppm BBP, respectively (14) (Table 7-2). Male Wistar rats (27-45 rats/sex/group) received doses of 151, 381, or 960 mg/kg bw/day; female doses were 171, 422, or 1,069 mg/kg bw/day. At the low dose, an increase in liver to body weight ratio was seen in both sexes. No histopathology or hematology changes were noted. At the mid-dose, a decrease in body weight was noted in both sexes and increases in liver and kidney to body weight ratios were seen. Pancreatic tissues showed islet cell enlargement, vacuolization, congestion, inflammation, and minor fibrosis. Less frequently, additional pancreatic changes were observed, such as acinar cell atrophy, inflammation, and pyknotic nuclei. A decrease was observed in urinary pH in male rats only. At the highest doses tested, 960 (M) and 1,069 (F) mg/kg bw/day, hepatic necrosis and anemia were observed in addition to the effects seen at lower doses. Cecal enlargement, a finding of uncertain toxicological importance, was reported in this study. The LOAEL for this study was 151–171 mg/kg bw/day based on weight change in the liver.

In this same study, Sprague-Dawley (SD) rats (10/sex/group) were tested at doses of 0, 188, 375, 750, 1,125, or 1,500 mg/kg bw/day. Sprague-Dawley rats were less sensitive to BBP than were Wistar rats, as no pancreatic, hepatic, or testicular lesions, or cecal enlargement were observed. There were no changes in urinary pH or hematological parameters. The NOAEL was set at 375 mg/kg bw/day and the LOAEL at 750 mg/kg bw/day based on increases in organ weight ratios for kidney (male) and liver (female) (14).

A 13-week inhalation study was also conducted in groups of 6-8 week-old SD rats (25/sex/group) (14) (Table 7-2). The rats were exposed to BBP mists (>90% of aerosol particles <10 μm) at concentrations of 51, 218, or 789 mg/m^3 for 6 hours/day, 5 days/week. Using EPA (16) assumptions for rat body weights and daily inhalation rates, estimated exposure doses were 9.2, 39.4, and 143 mg/kg bw/day for males and 9.8, 42.0, and 152 mg/kg bw/day for females. NOAELs of 39.4 (M) and 42.0 (F) mg/kg bw/day were identified in this study. A LOAEL was determined at the highest doses tested, 143 (M) and 152 (F) mg/kg bw/day; this LOAEL was based on increases in liver and kidney organ to body weight changes. Serum glucose levels were also reduced at this dose in male rats only. No body weight changes or histopathological changes were observed.

The NTP (17) reported results of a 26-week dietary exposure study in 6-week-old F344/N male rats (Table 7-3). Groups of 15 male rats were fed BBP in the diet at concentrations of 0, 300, 900, 2,800, 8,300, or 25,000 ppm for 26 weeks. The authors calculated doses of 30, 60, 180, and 550 mg/kg bw/day for the 4 lowest exposure levels. A dose was not calculated in the highest exposure group because food intake could not be measured due to an excess scattering of feed. However, a dose of 1,650 mg/kg bw/day was estimated by CERHR based on intake levels observed in the lower dose groups. In the high-dose group, decreases in total body weight (due to decreased food intake) were observed, as were increases in relative liver and kidney weights. An increased incidence of macrocytic anemia was observed on days 30-180. The testis was determined the primary target organ based on weight, sperm concentration, and histopathological findings at the high dose. De-

creases in relative testis, absolute epididymis, and absolute seminal vesicle weight were observed, as were atrophy of seminiferous tubules and degenerative changes in testis and epididymis. No histologic changes in other body tissues were seen at this dose. The testis from animals in the lower dose groups were examined histologically and no effects were observed; lowered sperm counts were not seen at the 60, 180, or 550 mg/kg bw/day doses. Absolute and relative liver weight was increased at 550 mg/kg bw/day. A NOAEL was established at 180 mg/kg bw/day[1]. The LOAEL of 550 mg/kg bw/day reflects increases in mean cell hemoglobin after 60-180 days of treatment that may be associated with the macrocytic anemia observed at the next higher dose.

In a 3-month feeding study, 3 adult male and female beagle dogs/group were fed diets with 10,000–50,000 ppm BBP (males: 400, 1,000, or 1,852 mg/kg bw/day; females: 700, 1,270, or 1,973 mg/kg bw/day, as calculated by study authors) (14). Food palatability complicated interpretation of reduced body weights in low-and high-dose males and mid-and high-dose females. No other changes were observed for hematological or urinalysis measurements. In high-dose animals there were no histopathological effects in liver, testes, or pancreas.

Chronic Exposure Studies

Two sets of chronic feeding studies have been performed by the NTP (17, 18).

Potential BBP carcinogenicity was examined in both B6C3F1 mice and F344/N rats (18). Four-to-five week-old B6C3F1 mice (50/sex/group) were dosed through feed at concentrations of 0, 6,000, or 12,000 ppm for 106 weeks. Using EPA assumptions for B6C3F1 mouse body weight and food intake (body weight: 0.03733 kg [M], 0.0353 kg [F]; food intake: 0.0064 kg/day [M], 0.0061 kg/day [F]), dose levels of 0, 1,029, and 2,058 mg/kg bw/day and 0, 1037, and 2,074 mg/kg bw/day were calculated for males and females, respectively. No treatment-related changes in survival or neoplastic developments were seen. Dose-related decreases in body weight were seen in both male and female mice. There were no lesions observed in male or female reproductive organs.

F344/N rats (50/sex/dose) were fed diets containing 0, 6,000, or 12,000 ppm BBP (18) for 106 weeks. Using EPA assumptions for F344 rat body weight and food intake, respectively (M:0.380 kg, 0.030 kg/day; F:0.229 kg, 0.021 kg/day), dose levels of 0, 474, and 948 mg/kg bw/day and 0, 550, and 1,100 mg/kg bw/day were estimated for males and females, respectively. Male rats were sacrificed 29-30 weeks into the study because of increases in premature death. Internal hemorrhaging was suspected as the cause of these deaths. Body weight gain and food intake were decreased in both males and females. The female rats were allowed to continue through the 106 weeks of exposure; at necropsy the females exhibited an increased incidence of mononuclear cell leukemia (MNCL). Spleens were examined in the high-dose group and were found to be congested and infiltrated with mononuclear cells. MNCL has been associated with splenomegaly and sometimes hepatomegaly. No evidence of hepatomegaly was reported in these studies.

In another 2-year NTP bioassay (17) groups of 60 male Fischer 344/N rats (6 weeks old) were fed

[1]The NTP (17) report stated that epididymal sperm concentration was determined for the lowest and two highest of the treated groups. CMA reports that an audit revealed the original laboratory report, that is the data source for the NTP Report, states that epididymal sperm counts were determined from the three highest dose groups. The data from the original laboratory report are used in this evaluation.

BBP in the diet at concentrations of 0, 3,000, 6,000, or 12,000 ppm (0, 120, 240, or 500 mg/kg bw/day) and 60 females (6 weeks old) per group were fed concentrations of 0, 6,000, 12,000, or 24,000 ppm (0, 300, 600, or 1,200 mg/kg bw/day) (Table 7-4) (17). After 2 years of exposure to BBP, increases in relative kidney weights were observed in male rats at 120 mg/kg bw/day and represented the lowest observable changes in this study (17). Additional dose-related increases included relative epididymis weights at the 240 mg/kg bw/day dose and relative liver weight at the 500 mg/kg bw/day dose in male rats, with total body weight changes in rats occurring only at the highest dose tested, 500 mg/kg bw/day. At the highest dose level, histopathological changes included renal tubule pigmentation, hepatic granulomas, and focal pancreatic hyperplasia with "some evidence" of pancreatic carcinogenicity based on increased incidence of acinar cell adenoma and adenoma or carcinoma (combined). No testicular changes were observed; however, decreases in red blood cells (RBC) and increases in hemoglobin were observed 6 months into the study.

Female F344/N rats exposed to BBP for 2 years showed nephropathy at the 2 lowest doses tested (300 and 600 mg/kg bw/day). At 1,200 mg/kg bw/day, the animals exhibited decreases in body weight and increases in liver and kidney organ to body weight ratios. They also exhibited renal tubule pigmentation (15-24 months), nephropathy, microcytic anemia (15 months), decreases in triiodothyronine, and "equivocal evidence of carcinogenicity" based on pancreatic acinar cell adenoma and urinary bladder transitional cell epithelial papilloma. Pancreatic effects may have been due to chronic stimulation of pancreatic lipase secretion.

In a parallel study at the same laboratory, BBP's ability to induce hepatic peroxisomes was evaluated in female F344/N rats (17). Two enzyme markers for peroxisome proliferation, palmitoyl CoA oxidase and carnitine acetyl transferase, were significantly elevated after 1 month and 1 year of exposure in animals exposed to 6,000 ppm BBP and higher (~300 mg/kg bw/day), although the level of induction was lower than that observed after a 3-week exposure to DEHP. The discussion in the NTP report highlights the fact that BBP is a mild peroxisome proliferator compared to DEHP or to hypolipidemic drugs such as clofibrate.

From these 2-year studies, LOAELs for non-cancer, general toxicity effects were determined at 120 (M) and 300 (F) mg/kg bw/day based on kidney organ weight changes in the male and nephropathy in the females. At 500 (M) and 1,200 mg/kg bw/day (F), the highest doses tested, respectively, "some to equivocal evidence" of pancreatic (male and female) and urinary bladder carcinogenicity (female) was observed in rats. No testicular changes were observed at any of the doses tested; however, increases in epididymal weight were seen at the 2 highest doses (240 and 500 mg/kg bw/day). This change in epididymal weight was observed in the absence of total body weight change at the 240 mg/kg bw/day exposure dose.

2.2 Toxicokinetics

Phthalate Moiety Toxicokinetics

Absorption

Dermal: In a study of dermal absorption of a series of phthalate diesters (10), ^{14}C-BBP (157 µmol/kg) was applied to the skin (clipped back) of male F344 rats and the area covered with a perforated

cap. Absorption was estimated by the radioactivity eliminated in urine and feces over 7 days, which equaled 27% for BBP. Most of the remainder of the radioactivity was found at the site of application.

Oral: Oral administration of 5 g of BBP/kg to dogs resulted in 10% absorption (19). Administration of single oral doses of 2, 20, 200, or 2,000 mg/kg to male Fischer 344 rats showed a dose-dependent increase in the fraction of dose eliminated via the feces (20% at doses from 2-200 mg/kg; 72% at 2,000 mg/kg) and a dose-dependent decrease in the fraction eliminated via the urine (75% at a dose of 2-200 mg/kg and 22% at 2,000 mg/kg), suggesting that absorption through the gut was limited at the high dose (20).

Inhalation: There are no reports of the absorption of BBP administered by inhalation. By analogy with other phthalates, di(2-ethylhexyl)phthalate and diisodecylphthalate, BBP would be expected to be absorbed from the lung as the parent compound (21, 22).

Biotransformation

Oral studies in rats indicate that BBP is rapidly metabolized by gut enterases to its monoester metabolites (monobutyl and monobenzyl phthalates), which are absorbed and are either excreted in urine as the ester or conjugated with glucuronic acid and then excreted via the urine as the glucuronate (19, 20, 23). Urinary metabolites in rats following oral administration of 3.6 mmol BBP/kg/day (1,125 mg/kg bw/day) for 3 days indicated that 70% of the metabolites were monoesters while the remainder were monoester conjugates. The monobutyl ester is generally present in the highest amount; in one study, the ratio of monobutyl to monobenzyl phthalate was 5:3 (23). The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats).

BBP and dibutyl phthalate (DBP) share a common metabolite, monobutyl phthalate (MBuP); information from DBP germane to the monoester, and therefore also to BBP, will be presented throughout this evaluation. In addition to the monoesters, the esterase cleavage products, phenol (from the benzyl moiety) and butanol (from the butyl moiety), will be included.

Distribution

Tissue distribution was non-specific for the small amount of dermally absorbed BBP (10).

Excretion

Excretion of absorbed BBP and its metabolites is rapid, with approximately 90% eliminated in 24 hours, approximately 80% in urine and 20% in feces, at low doses (2-200 mg/kg). The half-life of BBP in blood is 10 minutes. The blood half-life of the monoester metabolites of BBP is approximately 6 hours (20). Following intravenous (IV) administration of 20 mg/kg of ^{14}C-BBP, 55% of the dose was excreted into bile while 34% was excreted in the urine (20).

Side Chain-associated Toxicokinetics

Phenol and butanol are products of hydrolysis of the monoesters. Phenol metabolism to polyphenols is well known: butanol is a primary alcohol that is easily oxidized to butyric acid (n-butanoic acid) by alcohol dehydrogenase and aldehyde dehydrogenase. Further metabolism (by α-oxidation

pathways) converts butyric acid into acetyl-CoA conjugates in intermediary metabolism pathways with no toxicological importance (24).

2.3 Genetic Toxicity

The NTP (17) reviewed the genetic toxicity of BBP. An increase in mutations was not observed following treatment of Salmonella and L5178Y mouse lymphoma cells with BBP in the presence or absence of S9 activation. BBP treatment with and without S9 activation did not result in sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. However, induction of sister chromatid exchanges and increased chromosomal aberrations in bone marrow cells were observed following a single intraperitoneal (IP) injection of mice with 1,250–5,000 mg/kg bw BBP. There were no increases in sex-linked recessive lethal mutations in the germ cells of Drosophila fed or injected with BBP.

Subsequent to the NTP review, BBP tested negative in the L5178Y mouse lymphoma mutation assay with and without activation, and in the Balb/3t3 cell transformation assay (25). Ashby et al. (26) reported negative results in a micronucleus assay in rats. The IPCS (2) review included the publication of Ashby et al. and concluded: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

The summary for Section 2, including general toxicity, toxicokinetics, and genetic toxicity, is located in Section 5.1.2.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

There were no human data located for Expert Panel review.

3.2 Experimental Animal Toxicity

Eleven complete studies and two abstracts were evaluated. Two studies performed through the NTP, were standard prenatal assessment (segment II) studies of BBP administered in the diet of rats and mice. A third was an oral gavage Segment II study in rabbits. There were five studies by Ema et al. in Wistar rats where BBP was administered in the diet or by gavage. Three studies of BBP evaluated drinking water exposure to Wistar rats during gestation and lactation with assessment of adult F_1 males. One abstract evaluated BBP exposure by subcutaneous injection to two strains of male mice ($B6C3F_1$ and CD-1) with subsequent mating to unexposed females (dominant lethal assessment).

3.2.1 Prenatal Development

A dietary study in CD (Sprague-Dawley) rats (27) involved exposure of 30 pregnant rats per group to 0, 0.5, 1.25, and 2.0% BBP (0, 420, 1,100, and 1,640 mg/kg bw/day) on gestation day (gd) 6-15. The dams were killed on gd 20, necropsied, and pups examined and evaluated (Table 7-5). Maternal toxicity was expressed in reduced body weights and decreased weight gain, decreased absolute feed consumption (but increased relative feed consumption in g/kg/day), increased relative liver weight (with no histopathological changes), and increased relative water intake at the 1,100 and 1,640 mg/kg bw/day doses. Relative kidney weights were increased at the 1,640 mg/kg bw/day dose. However, the kidneys were not examined histologically. Clinical signs of maternal toxicity, including ataxia and abnormal gait, were also observed at this dose.

At 1,640 mg/kg bw/day, there were increased resorptions and concomitant reduced numbers of live fetuses per litter, reduced fetal body weight, and increased fetal malformations. Urogenital malformations, analyzed separately, were increased; they included distended ureters and distended or absent kidneys. Other fetal malformations at the high dose were anophthalmia (missing eyes), fused or malaligned vertebrae, and fused ribs. There were increased incidences of fetal variations per litter at both the 1,100 and 1,640 mg/kg bw/day doses.

Significant developmental toxicity occurred at the 1,100 and 1,640 mg/kg bw/day doses; teratogenicity was observed at 1,640 mg/kg bw/day. Maternal toxicity was observed at doses that caused developmental toxicity. The maternal and developmental NOAELs were identified at 420 mg/kg bw/day.

Ema et al. (28) exposed pregnant Wistar rats, 15-18/group, to BBP in the diet at 0, 0.25, 0.5, 1.0, and 2.0% (intakes of 0, 185, 375, 654, and 974 mg/kg bw/day, respectively) on gd 0-20. Dams were killed on gd 20 and evaluated in a Segment II study design (Table 7-6). There were also pair-fed controls matched with the animals in the highest dose group. No dams died in any group. Adjusted maternal body weight gains (not including gravid uterus weight) and feed consumption were reduced at doses of 654 and 974 mg/kg bw/day. All dams at 974 mg/kg bw/day had fully resorbed litters. There was no treatment-related pre-implantation loss or teratogenicity. The authors concluded that the maternal NOEL was 375 mg/kg bw/day and the developmental toxicity NOEL was 654 mg/kg bw/day. The Expert Panel did not agree with the author's identification of developmental

effect levels given that live litter size was reduced at 375 mg/kg bw/day (11.3 vs. control value of 13.9) and 654 mg/kg bw/day (12.3 vs. control value of 13.9); fetal body weights (by sex per litter) were significantly reduced at 654 mg/kg bw/day. The data did support a developmental NOAEL of 185 mg/kg bw/day.

In a second Segment II study, Ema et al. (29) treated 10 Wistar rats/group with BBP by gavage with 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–15 (Table 7-7). Dams and fetuses were evaluated following sacrifice on gd 20. Maternal body weight gains were reduced at doses of 750 and 1,000 mg/kg bw/day, but the corrected weight gain (maternal body weight excluding the gravid uterus) was decreased only at the high dose. Food intake was reduced at all dose levels. Four dams in the high-dose group died and entire litters were resorbed in the six surviving dams. Complete litter resorptions were observed in 3/10 dams in the 750 mg/kg bw/day group. Other effects at that dose included increased fetal death due to postimplantation loss, reduced fetal weight, and increased external, skeletal, and internal malformations. The malformations consisted primarily of cleft palate, fused sternebrae, and dilated renal pelves. The maternal and fetal NOAEL was identified as 500 mg/kg bw/day.

The Segment II dietary study in CD-1 mice (30) involved exposure of 30 pregnant mice per group to 0, 0.1, 0.5, and 1.25% BBP (0, 182, 910, and 2,330 mg/kg bw/day), on gd 6-15 (Table 7-8). Maternal toxicity was expressed as reduced weight gain at the two highest doses (910 and 2,330 mg/kg bw/day), and increased relative liver and kidney weights and increased relative water intake at the high dose. No histopathological changes were observed in the liver or kidneys.

Embryofetal effects included increased incidences of resorptions and late fetal deaths, with concomitant reductions in live fetuses per litter, and increased malformations (external and skeletal) at 910 and 2,330 mg/kg bw/day. Malformations included exencephaly, short tail, cardiovascular defects, fused ribs, and abnormal or fused sternebrae and vertebrae. Fetal body weight per litter was decreased and fetal variations were increased at the 2,330 mg/kg bw/day dose. As with rats, maternal and developmental toxicity was present at the two highest doses. The maternal and developmental NOAEL was 182 mg/kg bw/day.

A Segment II developmental toxicity study (31) was also performed in New Zealand white rabbits. The does, 17/group, were administered BBP (Santicizer 160) orally by gelatin capsule on gd 6-18 at 0, 3.0, or 10 mg/kg bw/day. Does were terminated on gd 29. There was no demonstrable maternal toxicity. There was no demonstrable developmental toxicity, such as effects on fetal body weight, 24-hour survival, or treatment-related external or visceral malformations. Skeletal findings in toto were considered equivalent across groups.

Mechanistic Studies

Ema et al. has published a series of articles that focus on three issues: 1) direct vs. indirect toxicity of BBP; 2) the dose and time dependency of the prenatal effects of BBP exposure; and 3) study of the toxic properties of the two monoester metabolites of BBP.

Direct vs. indirect toxicity:

Ema (32, 33) exposed Wistar rats to BBP at 2.0% in diet (974 mg/kg bw/day) on gd 0-20, gd 0-11

or gd 11-20. Pair-fed controls received the same amount of diet as treated rats. All dams exposed on gd 0-20 had fully resorbed litters. The pair-fed controls exhibited maternal weight gains comparable to the BBP group, but no treatment-related fetal malformations or resorptions were observed. Dams fed BBP on gd 0-11 also had fully resorbed litters. No increase in postimplantation loss was found in rats exposed on gd 11-20, but the fetuses in this group exhibited malformations, predominantly cleft palate and fused sternebrae. Thus, resorption does not appear to be related to decreased food consumption, but is an effect of the chemical, per se.

Time-and dose-dependency:
In another dietary study using 2.0 % BBP on gd 0–7, gd 7–16, and gd 16–20 (34), postimplantation loss was increased after exposure on gd 0-7 or 7-16; teratogenicity was observed (predominantly cleft palate and fused sternebrae) after exposure on gd 7–16 (34). Ema et al. (29) also dosed Wistar rats by gavage with BBP in olive oil at 0, 500, 750, or 1,000 mg/kg bw/day on gd 7-15. No live fetuses were present at 1,000 mg/kg bw/day and malformations (cleft palate, fused sternebrae, dilated renal pelves) occurred at 750 mg/kg bw/day accompanied by increased *in utero* death, decreased fetal body weight, and maternal toxicity (reduced weight gain and feed consumption). At 500 mg/kg bw/day, maternal feed consumption during the exposure period was reduced, but no embryofetal effects were observed.

To investigate further the observed embryolethality and teratogenicity, Ema et al. (35) exposed Wistar rats to BBP in the diet at 2.0% (954 mg/kg bw/day) during gd 0-7g, gd 0-9, or gd 0-11. Pre-implantation loss was equivalent across all groups. Postimplantation loss was highest for groups treated on gd 0-11. Uterine and ovarian weights were reduced, as was plasma progesterone in all groups (except that ovarian weight was unaffected on gd 7). The authors suggest that the post-implantation loss in early pregnancy was mediated by reduced plasma progesterone levels from impairment in luteal function.

It appears that postimplantation death or the development of malformations is dependent upon both the dose and time during gestation when the exposure occurs.

Studies on monoesters:
Ema et al. evaluated the developmental toxicity of the two metabolites of BBP: MBuP (36-38) and mono-n-benzyl phthalate (MBeP) (39) when administered by gavage to Wistar rats.

Ema et al. (38) gavaged Wistar rats with MBuP at 0, 250, 500, and 625 mg/kg bw/day on gd 7–15. Maternal toxicity was present at the two highest doses, expressed as reduced body weight gains and reduced feed consumption. At these doses there were also significant increases in postimplantation loss/litter, and decreases in live fetuses/litter and fetal body weight per litter. Fetal malformations were also increased at these doses, with cleft palate, deformed vertebral column, and dilated renal pelves the predominant findings.

Ema et al. (36) followed-up with evaluation of stage specificity studies. Wistar rats were dosed with MBuP at 0, 500, 625, or 750 mg/kg bw/day on gd 7–9, gd 10–12, or gd 13–15. Embryolethality was increased at all doses for all dosing intervals. No teratogenicity was observed from the gd 10–12 dosing interval. Increased incidences of fetal external malformations were present in the groups

treated with 500 and 750 mg/kg bw/day on gd 7–9 and 13–15. Increased skeletal malformations were observed in groups treated with 500, 625, and 750 mg/kg bw/day on gd 7–9, and with 625 and 750 mg/kg bw/day on gd 13–15. Deformed cervical vertebrae were predominant in groups treated on gd 7–9. Cleft palate and fused sternebrae were observed in groups treated on gd 13–15. These results are consistent with the findings for DBP and BBP, and imply that MBuP (and/or subsequent metabolites) may account for the developmental toxicity (embryolethality and malformations) for both DBP and BBP.

Ema et al. (39) also administered MBeP by gavage at 0, 250, 313, 375, 438, and 500 mg/kg bw/day to pregnant Wistar rats on gd 7–15. Decreased maternal weight gain during dosing was present at doses from 313 to 500 mg/kg bw/day, and reduced feed consumption was present from 250 to 500 mg/kg bw/day. Increased postimplantation loss was present at 438 and 500 mg/kg bw/day. Increased incidences of fetal external malformations were present at 438 and 500 mg/kg bw/day, skeletal malformations were present at 313–500 mg/kg bw/day, and visceral ("internal") malformations at 375–500 mg/kg bw/day. The most common fetal findings were effects on cervical and thoracic vertebrae, ribs, and kidney (dilated renal pelves at 375 and 438 mg/kg bw/day, and hypoplasia of the kidney at 500 mg/kg bw/day).

These studies establish a maternal and developmental NOAEL for MBuP of 250 mg/kg bw/day. For MBeP, no maternal NOAEL was identified (effects were observed at 250 mg/kg bw/day); the developmental NOAEL was 250 mg/kg bw/day under the conditions of the study. The finding of fetal kidney effects at 375–500 mg/kg bw/day for MBeP is of concern since the CD rat study (27) also found fetal kidney malformations at the high dietary dose (1,640 mg/kg bw/day) and the kidney is a known target organ in adult rats. Cervical ribs are also of concern due to their rarity and proposed mechanism of disruption in gene expression.

An additional study by Ema et al. (37) compared effects of BBP and DBP administered by gavage to pregnant Wistar rats at 0, 750, 1,000 or 1,250 mg/kg bw/day on gd 7–9, gd 10–12, or gd 13–15. Increased postimplantation loss was observed for both compounds at all doses from all exposure periods. Malformations were observed in groups treated with both phthalate esters at ≥750 mg/kg bw/day on gd 7–9 (vertebral column and ribs) and on gd 13–15 (cleft palate and fused sternebrae). No malformations were observed with either compound at any dose when they were administered on gd 10–12. The authors concluded that "the similarity in dependence of gestational days of treatment on the manifestations of developmental toxicity and on the spectrum of fetal malformations caused by BBP and DBP suggests that they may act by the same mechanism, possibly via a common metabolite of these two parent compounds."

3.2.2 Postnatal Development

Imajima et al. (40) gavaged pregnant Wistar-King A (WKA) rats with MBuP in sesame oil at 0 or 300 mg/day on gd 15–18 (equivalent to approximately 1,000 mg/kg bw/day) (Table 7-16). Male offspring were evaluated on gd 20 and on postnatal days (pnd) 30–40 to determine the position of the testes. In control males, all testes were located in the lower abdomen on gd 20 (19 pups, 3 litters) and had descended into the scrotum on pnd 30–40 (15 pups, 3 litters). In stark contrast, in males exposed *in utero* to MBuP, on gd 20 all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent. On pnd 30–40, MBuP exposed males ex-

hibited cryptorchidism (22/26 pups, 5 litters with uni-or bi-lateral undescended testes); 87% of the undescended testes were in the abdominal cavity, the remaining 13% were located at the external inguinal ring. Testis descent is under androgenic control; the authors suggest that phthalate esters may interfere with FSH stimulation of cAMP accumulation in Sertoli cells, resulting in the reduced secretion of Mullerian inhibiting substance, a putative mediator in transabdominal migration of the testis.

The Panel is aware of data indicating that DEHP, BBP, and diisononylphthalate (DINP), but not diethylphthalate (DEP), or dimethylphthalate (DMP), produced reproductive tract malformations in male offspring of rats gavaged with 750 mg/kg bw/day in corn oil on gd 14 to pnd 3 (41). DEHP and BBP are approximately equipotent, resulting in 91 and 84% of the male offspring with multiple malformations, respectively; DINP resulted in 7.7% of the offspring males affected ($p<0.04$) versus 0% in controls. DBP is also active as an anti-androgen with comparable potency to DEHP and BBP.

Since BBP and DBP share a common metabolite, MBuP, the study by Mylchreest et al. (42), in which pregnant rats were orally dosed on gd 12–21 with DBP at 0, 0.5, 5, 50, 100, or 500 mg/kg bw/day, is germane. The male offspring were evaluated until puberty. The maternal NOAEL was 500 mg/kg bw/day. The developmental NOAEL was 50 mg/kg bw/day, based on the presence of retained nipples and areolae in pre-weanling males at 100 mg/kg bw/day and malformations of the male reproductive tract, testicular lesions (Leydig cell hyperplasia and one Leydig cell adenoma), increased incidence of undescended testes, reduced anogenital distance, and retained nipples and areolae in males at 500 mg/kg bw/day.

3.2.3 Postnatal Function

This section discusses a series of studies in which pregnant rats were exposed to low doses in drinking water. Two primary issues emerged: effects on male reproductive organs and perinatal mortality.

Sharpe et al. (43) reported on adult male offspring from Wistar rat dams exposed 2 weeks prior to mating, and during gestation and lactation, to BBP (in ethanol) in drinking water at 1 mg/L (Table 7-9). This study combined data from the same dams bred twice, with exposure continuing, to assess the effects of BBP. At weaning, male offspring were reared to adulthood, with no further BBP exposure and assessed for reproductive effects. Maternal BBP intake was calculated by weighing water bottles for three 48-hour intervals. On pnd 1-2, pnd 10-12, and pnd 20-21, BBP intake was estimated at 0.126, 0.274, and 0.336 mg/kg bw/day (the latter two measurements were confounded by pups drinking the treated water). At 90-95 days of age, male offspring had significantly smaller testes, but exhibited no effects on body, kidney, or ventral prostate weights. Testicular morphology and seminiferous epithelial tubule cross-sections were unaffected, but the authors reported reduced daily sperm production when compared to controls. This laboratory subsequently reported unexplained fluctuation in testicular weight of control rats (44).

Ashby et al. (26) attempted to replicate the Sharpe et al. (43) findings with larger group sizes and better control and characterization of the dosing material. They exposed 18 AP (Wistar) rats during gestation and lactation to 1 mg/L BBP in drinking water and assessed the F_1 male offspring as adults (Table 7-10). They found no effects of BBP exposure on any endpoints assessed, including testis weights, daily sperm production, caudal epididymal sperm count, accessory sex organ

weights, or relative incidence of gonadotrophs (FSH-positive cells) in the pituitary for male or female offspring. This study employed only one dose level. Additional details about study results are included in Table 7-10.

Another replication of the Sharpe et al. (43) study was attempted by TNO (45) (Table 7-11). They exposed Wistar outbred (Crl:(WI)WU BR) rats, 28 females/group, to BBP in the drinking water at 0.1, 1, and 3 mg/L during premating, gestation, and lactation periods. Doses to dams were estimated at 0, 0.012, 0.14, and 0.385 mg/kg bw/day. No effects were observed on mating index, female fecundity or fertility, or on prenatal postimplantation loss in the parental generation. The study failed to reproduce any effects on F_1 male reproductive organ weights or daily sperm production rates when the F_1 offspring reached adulthood. A decreased number of normal epididymal sperm was found in the low-dose group, and was not considered treatment related. Epididymal sperm motility was normal. Preputial separation in males and estrous cyclicity in females were also unaffected by BBP treatment. Following an evaluation of the Sharpe et al, Ashby et al., and TNO studies, the Expert Panel recommended that the reproductive effects in F_1 males reported by Sharpe et al. (43) not be used in assessing the reproductive toxicity of BBP. The bases for the recommendation are:

1) lack of dose-response data (e.g., a single-dose study);
2) no analytical information of BBP levels in drinking water;
3) the original laboratory could not replicate their original findings; and
4) two other respected laboratories have been unable to replicate the effects.

Although no reproductive effects were observed in the TNO (45) study, an increase in postnatal pup mortality was noted. There was a significant decrease in postnatal pup survival (by total pups/group) in the 1 and 3 mg/L BBP groups and the DES-positive control (Table 2). According to the authors, the values for BBP were not statistically significant on a per litter basis. The same lab immediately repeated the study according to the same protocol, except that only controls and the 1 and 3 mg/L doses were tested. Pup losses in the second study were significantly decreased compared to control at the 1 mg/L dose and again significantly increased compared to control at the 3 mg/L level (Table 2). Again, statistical significance was reported by the authors as not being achieved when analyzed on a per litter basis. Interestingly, significant effects on decreased pup survival (by total pups/group) were reproduced at the 3 mg/L level. The Panel is aware that the concurrent control values for pnd 0–4 pup loss in these two studies exceeded the historical control values for this laboratory, and that other studies performed at this laboratory during this general time period also experienced high pup losses on pnd 0–4, even in the vehicle control groups.

As reported in an abstract, Parks et al. (47) dosed Sprague-Dawley rats by gavage with 750 mg/kg bw/day of BBP, DEHP, or corn oil (vehicle) from gd 14 through pnd 3. On pnd 2, anogenital distance (AGD), and testes weight were measured. Testes weights and AGD were significantly decreased, and the incidence of retained areolae on pnd 13 was increased for both DEHP-and BBP-exposed male pups.

Developmental effects were also reported in a reproductive screening study by Piersma (48) and are addressed in Section 4.

Table 2: Combined Postnatal Mortality in Two TNO Studies with Wistar Rats

Maternal BBP doses in Drinking Water: mg/L (mg/kg bw/day)	***0***	***0.1 (0.012)[a]***	***1.0 (0.14)***	***3.0 (0.385)***
Study 1 Pnd 0-4 pup loss/ total pups at birth[b]	17/252 (25)	2/233 (23)	30[c]/212 (23)	36[c]/248 (24)
Study 2 Pnd 0–4 pup loss/ total pups at birth[b]	42/299 (26)	Not determined	19[d]/248 (23)	70[c]/277 (26)
Combined pnd 0-4 Pup loss /total pups at birth[b]	59/551 (51)	2/233 (23)	49/460 (46)	106/525 (50)
% Pnd 0-4 pup loss	10.7%	0.86%	10.65%	20.19%

[a] This dose only tested in one study; all other doses tested in two studies.
[b] Number in parentheses equals total number of litters.
[c] Significant increase when analyzed by group not significant when analyzed by litter.
[d] Significant decrease when analyzed by group not significant when analyzed by litter.

Table 3: Pre and Postnatal Mortality in Bayer Study

	Drinking Water (ppm)			***Diet (ppm)***		
	0	***1.0***	***3.0***	***0***	***1.0***	***3.0***
BBP intake (mg/kg bw/day)	0	0.17	0.54	0	0.11	0.34
Postimplantation loss per group: number of resorptions (number of implantations)	24(269)	30(281)	40(300)	18(282)	33(316)	25(300)
% Postimplantation loss per group (not statistically significant)	8.92	10.68	13.33	6.38	10.44	8.22
Postnatal viability index % (pnd 0–4)	97.1	100.0	99.6	98.5	99.6	99.3

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data available on the reproductive toxicity of BBP alone. Occupational exposure to phthalate mixtures containing BBP in PVC production has been associated with increased incidence of menstrual disorders and spontaneous abortions among female workers (49).

4.2 Experimental Animal Toxicity

Six studies were reviewed in the evaluation of the reproductive toxicity of BBP. No study was definitive and no multigeneration-reproduction study has been published for BBP. Three studies measured reproductive performance. One other reported claims of low-level effects of BBP on reproductive development (discussed in Section 3), but these effects have not been reproduced by separate laboratories.

An assessment of the reproductive toxicity of BBP was reported by Piersma (48) (Table 7-13). This standard general and reproductive toxicity screen, conducted according to the OECD 421 protocol, provides useful indications as to major toxic effects. Male and female WU rats (10/sex/group), 10–11 weeks old at the start of exposure, were gavaged for 14 days with BBP in corn oil at dose levels of 0, 250, 500, or 1,000 mg/kg bw/day, and then paired (1:1) and allowed to mate for a maximum of 14 days while dosing continued. Once evidence of mating was observed, the animals were separated. Males continued to be dosed daily, and were then killed and necropsied after a total dosage period of 29 days. Reproductive organs were removed and placed in Bouins fixative. Dosing of females continued until pnd 6, after which the females were killed and necropsied and ovaries and uteri examined. Pups were counted, sexed, weighed, and examined for external malformations on pnd 1 and 6 and then killed.

Body weight gain for the F_0 males was reduced at the high dose (by 21%), whereas the body weight gain of the F_0 females was increased in the second week of dosing (12 g/week compared to 4 g/week for the controls). During pregnancy, the body weight gain of the dams was significantly reduced at the high dose (by 40%). The numbers of animals achieving a pregnancy were 9, 8, 7, and 4 (of 10) in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively. Postnatal pup mortality did not differ across dose groups, but average litter sizes at birth were 9.4, 11.4, 8.4, and 1.5 in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively, with statistical significance achieved at the highest dose. Absolute pup weight was significantly reduced at birth in the high-(29%) and mid-dose (7%) groups. Testicular degeneration accompanied by interstitial cell hyperplasia was significantly increased in the high-dose F_0 males. Ovary structure was not affected by treatment.

In Piersma et al. (48), the high-dose group had lower fertility (decreased numbers of litters and decreased numbers of pups per litter) in the F_0 generation with marked histopathology in the testes, but not in the ovaries. F_1 pup weight was reduced at birth in the mid-and high-dose groups and a developmental NOAEL of 250 mg/kg bw/day was identified. The reproductive NOAEL was identified as 500 mg/kg bw/day. The Expert Panel's confidence in the quality of the study is moderate to high; however, because of the design limitations, such as a lack of measures in the F_1 generation, there is uncertainty that these doses correctly represent the reproductive NOAEL.

A one-generation reproduction study following OECD guideline 415 was performed in Wistar rats that were mated twice and produced two litters (50) (Table 7-14). BBP was administered in the diet at levels of 0, 0.2, 0.4, and 0.8% to 12 male and 24 female rats per group for 10 and 2 weeks prior to the first mating, respectively. Seven to thirteen days after the first litter was weaned (at pnd 21), the study was repeated with the same rats. Average doses to males during the premating period were estimated by authors at 0, 108, 206, or 418 mg/kg bw/day. Average female doses during the premating, gestation, and lactation periods were estimated at 0, 106, 217, or 446 mg/kg bw/day; 0, 116, 235, or 458 mg/kg bw/day; and 0, 252, 580, or 1,078 mg/kg bw/day, respectively. There were no treatment-related clinical signs or mortality. There were periods of reduced body weight or weight change in females in the high-dose group during gestation and lactation in each of the two matings. A decrease in food consumption during the gd 0–14 period in both matings was considered a substance-related effect. A slight decrease in the number of treated females with litters observed in the first mating was not observed in the second mating. Mean pup weight was slightly decreased in the high-dose group during lactation; this decrease reached statistical significance at pnd 21 in the second litter. The authors attributed the pnd 21 finding to direct consumption of BBP in diet by the pups after pnd 14. All standard reproductive indices (fertility, implantation, and fecundity) were within normal ranges. At necropsy, tissues from male and female reproductive organs were collected and fixed in 4% buffered formalin. Microscopic examination of hematoxylin-and eosin-stained slides from these tissues was performed for control and high-dose rats. Relative liver weights were increased in high-dose females, but examination revealed that the liver and reproductive tissues were normal. The authors concluded that the NOAEL for reproductive performance was 418 mg/kg bw/day in males and 446 mg/kg bw/day in females, with the parental NOAEL being 206 mg/kg bw/day in males and 217 mg/kg bw/day in females.

The NTP (17) (Table 7-15) described a 10-week modified mating study. Male F344 rats, 6 weeks old at the commencement of the study, were exposed to BBP (15/group) in the diet at levels of 0, 300, 2,800, or 25,000 ppm for 10 weeks (which delivered approximate doses at 0, 20, 200, 2,200 mg/kg bw/day) and then allowed to recover for 2 days. The rats were then housed individually with two untreated females during a 7–day mating period and females were removed on the first day of a vaginal plug or sperm detection. Females were necropsied on gd 13. After the mating period, 10 and 11 days after receiving the last dose in feed, the males were necropsied and a full histological examination made at 0 and 25,000 ppm only. However, the testis and epididymis, seminal vesicle, and prostate were examined in all groups. The fixative used to preserve the testis was not indicated. Epididymal sperm analysis was also performed on the males; sperm samples were collected for evaluation at the end of the study.

Mean body weights of the high-dose males were 71% of control values at the end of the study, representing a significant reduction. Food consumption differences between the control and high-dose groups at the end of the study were only modestly decreased with treatment when proportionality to body weight is considered. Liver and thymus to body weight ratios were increased in the 2,200 mg/kg bw/day group, whereas absolute and relative testis and prostate weights were reduced. There was marked degeneration in the testis and epididymis at this dose. One animal in the low-dose group had marked testicular atrophy and others had fewer sperm in the epididymis. Epididymal sperm concentration was: 87, 70, and 0.1 % of control at the 20, 200, and 2,200 mg/kg bw/day groups, respectively. Other sperm parameters (motility, morphology) were not measured in the high-dose

group due to the absence of sperm; sperm motility and morphology were not different from controls in the other treatment groups. Although 10/30 females mated to high-dose males were sperm-positive during the mating trial, none were pregnant at necropsy. The pregnancy measures of the two lower dose groups were similar to control values.

In the NTP study, the high-dose group (2,200 mg/kg bw/day) had a high rate of infertility (decreased numbers of pregnancies) with marked histopathology in the testes and epididymides and a lowered sperm count. Effects in the 200 mg/kg bw/day group were restricted to a significant reduction in sperm count. However, it was subsequently noted that sperm counts might have been affected by a shorter recovery period from the time between mating to necropsy in the 200 mg/kg bw/day group compared to the other dose groups (51). Judd et al. provide the most recent example of a significant body of literature indicating that sperm levels in the cauda epididymis are significantly reduced by ejaculation; in some cases counts are reduced to <50% of control values (52, 53). Because epididymal sperm counts in rats have been found to require at least 4–7 days to return to normal after mating (54), and 13/15 rats in the 200 mg/kg bw/day group were killed less than 4 days after the detection of a vaginal plug in their mates, while only 7 control males were killed in this same period, the reduction in sperm count in the 200 mg/kg bw/day group in this 10-week study must be considered questionable. Additionally, an expert panel reviewing methods of sperm analysis stated that at least a week should transpire between mating and necropsy in order to avoid ejaculation-induced confounding of sperm count data (55). The effects at 2,200 mg/kg bw/day are considered both treatment-and dose-related. A NOAEL of 200 mg/kg bw/day was selected by the Expert Panel. This may not correctly represent the NOAEL because of the lack of measures to assess effects in females and the lack of assessment of reproductive systems in the F_1 generation.

Parallel to the 10-week modified mating study (17), a 26-week sub-chronic study was performed where male F344 rats received BBP in the diet at doses of 0, 300, 900, 2,800, 8,300, and 25,000 ppm (0, 30, 60, 188, 550, and 1,650 mg/kg bw/day). The results of this study are presented in the section on General Toxicity and in Table 7-3. While a mating sequence was not part of the 26-week-study design, all other protocol parameters associated with male effects (organ weights, tissues for microscopic evaluation, and epididymal spermatozoal parameters) were identical to the NTP 10-week study. A comparison of results shows similarity in effects on body weight gain, organ weights, histopathological findings, and sperm motility. Interestingly, while sperm concentration in the 200 mg/kg bw/day group was reduced by 30% in the 10-week study, the values for the 550 mg/kg bw/day group in the 26-week study were not reduced. All other measures at this dose were similar to controls. Results of the other two doses, compared to their contemporary controls, were similar.

Agarwal et al. (15) (Table 7-1) examined the effect of BBP on the male reproductive system of adult rats. Fischer F344 rats (10 males per group) aged 12–13 weeks were administered BBP at levels of 0, 0.625, 1.25, 2.5, and 5% (0, 447, 890, 1,338, and 1,542 mg/kg bw/day) in the diet for 14 days and killed on day 15. Details of the study and effects on systemic endpoints are provided in Chapter 2. Reproductive effects at the two highest doses included significant weight and histological changes to the testis and accessory sex glands accompanied by changes in circulating FSH and LH levels. An oral NOAEL for reproductive toxicity in this 14-day study in adult male F344 rats was 1.25 % (890 mg/kg bw/day). Expert Panel confidence in the quality of the study is moderate;

within design limitations, the study is well conducted and reported. Panel confidence is low that these dose levels correctly represent the NOAEL due to the short exposure time and because guidelines for this type of study do not require assessment of younger animals or the F_1 generation.

Studies on postnatal male fertility, with animals exposed indirectly through maternal consumption, as reported by Sharpe et al. (43), and subsequent publications by Ashby et al. (26) and TNO (45), that failed to reproduce the original findings are presented and discussed in Section 3.2.

According to an abstract, a dominant lethal study was performed (56) on $B6C3F_1$ and CD-1 male mice administered BBP by subcutaneous injections on days 1, 5, and 10 of the study at doses equivalent to 400–600, 1,280-1,840, and 3,200–4,560 mg/kg bw/day (triethylene melamine was the positive control). The males were then paired with untreated females every 4 days through day 49; female uterine contents were evaluated on gd 17. BBP did not affect prenatal deaths or fertility in either strain at any dose.

Mode of Action

Several studies have examined the ability of selected phthalate esters to compete with labeled estradiol (E2) for binding to the estrogen receptor (ER). Sources of ER protein included rat uterine cytosol (57), rainbow trout hepatic cytosol (58), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (59, 60), and rainbow trout ERs expressed in yeast (61). Tritiated 17ß-estradiol (E2) was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. BBP has been shown to bind to the estrogen receptor (ER) of rat (57) or trout (58). The relative binding affinity is approximately 10,000–100,000 times less than (E2).

Selected phthalate esters have been examined in a number of *in vitro* gene expression assays systems. The assays have used stably transfected cells (57), transiently transfected cells (57, 58), yeast based assays (57, 61-63), and vitellogenin induction in rainbow trout hepatocyte cultures (61). BBP induces weak activity in *in vitro* estrogen-mediated gene expression assays in mammalian cell transfection experiments at 10 µM, the highest concentration examined (57). In a yeast assay of estrogen-mediated gene expression, the potency of BBP was $1x10^6$–$5x10^7$ less than that of E2, but its metabolites MBuP and MBeP demonstrated no estrogenic activity (63). However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in 10 Sprague-Dawley rats/group gavaged with 20, 200, and 2,000 mg/kg bw/day for 4 days (57). Moore (64) reviewed the data on the estrogenic potential of phthalates and concluded that the estrogenic ability of phthalates identified in the *in vitro* studies is "not relevant to humans or to the environment."

The summary for Section 4 is located in Section 5.1.4.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

BBP is used in PVC construction materials, automotive materials, and food conveyor belts (1). There appears to be no significant use of BBP in toys or medical equipment. It is believed that only negligible amounts of BBP are present in air due to its low volatility; a limited number of air monitoring studies support this view. In a survey of California homes, the median air level of BBP was measured at 0.034–0.035 ng/m^3, and median outdoor air levels of BBP were below the detection limit of 0.051 ng/m^3. However, inhalation exposure to BBP in flexible PVC manufacturing facilities has been estimated at 286 µg/kg bw/workday (1). Exposure through contact of BBP-containing materials with skin is negligible due to the relatively slow absorption through skin (2, 10, 11).

The IPCS (2) concluded that consumption of food containing trace levels of BBP is the only significant source of exposure to the general population. Based on a survey of Canadian foods, IPCS estimated that exposure of adults to BBP is 2 µg/kg bw/day and that exposure levels in children could be up to three-fold higher. Exposures in children may be higher due to dietary differences and intake of BBP through mouthing of BBP-containing objects. MAFF estimated the BBP exposure of adults through diet at 0.11–0.29 µg/kg bw/day and exposure of infants through formula at 0.1–0.2 µg/kg bw/day. In all exposure estimates, it was evident that exposure to the general population, including children, is well below 10 µg/kg bw/day. Discrepancies in food exposure estimates may be due to the inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations.

5.1.1.1 Utility of Data to the CERHR Evaluation

BBP exposures resulting from food intake were estimated by two authoritative sources. There are limitations in these estimates. One agency used 12-15 year-old data which may not reflect current exposure, and the food data were collected in Europe and Canada and estimates may not accurately reflect US dietary patterns.

5.1.2 General Biological and Toxicological Data

Toxicity.

There are no human data on exposure to BBP alone. Exposures to BBP-containing phthalate mixtures have been associated with elevated respiratory/neurological morbidity and increased risk of cancer in occupationally exposed population groups (2); a single controlled epidemiological study has found an increased risk of bronchial obstruction in young children related to indoor exposures from PVC floor covering (13); BBP is a common component of PVC.

In animals BBP is not acutely toxic by the oral or dermal route as evidenced by the LD_{50} value exceeding 2 g/kg bw (2). Several subchronic and chronic dietary studies in rats reported consistent adverse effects on body weight and in kidney, liver, and testes (14, 15, 17). The earliest response was an increase in kidney or liver to body weight ratio(s) observed at doses of 120-151 mg/kg bw/day and higher. Histological changes in the liver were observed in some studies at doses of

960 mg/kg bw/day and higher and changes in kidneys were observed in the chronic study at doses of 500 (M) – 1,200 (F) mg/kg bw/day. Anemia was observed at doses of 500 mg/kg bw/day and higher. The pancreas may also be a target organ in rats, as pancreatic lesions were reported in a sub-chronic study at 381 mg/kg bw/day. Lesions in testes, seminal vesicles, epididymis, and/or prostate were noted after exposure of rats to 1,338 mg/kg bw/day or higher. In an inhalation study in rats, increases in liver and kidney weights were reported at the maximum dose of 789 mg/m^3 (~150 mg/kg bw/day) (14). BBP is considered a weak inducer of peroxisome proliferation in rats.

In repeat-dose studies, mice were less sensitive to toxic effects than were rats. Dietary studies of up to 2-years' duration in B6C3F_1 mice showed dose-related reductions in body weight at doses of 1,029 mg/kg bw/day and higher (18). There was no clinical or histological evidence of toxicity in tissues, including male and female reproductive organs. Male dogs also appear to be less sensitive than rats because oral doses up to 1,852 mg/kg bw/day for 90 days resulted in reduced body weight but produced no histopathological effects in testes or liver (14).

A 2-year dietary study found no evidence of carcinogenicity in B6C3F_1 mice and only a marginal increase in mononuclear cell leukemia in F344 female rats (18). In a second study in F344 rats, there was some evidence of pancreatic carcinogenicity in males exposed to 500 mg/kg bw/day and equivocal evidence of pancreatic and urinary bladder carcinogenicity in females exposed to 1,200 mg/kg bw/day (17).

Toxicokinetics.
There are no data from studies in humans. There are no inhalation studies in any species. BBP is rapidly absorbed (at least 75% at doses of 2-200 mg/kg) in orally-dosed rats; this dropped to 22% at 2,000 mg/kg, suggesting saturation at high doses (20). BBP is absorbed slowly through the skin (27% in 7 days) of rats (10). BBP is rapidly metabolized to monobutyl and monobenzyl esters; by analogy to other phthalate esters, this probably occurs by pancreatic lipase and esterases in the small intestine. The monobutyl ester is usually present in higher amounts, 5:3, than is the monobenzyl ester (2). These monoesters are typically conjugated with glucuronic acid and then excreted in the urine (19, 20, 23). The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats). There is no evidence of accumulation in tissues. Excretion of the absorbed BBP and its metabolites is rapid, with approximately 90% elimination in 24 hours. The half-life of BBP in blood is 10 minutes; the blood half-life of the monoester metabolites of BBP is approximately 6 hours.

Genetic toxicity.
A recent review by the IPCS (2) stated: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

5.1.2.1 Utility of Data the CERHR Evaluation

The oral subchronic studies in rats and mice are adequate for the evaluation of general toxicity induced by BBP. The database is adequate to determine that the liver is a target organ of toxicity. Some studies were conducted according to GLP standards and relevant exposure routes were utilized. The examination of hepatic effects was adequate and included a limited evaluation of peroxisomal proliferation in rats. There is an inhalation study in rats.

There are acceptable toxicokinetic data for BBP, consisting of absorption, distribution, metabolism, and excretion data following oral and dermal exposure in the rat.

Table 4: Summaries of NOAELs and LOAELs and Major Effects in Oral General Toxicity Studies

Protocol and BBP Doses (mg/kg bw/day)	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day) and Effects*	*Major Effects at Higher Doses*
14-day repeat dose dietary study in adult male Fischer 344 rats. 10 rats/group. Doses 0, 447, 890, 1,338, or 1,542 (15)	None	447 ↑LH ↑Liver and kidney weight.	↓Testes, seminal vesicle, epididymis weight Histopathological effects in testes, seminal vesicles, and prostate ↑LH and FSH ↓Testosterone ↑Liver, kidney, and thymus weight Histological changes in liver and thymus ↓Bone marrow cellularity.
3-month repeat dose dietary study in Wistar rats 4–6 weeks old at start of study 27–45 rats/sex/group Doses – M: 0, 151, 381, 960 F: 0, 171, 422, 1,069 (14)	None	M: 151; F: 171 ↑Liver (4%) weight	↑Liver and kidney weight Liver lesions Pancreatic lesions Anemia ↓Urine pH (M) No testicular lesions.
90-day repeat dose dietary study in adult Beagles. 3/sex/group Doses – M: 0, 400, 1,000, 1,852 F: 0, 700, 1,270, 1,973 (14)	M: None F: 700	M: 400 F: 1,270 Decreased body weight.	No histological effects in liver or testes.
26-week dietary study in adult male Fischer 344/N rats 6-weeks-old at start of study 15 rats/group Doses – 0, 30, 60, 180, 550, 1,650 (17)	180	550 ↑Liver weight ↑Hemoglobin	↓Testis, seminal vesicle, & epididymis weight Lesions in testis and epididymis. ↓ Sperm counts ↑ Liver and kidney weight Anemia
2-year dietary study in Fischer 344/N rats. 6-weeks-old at start of study 60 rats/sex/group Doses – M: 0, 120, 240, 500 ; F: 0, 300, 600, or 1,200 (17)	None	M: 120; F: 300 ↑Kidney weight (M) Nephropathy (F).	↑ Liver weight ↑ Kidney weight Nephropathy (F) Anemia ↓ Thyroid hormone (F). Some evidence of pancreatic cancer (M) Equivocal evidence of urinary bladder and pancreatic cancer (F) No testicular lesions.
2-year dietary study in B6C3F_1 mice. 4–5 weeks old at start of study. 50 mice/sex/group. Doses – M: 0, 1,029, 2,058 ; F: 0, 1,037, 2,074 (18)	None	M: 1,029; F: 1,037 ↓Weight gain.	↓ Weight gain. No changes in survival or neoplasm development. No lesions in male or female reproductive organs.

5.1.3 Developmental Toxicity

Studies of prenatal development consistently show BBP to be embryolethal and teratogenic following exposure to high oral doses in rats and mice on gd 6–15 or 7–15. The incidence of these effects is dependent on dose and developmental age. A maternal and developmental NOAEL in CD-1 mice was 182 mg/kg bw/day (30). The Expert Panel noted that there was wide spacing between the NOAEL and the LOAEL of 910 mg/kg bw/day in this study. Effects at the LOAEL and higher doses included increased resorptions and late fetal deaths, reduced number of live fetuses per litter, and increased external and skeletal malformations. The developmental NOAELs in Sprague Dawley and Wistar rats ranged from 420 to 500 mg/kg bw/day, respectively (27, 29). Effects at doses of 750 mg/kg bw/day and higher included increased prenatal mortality, reduced fetal growth, and increased fetal variations and skeletal, visceral, and external malformations. Extending the exposure period to gd 0–20 in Wistar rats resulted in a developmental NOAEL of 185 mg/kg bw/day. An oral prenatal study in rabbits revealed no maternal or developmental toxicity at doses up to 10 mg/kg bw/day; however, utility of the results is limited since no maximum tolerated dose was established (31).

Using a prenatal study design similar to that used with BBP (29), the monoesters MBuP and MBeP were investigated (38, 39). The developmental toxicity observed with the monoesters was qualitatively similar to that produced by BBP. These data suggest that both monoesters can contribute to the developmental toxicity associated with BBP. Differences in the doses selected for study do not permit a close quantitative comparison of the dose-response relationship between the two monoesters or with BBP. A rat study, using an MBuP dose of 1,000 mg/kg bw/day, reported a subsequent interference with testicular migration and descent (40).

Studies in rats indicate that prenatal effects are directly related to the chemical and are not due to decreased food consumption (32). The mechanism of action for resorption has been proposed as reduced circulating progesterone due to impaired luteal function (35).

The effect of low-dose exposure during mating, gestation, and lactation in Wistar rats has been studied. An increase in postnatal pup mortality was reported (45) for rats treated with BBP through drinking water at 1 and 3 mg/L (0.14 and 0.385 mg/kg bw/day). The study was immediately repeated at the same laboratory and only the result at the highest dose (3 mg/L) was replicated. In both studies, statistical significance was not achieved with the litter as the unit of analysis. The Panel noted that concurrent control values for pnd 0–4 pup loss in these two studies exceeded the historical control values for this laboratory. Further, other studies performed at this laboratory during this time period also experienced high pup losses on pnd 0–4, even in the vehicle control groups. Increased pup mortality was not observed in similar studies by Sharpe et al. (43) and Ashby et al. (26) who dosed Wistar rats with 1 mg/L BBP in drinking water. In addition, a subsequent study by Bayer (46) did not result in increased postnatal pup loss at BBP doses of 1 or 3 ppm in drinking water or feed. For the Bayer study, maternal BBP intakes during the first week of lactation (the time of pup losses in the TNO studies) were 0.11 µg/kg bw/day and 0.34 µg/kg bw/day through diet and 0.170 µg/kg bw/day and 0.540 µg/kg bw/day through drinking water.

The Panel is therefore presented with a developmental LOAEL from the two TNO (45) studies of approximately 0.385 mg/kg bw/day (3 ppm) and a NOAEL of 0.140 mg/kg bw/day (1 ppm) based

on F_1 pup losses on pnd 0–4. There is very low confidence in these values due to the discrepancy between the results of the data when analyzed by group (statistically significant) versus by litter (not statistically significant) and due to the lack of effects in the Bayer (46) study. The NOAEL/LOAEL values from the TNO study are approximately 3 orders of magnitude lower (~0.3–0.4 mg/kg bw/day) than the NOAEL values for reproductive/developmental NOAELs in other studies (~185 mg/kg bw/day for developmental toxicity and 500 mg/kg bw/day for reproductive toxicity).

5.1.3.1 Utility of Data to the CERHR Evaluation

The data in rats and mice are adequate for a prenatal assessment of fetal growth, lethality, and teratogenicity. One study examined prenatal effects following exposure during late pregnancy. None of the studies included a postnatal evaluation of androgen-regulated effects (e.g., nipple retention, testicular descent, or preputial separation) that were the most sensitive indicators of developmental toxicity with DBP. BBP and DBP share a common monoester metabolite. Prenatal studies with BBP monoesters (MBuP and MBeP) were sufficient to determine that both metabolites contribute to developmental toxicity. Because of differences in doses administered to the mice and rats, it is not possible to compare sensitivity between the two species.

Table 5: Summaries of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol and Study	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day)*		*Developmental Effects Observed at Higher Dose Levels*
		Maternal	*Developmental*	
Prenatal dietary study in Sprague-Dawley rats. 30 per group received 0, 420, 1,100, or 1,640 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (27)	Maternal: 420 Developmental:420	1,100 ↓ Body weight gain. ↑ Liver weight.	1,100 ↑ Variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ Visceral, skeletal, and external malformations. ↑ Variations.
Prenatal dietary study in Wistar rats. 15–18/group received 0, 185, 375, 654, or 974 mg/kg bw/day on gd 0–20. Dams and pups examined late in gestation. (28)	Maternal: 375 Developmental: 185	654 ↓ Weight gain.	375 ↑ Prenatal mortality.	↑ Prenatal mortality. ↓ Decreased fetal weight.
Prenatal gavage studies conducted in Wistar rats. 10/group received BBP 0, 500, 750, or 1,000 mg/kg bw/day (0, 1.60, 2.40, 3.20 mmol/kg bw/day) on gd 7–15. Dams and pups examined late in gestation. (29)	Maternal: 500 Developmental: 500 (1.60 mmol/kg bw/day)	750 (2.40 mmol/kg bw/day) ↓ Weight gain.	750 (2.40 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations.	Complete prenatal mortality.
The same study was conducted with MBuP at doses of 0, 250, 500, or 625 mg/kg bw/day (0, 1.13, 2.25, 2.81 mmol/kg bw/day). (38)	Maternal: 250 Developmental: 250 (1.13 mmol/kg bw/day)	500 (2.25 mmol/kg bw/day) ↓ Weight gain.	500 (2.25 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.
The same study was conducted with MBeP at doses of 0, 250, 313, 375, 438, or 500 mg/kg bw/day (0, 0.976, 1.22, 1.46 mmol/kg bw/day). (39)	Maternal: None Developmental: 250 (0.976 mmol/kg bw/day)	250 (0.976 mmol/kg bw/day) ↓ Food intake.	313 (1.22mmol/kg bw/day) ↑ Skeletal malformations.	↑ Prenatal mortality. ↑ Internal, external, and skeletal malformations.

Table 5 (continued)

Protocol and Study	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day)*		*Developmental Effects Observed at Higher Dose Levels*
		Maternal	*Developmental*	
Prenatal dietary study in CD-1 mice. 30 per group received 0, 182, 910, or 2,330 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (30)	Maternal: 182 Developmental: 182	910 ↓ Weight gain.	910 ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations.	↓ Fetal weight. ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations. ↑ Variations.
Postnatal drinking water study in Wistar rats. 28/group received 0, 0.012, 0.140, or 0.385 mg/kg bw/day from 2 weeks prior to mating throughout gestation and lactation. Pups were examined postnatally	Maternal: 0.385 Developmental: 0.14	No adverse maternal systemic or reproductive effects.	0.385 ↑ Pup death on pnd 1–4 (12% in treated versus 0.8% in control).	No higher doses.
The experiment was repeated with the mid and high dose to verify increased postnatal mortality. (45)			↑ Pup death on pnd 1–4 (17% in treated versus 10% in control) in repeat experiment.	
Postnatal drinking water and dietary study. 21-25/group were treated from 2 weeks prior to mating throughout gestation and lactation. Lactational doses were 0, 0.11–0.16, and 0.34–0.49 through diet and 0, 0.17–0.24, and 0.54–0.80 through drinking water. Pups were examined postnatally. (46)	Maternal: 0.34–0.49 (diet), 0.54–0.80 (drinking water) Developmental: 0.34–0.49 (diet), 0.54–0.80 (drinking water)	No adverse maternal systemic or reproductive effects.	No significant effects on development including pup mortality.	No higher doses.

5.1.4 Reproductive Toxicity

There are no conclusive data in humans that assess the reproductive effects from exposure to BBP alone. All experimental animal studies that assess reproduction have been performed in the rat.

Male reproductive toxicity

Male reproductive performance was evaluated in three rat studies by the oral route of exposure (17, 48, 50). There were no effects in reproductive performance in 10 WU rats exposed to up to 500 mg/kg bw/day by gavage for 2 weeks prior to mating. Decreased fertility and testicular histopathology were seen at 1,000 mg/kg bw/day (48). No adverse effects were noted in Wistar rats exposed through diet with up to 418 mg/kg bw/day from 10 weeks prior to mating until the birth of a second litter (50). Reduced sperm counts were noted in F344 rats exposed to 200 mg/kg bw/day through diet for 10 weeks, but reproductive performance was not affected (17). The sperm count effects in the 200 mg/kg bw/day group were considered questionable and not used to determine a NOAEL because: 1) that group had a shorter recovery time from mating to necropsy and the required time to restore cauda epididymal sperm counts following ejaculation was not reached for most animals (51, 54); and 2) no effects on sperm count were reported following exposure to 550 mg/kg bw/day in a 26-week study by the same laboratory (17).

Histopathology of male reproductive organs has also been examined in subchronic and chronic F344 rat studies by the oral route; the lowest dose that produced testicular lesions was 1,338 mg/kg bw/day in rats exposed through diet (15). The reproductive organs of male B6C3F1 mice were unaffected at dietary doses up to 2,058 mg/kg bw/day and testes of beagle dogs were not affected at dietary doses up to 1,852 mg/kg bw/day. The Expert Panel selected a reproductive NOAEL of 500 mg/kg bw/day for adult male rats. There is uncertainty as to the dose that is without effect on the developing male reproductive tract. The Expert Panel noted that a primary BBP metabolite, MBuP, is likely the active toxicant in DBP studies where exposure during *in utero* development or during the neonatal period of life led to reproductive effects (42). Given that MBuP is a metabolite of BBP, the DBP data are relevant to BBP. Similar studies with MBeP, the other metabolite of BBP have not been performed. The existing studies with BBP did not critically examine pups during the sensitive postnatal phases of life. It is probable that such studies would likely result in a lower NOAEL.

Female reproductive toxicity

In a reproductive toxicity screening study in WU rats, decreases in the number of females conceiving and in the number of live pups per litter were observed at an oral gavage dose of 1,000 mg/kg. Clear testicular effects in males suggest that the effect may be due in part to toxicity in the male (48). Five hundred mg/kg bw/day was a NOAEL for female fertility in this study, but these data were from a screening study. No effects on implantation, reproductive organ morphology, fertility, or fecundity were seen in a one-generation reproductive toxicity study in Wistar rats that received the highest dietary dose (0.8%), comparable to a BBP intake value 446 mg/kg bw/day in diet during the mating phase of the study (50).

Mode of Action

BBP has been shown to bind to the estrogen receptor (ER) of rat and trout (57, 58). The relative binding affinity is approximately 10,000-100,000 times lower than 17 -estradiol (E2). BBP also induces weak activity in *in vitro* estrogen-mediated gene expression assays at 10 μM, the highest

concentration examined (57). In a yeast assay of estrogen-mediated gene expression, the potency of BBP was 1x106–5x107 less than that of E2, but its metabolites MBuP and MBeP demonstrated no estrogenic activity (63). However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in Sprague-Dawley rats gavaged with 20, 200, and 2,000 mg/kg bw/day for 4 days. Moore (64) reviewed the data on the estrogenic potential of phthalates and concluded that the estrogenic ability of phthalates identified in the *in vitro* studies is "not relevant to humans or the environment."

5.1.4.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of reproductive toxicity in adults. Studies are available that evaluate both structure and reproductive function. In studies with DBP, a phthalate that is also metabolized to MBuP, male rats exposed while in utero and during lactation were most sensitive to DBP-induced effects on reproductive structure and function (65). Therefore, the most sensitive age for reproductive toxicity was not addressed for BBP. The data was sufficient to demonstrate the testes as a target organ.

Table 6: Summaries of NOAELs, LOAELs, and Major Effects in Reproductive Toxicity Studies

Protocol & Study	*NOAEL (mg/kg bw/day)*	*Reproductive LOAEL (mg/kg bw/day) and effects*	*Systemic LOAEL (mg/kg bw/day) and Effects*	*Reproductive Effects at Higher Doses*
One-generation reproductive screening assay in WU rats. 10 pairs/group received 0, 250, 500, or 1,000 mg/kg bw/day by gavage from 2 weeks prior to mating for a total of 29 days (males) or until pnd 6 (females). (48)	Reproductive: 500 Systemic: 500	1,000 ↓Fertility Testicular lesions ↓Litter size	1,000 ↓Weight gain	No higher doses in study
One-generation dietary reproductive toxicity assay in Wistar rats with 12 males and 24 females/group. Males were treated 10 weeks prior to mating with 0, 108, 206, or 418 mg/kg bw/day. Females were treated from 2 weeks prior to mating (0, 106, 217, or 446 mg/kg bw/day), through gestation (0, 116, 235, or 458 mg/kg bw/day) and lactation (0, 252, 580, or 1,078 mg/kg bw/day). (50)	Reproductive: 418 (M); 446 (F) Systemic: 206 (M); 217 (F)	No structural or functional effects at any dose	418 (M); 446 (F) ↓Weight gain (F) ↑Liver weight	No higher doses in study
One-generation modified mating study in male F344 rats. 15 males/group treated with BBP through diet at 0, 20, 200, or 2,200 mg/kg bw/day for 10 weeks and then mated with untreated females. (17)	Reproductive: 200 Systemic: 200	2,200 ↓Sperm counts ↓Fertility Testicular and epididymal lesions ↓Testis and prostate weight	2,200 ↓Weight gain ↑Liver weight Anemia	No higher doses in study

5.2 Integrated Evaluation

BBP is primarily used in PVC utilized in the manufacture of construction materials, automotive materials, and food conveyor belts. Exposure of the general population through inhalation is negligible due to the low volatility of BBP. Inhalation exposure to BBP in flexible PVC manufacturing facilities has been estimated at 286 μg/kg bw/workday. Exposure through contact with skin is negligible due to the relatively slow absorption. The IPCS has concluded that consumption of food containing trace levels of BBP is a significant source of exposure to the general population. Estimates based on BBP levels in Canadian and UK foods indicate that exposure to the general population, including children, is below 10 μg/kg bw/day.

There are no human toxicokinetic or toxicity studies for BBP. Studies in rats demonstrate that orally-administered BBP is rapidly converted to the monoester metabolites, MBuP and MBeP, and their respective alcohols within the gut. At low doses, 2–200 mg/kg, approximately 80% of the administered dose is metabolized and the metabolites are absorbed into systemic circulation. The remainder of the dose is excreted in feces unchanged. Absorbed metabolites are glucuronidated and rapidly excreted in urine with no evidence of accumulation. The Expert Panel assumes the toxicokinetic studies in rats to be relevant to human exposure of BBP through food. There are no inhalation toxicokinetic studies.

Prenatal exposure studies in rats and mice have indicated that oral exposure on gd 6–15 or 7–15 to high doses of BBP (> 500 mg/kg bw/day) results in reduced fetal growth, prenatal mortality, and visceral, skeletal, and external malformations. NOAELs of 182 mg/kg bw/day and 500 mg/kg bw/day were identified for mice and rats exposed on gd 6- or 7-15, respectively; however, a comparison of sensitivity between species is not possible due to variations in doses administered. Exposure of Wistar rats during the entire gestation period resulted in a developmental NOAEL of 185 mg/kg bw/day. Oral prenatal studies with the BBP metabolites MBuP and MBeP have demonstrated qualitatively similar results to BBP and suggest that the metabolites are associated with the observed developmental toxicity. None of the studies examined the postnatal effects on the male reproductive system. This is of concern because standard prenatal studies do not detect effects such as altered anogenital distance, retained nipples, delays in acquisition of puberty (preputial separation), and malformation of the post-pubertal male reproductive system. Such effects have been observed with DBP, the monoester metabolite of which is the same as one of the metabolites of BBP. Therefore, the Expert Panel is not confident in the NOAELs obtained from the existing BBP developmental studies. In studies using DBP, a NOAEL of 50 mg/kg bw/day was identified with male reproductive tract anomalies observed at higher doses.

The data indicate that BBP is a reproductive toxicant in adult male rats as evidenced by testicular lesions, reduced sperm counts, and increased infertility following exposure to oral doses exceeding the NOAEL of 500 mg/kg bw/day. Effects on the reproductive system of adult female rats are less certain. There were no reproductive effects in female rats exposed orally to 446–1,078 mg/kg bw/day from 2 weeks prior to mating through lactation. However, in a second study, the number of females conceiving litters was reduced following exposure to 1,000 mg/kg bw/day by gavage. The data do not permit clear delineation as to whether this was male-or female-related, although clear evidence of testicular toxicity was seen. The Expert Panel notes that the database does not allow for a complete evaluation of reproductive effects due to the lack of a multigeneration study

that exposes animals during the development of the reproductive system. Lower NOAELs may be observed in studies with late gestational exposure and complete postnatal examination of the male reproductive system.

The Expert Panel believes the database is sufficient to judge that oral exposure to BBP can cause reproductive toxicity in adult rats and developmental toxicity in rats and mice. These data are assumed to be relevant to humans. The Panel is not confident that the lowest dose at which developmental toxicity, specifically effects on the developing male reproductive tract, has been established.

Lastly, the Panel is aware of studies performed at CDC using urine from human subjects. Results of these studies were given in an oral presentation in Copenhagen, Denmark, in May, 2000. MBuP values in the urine of women of child-bearing age were among the higher values. Such data, when published, should serve to improve our ability to assess phthalate exposure in the general population.

5.3 Expert Panel Conclusions

BBP is used in the manufacture of vinyl tile and PVC to make food conveyor belts, carpet tile, tarps, weather stripping, and, to a limited extent, vinyl gloves and adhesives. BBP can be released into the environment during production, incorporation into products, use, and disposal.

The best estimate of exposure to the general public is 2 μg/kg bw/day from food in adults, with exposures to infants and children possibly up to three-fold higher, with negligible exposures from infant formula, dermal absorption, drinking water, or soil intake. Occupational exposure is estimated at 286 μg/kg bw/workday. Median indoor air levels (from 1 study of 125 southern California homes) were 0.034–0.035 ng/m^3, outdoor ambient air levels from 65 of these homes were 5.3–6.7 ng/m^3 for the 90th percentile, and below the estimated detection limit of 0.051 ng/ m^3 for the median BBP level. The Expert Panel has low-to-moderate confidence in the completeness of the exposure database from which these estimates were made, based on the range of values provided by different sources for the same route of exposure and on the age of the data available for exposures for food and food packaging.

With regard to developmental and reproductive toxicity, the database is sufficient to judge that oral exposure to BBP can cause developmental toxicity in rats and mice, and reproductive toxicity in rats. The current database is insufficient to fully characterize the potential hazard. The lowest NOAELs identified by the Panel for developmental toxicity were 182 mg/kg bw/day in CD-1 mice and 185 mg/kg/day in Wistar rats. Given the low exposures to adults and the high dose designated as the NOAEL, the Panel agrees that there is an adequate database to provide negligible concern for male reproductive effects from adult exposure. There is not an adequate database to determine NOAELs/LOAELs for male or female reproductive effects from perinatal exposure. BBP and DBP have a common metabolite, MBuP, and the Panel noted that orally-administered DBP causes male reproductive tract malformations at 100 mg/kg bw/day (LOAEL). Data gaps did not permit the Panel to ascribe a level of concern for postnatal consequences from perinatal exposure to BBP. Multigeneration studies now in progress include endocrine-sensitive endpoints and should provide a robust dataset from which to determine the LOAEL/NOAEL and allow subsequent assignment of the level of confidence in these values, and of the level of concern.

5.4 Critical Data Needs

Critical data needs are discussed under two categories: experimental studies and human exposure.

Experimental Studies

1) Multigeneration study. There is a priority need for a multigenerational study that evaluates effects on reproductive development, fertility, and reproductive system structures, including endocrine sensitive parameters, with continuous exposure across multiple generations. Female reproductive effects need to be evaluated explicitly.

The Expert Panel is aware that a two-generation study under current testing guidelines and with evaluation of endocrine-sensitive endpoints in rats was recently completed in Japan and that a similar study is underway in the United States. It is likely that data needs cited in 1) above would be fulfilled by the results from these studies.

Human Exposure

1) No studies of humans were found. Occupationally-exposed cohorts might be located, but would be of limited utility if the major exposure source is food. Priority should be given to studies on occupational exposures and general population indoor exposures from BBP-releasing materials.

2) Better exposure data. The Panel is aware of emerging data for human exposure (by analysis of urinary phthalate metabolites from a human reference population) that may alter existing exposure estimates, particularly for women of child-bearing age.

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Table 7-1: BBP General Toxicity, Male Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose**	*Body Weight*	*Organ Weight*	*Histopathology*	*Hematopoietic System*	*Chemistry*	*Other*
Fischer 344 Rat Agarwal 1985 (1)	Adult male rats were fed diets with BBP at 0, 0.625, 1.25, 2.5, or 5.0% for 14 days, then were sacrificed and necropsied.	10	0						
		10	447[a]	NE	↑Li and Ki	NE	NE	↑LH	LOAEL
		10	890[a]	NE	↑Li and Ki	NE	NE	NE	NE
		10	1,338[a]	↓	↓Te and SV ↑Li and Ki ↓Th	Dose-related increase in severity of morphological changes in seminal vesicles, testes and prostate	↓Bone marrow cellularity	↑FSH ↑LH	↓Food consumption
		10	1,542[b]	↓	↓Te, SV, Ep ↑Li, Ki ↓Th	Mild multifocal chronic hepatitis in liver Cortical lymphocytolysis in thymus (atrophy)	↓Bone marrow cellularity	↓Test ↑FSH ↑LH	↓Food consumption

*Dose in mg/kg bw/day.
[a]Doses calculated using pre-treatment body weights (200 g) and average food consumed per group during 14-day study.
[b]Dose calculated from average body weight during study (since there was a weight loss) and food consumed during the 14-day study.

NE = No Effects
↑= Statistically significant increase
↓=Statistically significant decrease
Li = Liver
Ki = Kidney
Th = Thymus
Te = Testes
Ep = Epididymis
SV = Seminal Vesicle
FSH=Follicle Stimulating Hormone
LH = Luteinizing Hormone
Test=Testosterone

Table 7-2: BBP General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose***	*Body Weight*	*Organ Weight*	*Histopathology*	*Hematology*	*Chemistry*	*Other*
Sprague Dawley Rat	4–6 week-old rats were fed diets with BBP at 2,500–20,000 ppm for 3 months, then were sacrificed and necropsied.	10	0						
		10	188	NE	NE	NE	NE	NA	
		10	375	NE	NE	NE	NE	NA	NOAEL
		10	750	NE	↑Ki(M), Li(F)	NE	NE	NA	LOAEL
		10	1,125	↓(M)*	↑Ki(M), Li	NE	NE	NA	
		10	1,500	↓*	↑Ki(M), Li	NE in liver, testes, or pancreas	NE	NA	
Wistar Rat	4–6 week-old rats were fed diets with BBP at 2,500–12,000 ppm for 3 months and sacrificed and necropsied.	27–45	0						
		27–45	151(M)–171(F)	↓(M)*	↑Li and Ce(F)	NE	NE	NE	LOAEL
		27–45	381(M)–422(F)	↓*	↑Li and Ce(F), Ki	Pancreatic lesions	NE	NE	↓Urinary pH (M)
		27–45	960(M)–1,069(F)	↓*	↑Ce(F), Li, Ki	Hepatic necrosis and pancreatic lesions	Anemia(M)	NE	↓Urinary pH (M)
Sprague-Dawley Rat Hammond 1987 (2)	6–8 week-old rats inhaled BBP mists at 50, 218, or 789 mg/m^3 for 6 hours/ day, 5 days/week for 13 weeks, then were sacrificed and necropsied.	25	0						
		25	9.2(M)/9.8(F)	NE	NE	NE	NE	NE	
		25	39.4(M)/42(F)	NE	NE	NE	NE	NE	NOAEL
		25	143(M)/152(F)	NE	↑Li, Ki	NE	NE	↓Serum glucose (M, 13wk)	LOAEL

↑= Statistically significant increase
↓=Statistically significant decrease
*Statistical significance is unknown
**Dose in mg/kg bw/day

F=Female
M=Male
NA=Not Analyzed
NE=No Effect

[a]Organ to body weight ratio
Ce=Cecum
Ki=Kidney
Li=Liver

wk=Week

Table 7-3: BBP General Toxicity, Male Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose**	*Body Weight*	*Organ Weight*	*Histopathology*	*Epididymal Sperm Count*	*Hematology*	*Other*
Fischer 344/N Rat NTP 1997 (3)	Sub-chronic study (26 wk) 6-week-old male rats fed diets with BBP at 0, 300, 900, 2,800, 8,300, and 25,000 ppm. Hematological measurements taken every 30 days. Rats were killed and necropsied at the end of the study, epididymal sperm counts were taken.	13	0						
		14	30	NE	NE	NA	NA	NE	
		14	60	NE	NE	NA	NE	NE	
		14	180	NE	NE	NA	NE	NE	NOAEL
		15	550	NE	↑Li[b]	NE	NE	↑Hb day 60–180	LOAEL
		11	1,650[a]	↓	↑Li, Ki[b] ↓Te[b] ↓SV, Ep[c]	Testicular and epididymal degeneration and seminiferous tubule atrophy.	↓ Sperm counts	↑Macrocytic anemia days 30–180	

*Dose in mg/kg bw/day.
[a]The dose for the highest exposure level could not be calculated but was estimated from lower doses, assuming equal body weight and food intake.
[b]Organ to body weight ratio.
[c]Absolute organ weight.

↑= Statistically significant increase
↓=Statistically significant decrease

NA=Not analyzed
NE=No effects

Ep=Epididymis
Ki=Kidney
Li=Liver

Hb=Hemoglobin
SV=Seminal Vesicle
Te=Testes

Table 7-4: BBP General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose***	*Body Weight*	*Organ Weight**	*Histopathology*	*Hematology*	*Chemistry*	*Other*
Fischer 344/N Rat NTP 1997 (3)	6-week-old rats were fed diets with BBP at 0, 3,000, 6,000, and 12,000 ppm (M); 0, 6,000, 12,000, and 24,000 ppm (F) for 2 years. Hematological analysis was conducted at 6, 8, and 15 months and hormone levels were measured at 6, 15, and 24 months. Organ weights were measured at 15 months and histopathology was evaluated at 15 and 24 months.		0						
		60	Male: 120	NE	↑Ki	NE	NE	NE	
		60	Male: 240	NE	↑Ki ↑Ep	NE	NE	NE	
		60	Male: 500	↓	↑Ki, Li ↑Ep	Renal tubule pigmentation (15–24 mo) Hepatic granuloma (24 mo) No testicular effects Focal pancreatic hyperplasia and some evidence of pancreatic carcinogenicity	↓RBC (6 mo) ↑Hb (6 mo)	NE	↑Skin lesions
		60	Female: 300	NE	NE	Nephropathy (24 mo)	NE	NE	
		60	Female: 600	NE	NE	Nephropathy (24 mo)	NE	NE	
		60	Female: 1,200	↓		Renal tubule pigmentation (15–24 mo) Nephropathy (24 mo) Equivocal evidence of pancreatic and urinary bladder carcinogenicity	↑Microcytic anemia (15 mo)	↓Triiodothyronine (6–15 mo)	

*Organ to body weight ratio
**Dose in mg/kg bw/day
↑= Statistically significant increase
↓=Statistically significant decrease
NA=Not analyzed
NE=No effects
mo=Month
Ep=Epididymis
Ki=Kidney
Li=Liver
Hb=Hemoglobin
RBC=Red Blood Cell

Table 7-5: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Num-bers[a]*	*Dose**	*Maternal effects*	*Fetal effects*
CD Rat Field 1989 (4)	Prenatal developmental toxicity study. BBP administered in feed on gd 6–15, at 0, 0.5, 1.25, 2.0%. Sacrificed on gd 20. Dams weighed on gd 0, 3, 6, 9, 12, 15, 18, and 20. Maternal liver, kidney, and intact uterus were weighed, corpora lutea were counted and implantation sites examined. All fetuses were weighed and examined for gross external, visceral, and skeletal malformations.	28	0		
		27	420	NOAEL	NOAEL
		30	1.100	↓ Weight gain (37%) ↑ Liver to body weight ratio ↑ Food and water intake	Fetuses with variations/litter (41 vs 19%)
		29	1,640	↓ Weight gain (93%) ↓ Corrected weight gain (17%) ↑ Liver to body weight ratio with no pathological effects ↑Kidney to body weight ratio ↑ Food and water intake Clinical signs of toxicity	↓ Fetal Weight (20%) ↓ Live fetuses/litter (n=10 vs 15) ↑ Resorptions/litter (40 vs 4%) and litters with resorptions (86 vs 32%) ↑ Fetuses with variations/litter (71 vs 19%) ↑ Fetuses with malformations (53 vs 2%); ↑ Litters with malformations (visceral, external, and skeletal, especially of the urinary tract, eyes, and spine) (96 vs 25%)

*Dose in mg/kg bw/day.
[a]Number of dams pregnant at sacrifice.
↑=Statistically significant increase
↓=Statistically significant decrease
gd=gestation day

Table 7-6: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Num-bers[a]*	*Dose **	*Maternal effects*	*Fetal effects*
Wistar Rats Ema 1990 (5)	Prenatal developmental toxicity study. Rats were fed diets with DBP at 0, 0.25, 0.5, 1.0, 2.0% from gd 0–20. Body weights and food intake were measured daily. Dams were sacrificed on gd 20. Implantation sites were examined. Pups were sexed, weighed, and evaluated for external malformations. Two-thirds of fetuses were examined for skeletal malformations and 1/3 for visceral malformations.	15 (15)	0		
		17 (17)	185	NE	NOAEL
		15 (15)	375	NOAEL	↓Live fetuses/litter (n=113 vs 139)
		13 (13)	654	↓Weight gain (35%) ↓ Adjusted weight gain (96%) ↓Food Intake	↓Fetal weight (7%) ↓Live fetuses/litter (n=123 vs 139)[c]
		13 (0)	974	Weight loss (15 g) Adjusted weight loss (21 g)[b] ↓Food Intake	Complete postimplantation loss in all litters Treatment-related increases in malformations, variations, or retardations were not seen at any dose

*Dose in mg/kg bw/day.
[a]Number of pregnant rats (Number of litters evaluated).
[b]Body weight not including gravid uterus weight.
[c]Not statistically significant

NE=No Effect
n=Number
gd=gestation day

↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-7: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Numbers*[a]	*Dose **	*Maternal effects*	*Fetal effects*
Wistar Rats Ema 1992 (6)	Prenatal developmental toxicity study. Rats were gavaged with BBP on gd 7–15. Body weights and food intake were measured daily. Dams were sacrificed on gd 20. Implantation sites were examined. Pups were sexed, weighed, and evaluated for external malformations. Two-thirds of fetuses were examined for skeletal malformations and 1/3 for visceral malformations.	10 (10)	0		
		10 (10)	500	NOAEL	NOAEL
		10 (7)	750	↓ Body weight gain ↓ Food intake	Complete resorption in 3/10 litters ↑ Fetal death/litter (n=11 vs 1) ↑ Postimplantation loss/litter (82 vs 8%) ↓ Fetal weight (18%) ↑ Malformations: • external (12 fetuses/7 litters vs 0), • skeletal (5 fetuses/4 litters vs 1) • internal (3 fetuses/3 litters vs 0)
		10 (0)	1,000	↑ Death (4 dams) ↓ Corrected body weight gain[b] ↓ Food intake	Complete resorption in 6/6 litters

*Dose in mg/kg bw/day.
[a]Number of pregnant rats (Number of litters evaluated).
[b]Body weight not including gravid uterus weight.

n=Number
gd=gestation day

↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-8: BBP Developmental Toxicity, Mice

Species, Strain, and Source	*Experimental Regimen*	*Num-bers*[a]	*Dose**	*Maternal effects*	*Fetal effects*
CD-1 Mice Price 1990 (7)	Prenatal developmental toxicity study. BBP administered in feed at 0, 0.1, 0.5, 1.25% on gd 6–15. Sacrificed on gd 17. Dams weighed on gd 0, 3, 6, 9, 12, 15, and 17. Maternal liver, kidney, and intact uterus were weighed, corpora lutea were counted and implantation sites examined. All fetuses were weighed and examined for gross external, visceral, and skeletal malformations.	29			
		28	182	Maternal NOAEL	Developmental NOAEL
		30	910	↓ Weight gain (15%)	↑ Late fetal deaths/litter (29 vs 07%) ↑ Non-live implants/litter (15 vs 8%)[b] ↓ Live fetuses/litter (n=12 vs 13) ↑ Fetuses/litter with malformations (14 vs 4%) ↑ Litters with malformations (60 vs 31%)
		27	2,330	↓ Weight gain (71%) ↓ Corrected weight gain (25%) ↑ Water intake ↑ Liver and kidney to body weight ratio with no pathological effects	↑ Resorptions/litter (91 vs 7%); ↑ Litters with resorptions (100 vs 55%) ↑ % Non-live implants/litter (93 vs 8%) ↑ Litters with non-live implants (100 vs 59%)[b] ↓ Live fetuses/litter (n=3 vs 13) ↓ Fetal weight (17%) ↑ Fetuses/litter with malformations (89 vs 4%) ↑ Litters with malformations, especially external and skeletal defects of the tail, ribs, sternebrae and vertebrae (100 vs 31%) ↑ Fetuses with variations/litter (98 vs 29%)

*Dose in mg/kg bw/day
[a]Number of pregnant dams evaluated at sacrifice
[b]Non-live implants include resorptions and late fetal deaths

n=Number
gd=gestation day

↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-9: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Num-bers[a]*	*Dose**	*Maternal Effects*	*Fetal effects*
Wistar Rat Sharpe 1995 (8)	Pre- and post-natal developmental toxicity study. Female rats were exposed to BBP through drinking water at 0 or 1 mg/L for 2 weeks prior to mating, and during mating, gestation and lactation. Rats were mated to untreated males. Dams were allowed to litter. Litter sizes were evaluated at birth. At 90–95 days of age, male offspring were sacrificed and organ weights were determined.	5	0		
		5	0.126–0.336	NA	↑Body weight on pnd 22 (11%). ↓Absolute testes weight (10%) and testes to body weight ratio (8%).
		6	*0.0011 DES[b]*		*↓Body weight on pnd 22.* *↓Absolute testes weight and testes to body weight ratio*
	After the first litters were weaned, the experiment was repeated in the same dams. Additional parameters monitored included testicular morphology in 2 pups/group and sperm counts in 7–12 pups/group.	6	0		
		5	0.126–0.336	NA	↑Body weight on pnd 22 (14%). ↓Absolute testes weight (7%) and testes to body weight ratio (7%). ↓Daily sperm production (~10–21%).
		5	*0.0011 DES[b]*		*↑Body weight on pd 22.* *↓Absolute testes weight and testes to body weight ratio.* *↓Daily sperm production.*

*Dose in mg/kg bw/day
[a]Total litters evaluated. The number of treated dams was not stated.
[b]Positive DES control, dose estimated by CERHR.

NA=Not Analyzed
↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-10: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Numbers*[a]	*Dose**	*Maternal effects*	*Offspring effects*
Wistar AP Rat Ashby 1997 (10)	Pre- and post-natal developmental toxicity study. Rats were exposed to BBP through drinking water at 1 mg/L during gestation and lactation (gd 1–pnd 20). Body weights were measured on gd 1, 4, and 22 and pd 3, 7, 14, and 20. Water intake was measured daily. Dams were allowed to litter and following weaning of pups, were killed and necropsied. Liver enzyme activity, hematology, and micronucleated erythrocytes were assessed. Pups were sexed, weighed, and evaluated for sexual maturation. Uterotrophic effects were examined in groups of 10 female rats on pnd 21 and 24. The majority of pups were sacrificed and necropsied on pnd 90 and 10 males/group were sacrificed on pnd 137. Sperm analysis was conducted at necropsy. FSH-positive pituitary cells were counted in 9 rats/sex.	19			
		18	0.183	NE	↑ Male pup weight on pnd 2 (13%). ↑ Anogenital distance in males on pnd 2 (4%)[b]. ↓ Age of vaginal opening (34 vs 35.1 days)[b]. ↑ Liver to body weight ratios in males (4%). No effects on sperm counts, testes weight, or uterotrophic response.
		5	*0.0086 DES*[a]	↓*Body weight*	↓*Body weight.* ↑*Uterine weight and uterotrophic response.* ↑*Absolute ovarian weight.* ↓*Anogenital distance in males and females.* ↓*Age of vaginal opening.* ↑*Age of preputial separation.* ↓*Decrease testis, epididymis, seminal vesicle, and prostate weight.* ↓*Decreased sperm count.*

*Dose in mg/kg bw/day
[a]Positive DES control
[b]Authors considered effects to be related to increased pup weight.

gd=gestation day
pnd=postnatal day
NE=No Effect

↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-11: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Numbers[a]*	*Dose**	*Maternal effects*	*Fetal effects*
Wistar Rat TNO 1998 (9)	Pre- and post-natal developmental toxicity study. Female rats were exposed to BBP through drinking water at 0.1, 1, or 3 mg/L for 2 weeks prior to mating, and during mating, gestation and lactation. Rats were mated for 1 week to untreated males, that were only exposed to BBP while breeding. Body weights and food intake were measured weekly and water intake was measured daily. Dams were allowed to litter and following weaning of pups, were killed, necropsied, and implantation sites were examined. Pups were weighed, examined for abnormalities, evaluated for sexual maturation and function, and necropsied at 89–101 days of age.	25	0		
		23	0.012	NE	NE
		22	0.140	NE	↑Pup death on pnd 1–4 (14 vs 08%) *(Pup death/litter not significant)* ↑Large pups (pnd 4)
		24	0.385	NE	↑Pup death on pnd 1–4 (12 vs 08%) *(Pup death/litter not significant)* ↑Cold pups (pnd 1) ↑Large pups (pnd 4) ↑Hair loss No effects on sperm morphology, number, or motility; estrous cycles; or sexual maturation at any dose level
		21	0.0011–0.0055 DES[a]	↓Gestational weight gain ↑Duration of pregnancy	↑Pup death (pnd 1–4) ↓Live pups/litter ↓Decreased weight gain ↑Age of preputial separation ↓ Normal sperm ↓Sperm count (significance not known) ↓Testes weight
	The study was repeated with BBP to verify postnatal pup deaths	26	0		
		22	0.140		↓Pup death on pnd 1–4 (46 vs 10%)
		24	0.385		↑ Pup death on pnd 1–4 (17 vs 10%) ↑Stillborn pups (n=28 vs 13) *(Both effects/litter were insignificant)*

*Dose in mg/kg bw/day
[a]Positive DES control, dose estimated by CERHR
NE=No Effect
n=Number
↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-12: BBP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Numbers**	*Dose***	*Maternal effects*	*Fetal effects*
Wistar Rat Bayer 1998 (11)	Pre- and post-natal developmental toxicity study. Female rats were exposed to BBP through drinking water or diet at 0, 1, or 3 ppm for 2 weeks prior to mating, and during mating, gestation and lactation. Rats were mated for up to 3 weeks to untreated males, that were only exposed to BBP while breeding. Body weights and food and water intake were measured every 3−7 days. Dams were allowed to litter and following weaning of pups, were killed, necropsied, and examined for implantation sites. At birth, pups were counted, weighed, and examined for abnormalities. Pups were evaluated for survival and weight gain until pnd 21, when they were sacrificed and necropsied.	21−22	0		
		22−25	0.08−0.09/0.06−0.07/0.11−0.06[a] 0.10−0.12/0.11−0.11/0.17−0.24[b]	No significant effects on fertility, body weight gain or food and water intake.	Non-significant increase in resorptions in both dose groups. No significant effects on litter size, pup viability from birth to pnd 4, and pup weight.
		24	0.27−0.28/0.19−0.25/0.34−0.49[c] 0.34−0.35/0.35−0.35/0.54−0.80[d]		

*Number of females that gave birth to a live litter/exposure media.
**Dose in mg/kg bw/day.
pnd=postnatal day

[a]Exposure through 1 ppm diet during prebreed/gestation/lactation.
[b]Exposure through 1 ppm drinking water during prebreed/gestation/lactation.
[c]Exposure through 3 ppm diet during prebreed/gestation/lactation.
[d]Exposure through 3 ppm drinking water during prebreed/gestation/lactation.

Table 7-13: BBP Reproductive Toxicity Screening Study, Rats

Species, Strain, and Source	*Experimental Regimen*	*Dose**	*Effects Paternal*	*Effects Maternal*	*Litters*
WU Rat Piersma 1995 (12)	Reproduction screening study. BBP administered by gavage to male and females rats 10–11 weeks old for 2 weeks prior to mating. Males were dosed for a total of 29 days and females were dosed until pnd 6. Rats were housed together 1:1 for a maximum of 2 weeks. Body weight and food intake were measured weekly. Dams delivered and nursed pups. F_0 were evaluated for fertility and reproductive function, and were killed and necropsied at end of dosing period. Implantation sites were examined and histopathology was conducted. Litters were examined for external malformations, counted, sexed, weighed, and sacrificed and discarded on pnd 6.	0		9/10 females conceived.	
		250	NE	8/10 females conceived.	
		500	NE	7/10 females conceived.	↓Pup weight on pnd 1 (7%).
		1,000	↓Weight gain (21%). ↓ Testis and epididymis weight in F_0 males (14%). ↑Leydig cell hyperplasia and testicular degeneration.	4/10 females conceived. ↓Gestational weight gain (42%).	↓Live pups/litter at birth (n=2 vs 9) and pnd 6 (n=1 vs 9). ↓Pup weight on pnd 1 and 6 (29% and 43%).

*Dose in mg/kg bw/day
↓=Statistically significant decrease
↑=Statistically significant increase
NE=No Effect
n=Number
pnd=postnatal day

Table 7-14: BBP Reproductive Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose* [b]	*Effects*
Wistar Rat TNO 1993 (13)	One generation reproductive toxicity study. BBP administered in feed at 0, 0.2, 0.4, 0.8% for 10 weeks and 2 weeks before mating in males and females, respectively, and throughout rest of study. Body weight and food intake measured weekly. One male and two females housed together for 3 weeks. Dams nursed pups through pnd 21. Dams were rebred after first litter was weaned. Study was repeated in the same rats. Litters examined counted, sexed, and weighed. After weaning, F_1 examined for external abnormalities and sacrificed. F_0 rats were killed and necropsied. Histopathology examined in liver and reproductive tissue of control and high-dose group.	12(M)/ 21–20(F)[a]	0	
		12(M)/ 17–22(F)	108/106 116/252	NE
		12(M)/ 20–21(F)	206/217 235/580	NE
		12(M)/ 17–22(F)	418/446 458/1,078	↑Liver to body weight ratios in F_0 females. ↓Weight gain of F_0 females during gestation and lactation. ↓F_{1b} pup weight on pnd 21 (12%). No effects on implantations, reproductive organ morphology, or fertility, fecundity, and gestation indices.

[a]Number of males and females delivering first and second litter, respectively.
[b]Doses (in mg/kg bw/day) for males during premating / females during premating / females during gestation / females during lactation.
NE=No Effect
pnd=postnatal day
↑=Statistically significant increase
↓=Statistically significant decrease

Table 7-15: BBP Reproductive Toxicity, Male Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose**	*Effects*
F344/N Rat NTP 1997 (3)	Sub-chronic reproductive toxicity study (10 wks), in 6-week-old males. BBP was administered in feed at 0, 300, 2,800, and 25,000 ppm for 10 weeks prior to mating. Body weight and food intake were measured weekly. Each male was mated to 2 untreated females for 7 days. Reproductive parameters included fertility and fetal mortality. Males were then killed and examined for hematological, sperm, and histopathological effects. Females were killed and examined for corpora lutea and implantation sites on gd 13 or 13 days after mating.	15	0	
		15	20	NE
		15	200	NOAEL
		15	2,200	↓ Sperm concentration (>99%). Evidence of mating in 10/13 females; no pregnancies. ↓Prostate and testes to body weight ratio. ↓Epididymis and seminal vesicle weight. Testicular and epididymal degeneration. ↓Body weight gain (29%). ↑Liver and thymus to body weight ratio. Mild macrocytic anemia response.

*Dose in mg/kg bw/day
gd=gestation day
↓=Statistically significant decrease
↑=Statistically significant increase

Table 7-16: MBuP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Numbers*[a]	*Dose**	*Maternal effects*	*Developmental effects*
Wistar-King A rats.	Pre-and post-natal developmental toxicity study with prenatal exposure.	19/15	0		
Imajima et al. (1997) (14)	Rats were gavaged with 0 or 300 mg/day MBuP in sesame oil from gd 15–18. Testicular descent was evaluated in male offspring on gd 20 or pnd 30–40.	15/26	1,000	Not reported.	Testicular ascent on gd 20. ↑ Chryptorchidism in 22/26 male pups on pnd 30–40 with 87% of the undescended testes in abdominal cavity and 13% in the inguinal ring.

*Dose in mg/kg bw/day
[a]Number of male fetuses evaluated on gd 20 / pnd 30–40
gd=gestation day
pnd=postnatal day
↑=Statistically significant increase

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National Toxicology Program
U.S. Department of Health and Human Services



Center For The Evaluation Of Risks To Human Reproduction

PUBLIC COMMENTS ON THE PHTHALATES EXPERT PANEL REPORTS

Appendix III

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AdvaMed
Advanced Medical Technology Association

December 11, 2000

Michael D. Shelby, Ph.D.
Director, CERHR
National Toxicology Program B3-09
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709-2233

Dear Dr. Shelby:

The Advanced Medical Technology Association (AdvaMed) would like to comment on NTP's CERHR Expert Panel Report on di(2-ethylhexyl) phthalate (DEHP), dated October 2000 (*Fed. Reg.*, vol. 65, no. 196, p. 60206). Our comments are limited specifically to your review, conclusions, and recommendations regarding DEHP exposure through medical products.

AdvaMed is the largest medical technology trade association in the world, supported by more than 800 medical device, diagnostic products and health information systems manufacturers of all sizes. AdvaMed member firms provide nearly 90 percent of the $68 billion of health care technology products purchased annually in the United States, and nearly 50 percent of the $159 billion purchased annually around the world.

We are pleased that the CERHR panel has adhered to current, relevant, scientific data in its review of potential human reproduction and developmental risks due to DEHP exposure. We especially applaud the CERHR panel for your recognition that concern for the immediate welfare of patients – particularly for critically ill infants – should override any theoretical or unproven risk associated with medical therapies.

The final draft reflects the substantial efforts of the expert panel as well as input from interested parties. CERHR has received correspondence from AdvaMed as well as member companies. We still believe that there are several key issues that have not been adequately addressed in the current monograph:

- The absence of clinical indication of health risks from DEHP plasticized vinyl medical products needs to be clearly stated and given prominent status in the document, not simply mentioned in a few sentences that minimize the importance of this reality.

- Exposure does not equal risk, and should not be described as such. This is a fundamental concept in toxicology, but a point that may be lost on readers less familiar with the science. Accordingly, it is a point that should be clearly reinforced throughout the document.

- The CERHR panel has not reviewed all relevant, product-specific, pre-clinical testing that occurs with product submissions to regulating agencies. At least one member company has provided the panel with clinically relevant studies conducted by non-oral routes of exposure (e.g., intravenous) which have not been fully considered in the review and drafting process.

- When the CERHR review moves from oral dosing studies in sensitive rodents to clinical, non-oral exposures, the public needs to clearly understand that the panel is applying default assumptions that may or may not reflect clinical reality. To date, we are not aware of *any* animal studies conducted by non-oral routes, and at clinically relevant DEHP or MEHP exposure levels, that demonstrate adverse effects. The general public, and especially the patient population, has the right to be clearly informed of this, especially since there are demonstrated differences in sensitivities within, and between, species. While the data may not prove the negative, they do strongly suggest that the application of default assumptions may *not* be consistent with biological reality.

Given the panel's identification of data gaps/needs, we believe the CERHR would be particularly interested in updating the DEHP evaluation as additional data that specifically addresses these identified gaps/needs becomes available. AdvaMed encourages CERHR to identify a timely process in which relevant data, as it becomes available, could be considered and incorporated in the assessment. We believe this could be one of the most important ways that the CERHR contributes to public health policies that reflect the highest adherence to current scientific evidence.

AdvaMed is aware of several new studies that will yield data specifically responsive to the data needs identified by the CERHR panel:

1. AdvaMed is co-sponsoring, with the U.S. Food and Drug Administration, a medical device utilization study that will collect usage data on the most commonly used device categories, therapies, and certain disease conditions. Such utilization information, expected within two years, is important in completing a risk/benefit review of any medical products, including those made with DEHP/vinyl.

2. Another study is underway to examine the developmental effects of intravenous (IV) exposure to DEHP in newborn rats. The study started in late November 2000, and includes oral dosing groups as well three IV groups. This study will be the only publicly available investigation we are aware of that compares oral vs. IV dosing at doses up to 600 mg/kg/day, starting at post-natal day 3-5. Notably, AdvaMed contacted a CERHR phthalate expert panel member for input on the study design, which proved invaluable.
   In addition, a US FDA toxicologist with significant expertise in DEHP has reviewed the protocol, encouraged conduct of the study, and provided highly useful comments/suggestions.

3. Finally, we are confident the CERHR is aware of the American Chemistry Council's (ACC) intended study to examine the effects of relatively high oral exposure to DEHP on sexually immature primates and the multigenerational studies in rodents (oral exposure) that are on-going. We believe the ACC sponsored studies will provide new and important information on the basic reproductive and developmental toxicology of DEHP, just as the AdvaMed studies will provide invaluable information relevant to medical products.

Support for clinically relevant, sound scientific data remains the cornerstone of the medical device industry's interest that appropriate materials are available to meet the performance, storage, and sterilization demands placed on medical products. Given the valuable data the AdvaMed studies and ACC's studies will yield, as well as likely future data from other qualified studies, we reiterate our request that CERHR identify a process to incorporate this data into its evaluation of DEHP so that public health policies reflect the most relevant, current data available.

The NTP, FDA, and other national and international regulators bear a heavy responsibility for ensuring that sound, appropriate science – never conjecture and certainly not emotional debate – drive the public health policies that make safe and effective vinyl medical devices available to patients. No corroborated

clinical observations, case reports, or patient monitoring data have indicated a need for extensive clinical or epidemiological evaluation of DEHP, yet medical technology companies constantly evaluate the performance of their products, each of which has been designed with a specific material to meet a specific set of rigorous performance requirements. This is particularly important in light of the need to preserve patient access to technology where there is a notable absence of demonstrably "safer" alternative materials for vinyl medical applications. Any alternative materials should be held to the same level of scrutiny and scientific review as DEHP plasticized vinyl, which has certainly been more extensively studied than any other available medical grade material.

AdvaMed and member companies are committed to providing the best overall products for many diverse applications. We look forward to on-going dialogue with CERHR and other expert communities reviewing scientific data related to medical technologies, and we appreciate this opportunity to comment on your evaluation of DEHP.

Sincerely,

[redacted]

James S. Benson
Executive Vice President
Technology & Regulatory Affairs

[redacted]

Jon Cammack, Ph.D., D.A.B.T.
Chair, AdvaMed PVC Issue Working Group

cc: Ron Brown, FDA/CDRH
Jaro Vostal, FDA/CBER
John Moore, D.V.M., D.A.B.T.

Attachment 1

Evaluation of Reproductive Organs Following 21 Days of Repeated Intravenous and Oral Administration in Male Neonatal Rats

Type of Study: GLP

Table 1. Study Design

Treatment	Number of Animals and Sex	
	Sac at 24 d of age	Sac at 90 d of age
IV Vehicle Control	7M	9M
IV 60 mg/kg	7M	9M
IV 300 mg/kg	7M	9M
IV 600 mg/kg	7M	9M
PO Vehicle Control	7M	9M
PO 300 mg/kg	7M	9M
*PO 1000 mg/kg	7M	9M

*Dose had to be decreased to 600 mg/kg

Total Number of Animals: 112 pups

Dosing: IV; once daily for 21 consecutive days starting at 3 ± 1 days of age

Observations: Daily

Body Weight: Daily for dosage calculation (non-fasted), weekly after dosing (non-fasted) and at necropsy (non-fasted 24 day and fasted 90 day)

Organ Weights: Testes, Brain, Liver, Kidney, Spleen, Heart at 24 and 90 day

Sperm Count: At 90 day

Statistics: Body weight (i.e., weekly)
Organ weight
Organ relative to brain weight
Organ relative to body weight
Sperm Morphology/Motility and Count

Necropsy: Gross observations

Clinical Pathology: None

Histopathology: Testes (one) at 24 and 90-day
Epididymis at 90 day
Prostate at 90 day
Seminal vesicle at 90 day
Any gross pathological lesions
Sperm Morphology/Motility and Count

Tissues Preserved: Brain, Liver, Kidney, Spleen, Heart at 24 and 90 day sac

DEC - 7 2000

December 1, 2000

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council *Good Chemistry Makes It Possible*

Ms. Kate Rawson
Editor, The Rose Sheet
5550 Friendship Blvd., Suite One
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Dear Sir/Madam:

I am writing on behalf of the Phthalate Esters Panel (Panel) of the American Chemistry Council regarding the article entitled "Phthalates Carcinogenicity Potential In Consumer Products, CDC Study," which appeared in the October 23 edition of *The Rose Sheet*. As you may know, phthalates are a key ingredient found in many products that have improved the quality of life for families, businesses and hospitals for over 50 years. As such, I am very concerned by the inaccurate and potentially misleading nature of this article as it could result in raising undue concern on the part of your readership. I'd like to address my concerns more specifically in this letter, and I would strongly encourage you to contact a representative of the Panel in the future prior to any additional articles on phthalates.

The article is inaccurate regarding its main premise, the "planned carcinogenicity testing" of phthalates. The Panel has verified with both the National Institute of Environmental Health Sciences (NIEHS) and Centers for Disease Control (CDC) that neither organization plans any carcinogenicity studies on phthalates. For your information, most of the major phthalates have already undergone carcinogenicity testing. In February of this year, the International Agency for Research on Cancer (IARC), the world's leading authority on cancer, concluded that, DEHP, the most widely used phthalate, cannot be classified as being carcinogenic to humans.

The Rose Sheet article further misleads by failing to provide a context for the phthalate levels reported in the CDC biomonitoring study, as reported in the October issue of *Environmental Health Perspectives*. Such context, however, was provided in letters to the editor published in that same issue of EHP — one from researchers at NIEHS and CDC, the other from Dr. Raymond David of the Phthalate Esters Panel (see Attachments 1 and 2). These letters note that exposures to the most commonly used phthalates are consistent with previous estimates and are within safe limits derived by the U.S. Environmental Protection Agency (EPA). Using separate methodologies, both sets of authors used the CDC biomonitoring data to assess actual exposures. Although the exposure assessments were independently derived, the median, 95th percentile and maximum exposures to the various phthalates determined by each group are very similar to each other (see Table 1 of the Panel letter and Table 2 of the NIEHS/CDC letter). As pointed out in the Panel letter, the maximum exposures are at or within EPA — determined "safe" levels (known as RfD's). Those EPA levels incorporate conservative margins of safety so that even exposures at or slightly above the RfD does not necessarily indicate risks to health.

The broad comments indicating that phthalates cause "cancer, birth defects and adverse hormone reactions in laboratory animals" do not take into account the very large doses of phthalates that are required to induce effects in rodents, or the differences between rodents and humans in responding to phthalates, or the scientific uncertainties, which government and the scientific community are currently addressing concerning hormone disruption.

Since its inception 27 years ago, the Panel and its members have sponsored health and safety research on phthalates. This cutting-edge research always follows the strictest government and scientific standards to promote reproducibility, reliability and accuracy. Resulting data are peer-reviewed and published in respected scientific journals. The Panel shares its data with government agencies around the globe, including the U.S. EPA, the U.S. Food and Drug Administration, the National Toxicology Program, the Consumer Product Safety Commission and IARC. I have asked Marian Stanley, Manager of the Phthalate Esters Panel (703-741-5623), to call you to arrange for a full briefing about health and safety research on phthalates.

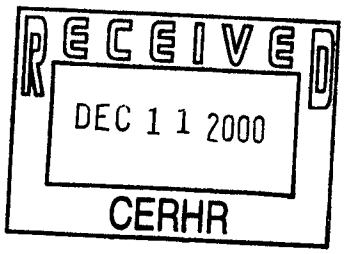
In summary, independent scientists, international government bodies and phthalate producers have conducted extensive studies about the safety, health and environmental effects of phthalates. This substantial body of scientific data does not present credible evidence that people are harmed by phthalates. There have been no confirmed reports of adverse health effects (including no human reproductive or developmental effects), in children or adults. Consumers and downstream customers can remain confident about using products that contain phthalates.

Sincerely yours,

[redacted]

Courtney M. Price
Vice President, CHEMSTAR

cc: Dr. John Brock, Centers for Disease Control and Prevention
Dr. Michael Cunningham, National Institute of Environmental Health Sciences
Dr. Michael Shelby, National Institute of Environmental Health Sciences
Mr. Gerald McEwen, Cosmetics, Toiletry and Fragrance Association
Mr. Glenn Roberts, Fragrance Manufacturers Association



December 11, 2000

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Re: Evaluations of Seven Phthalate Esters

Dear Dr. Shelby:

The American Chemistry Council Phthalate Esters Panel (PE Panel)[1] is submitting comments on the evaluations of seven phthalate esters made available by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP CERHR) on its website in October, 2000. Issues specific to each phthalate are addressed in Attachments 1-7 to this letter. In addition, the PE Panel would like to offer two general comments.

First, the PE Panel commends the NTP CERHR Expert Panel and the CERHR staff for the great effort reflected in these documents. In general, the PE Panel believes that the CERHR evaluations are well-written and provide generally accurate summaries of the data. We appreciate the opportunities that have been provided for interested parties to provide scientific input to the CERHR evaluations.

Second, the PE Panel wishes to express concern about CERHR's unwillingness in the final reports to place hazard information into context with qualitative statements of likely risk. CERHR's mission is to provide "timely and unbiased, scientifically sound assessments of reproductive health risks associated with human exposures to naturally occurring and man-made chemicals."[2] The Phthalates Expert Panel was asked to, "Rigorously evaluate all relevant data and reach a conclusion regarding the strength of scientific evidence that exposure to a chemical

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1 Formerly, the American Chemistry Council was known as the Chemical Manufacturers Association. The PE Panel includes the major U.S. producers and some processors of phthalate esters, as follows: Aristech Chemical Corporation, BASF Corporation, Eastman Chemical Company, ExxonMobil Chemical Company, Ferro Corporation, The Geon Company, and Teknor Apex Company.

2 "About CERHR," http://cerhr.niehs.nih.gov/aboutCERHR/index.html (emphasis added).

agent(s) may or may not present a risk to human reproduction or development."[3] Indeed, the word "risk" is used four additional times in the complete charge to the Expert Panel, and the Expert Panel was specifically directed to, "Provide judgments, including qualitative statements of the certainty of the judgments, that an agent presents a potential risk to human reproduction and/or development."[4] One would expect such judgments from a Center for the Evaluation of Risk to Human Reproduction.

During the first two rounds of Expert Panel deliberations, the Expert Panel stayed on this course and attempted to assess potential hazards, exposures and risks to human reproduction. In December 1999, the Expert Panel stated that it had completed its evaluation for DINP, and CERHR posted a summary on its website that stated, "Hence, available research and testing data make it unlikely that current estimated exposure levels constitute a risk to human reproduction or development." At the Expert Panel meeting in July 2000 however, it was announced that statements of risk would not be included in the CERHR evaluations, and a different hierarchy of nomenclature (based on expressions of "concern," from "negligible concern" to "serious concern") was developed. In the preface to each Expert Panel final report, the objectives of the Expert Panel have been restated, and the word "risk" has been removed entirely, although there is no acknowledgement that a change in approach has occurred.

The American Chemistry Counsel Phthalate Esters Panel disagrees with NTP's decision to alter the charge to the Expert Panel. We believe the alternative language that was developed is less scientific, less familiar to regulatory agencies, and less clear. We also believe it gives an inflated impression of the likelihood of a human risk or the strength of the evidence that indicates a possible risk, and we believe this bias is evident at both ends of the continuum, i.e., whether the expression of concern is "minimal" or "serious." Finally, we believe the hierarchy of language that was chosen invites incorporation of value judgments or policy considerations that are not suitable to the purely scientific assessments that we believe the CERHR Expert Panel was asked to render.

We urge the NTP CERHR to do three things: first, explain publicly why it changed the charge to the Expert Panel during the third round of deliberations; second, invite public discussion on the appropriateness of the approach adopted for the phthalate esters final reports; and third, return to the approach reflected in the original charge to Expert Panel, which we believe is the best approach.

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3 Charge to Expert Panel (emphasis added).

4 *Id.*

The PE Panel appreciates your consideration of this letter and the attached chemical-specific comments. If you have any questions, please call Marian K. Stanley, Manager of the Phthalate Esters Panel, at 703-741-5623.

Sincerely yours,

Courtney M. Price
Vice-President, CHEMSTAR

cc: John A. Moore, D.V.M., CERHR

ATTACHMENT 1

COMMENTS ON NTP CERHR
EVALUATION OF DI-n-BUTYL PHTHALATE (DnBP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DnBP (or DBP) dated October, 2000.[1] We offer the following general and specific comments.

General Comments

1. Generally, the Panel believes the DBP monograph is not as balanced or objective in presentation as some of the other monographs. The Panel's reasons for reaching this conclusion are reflected in several of the specific comments presented below.

2. The CERHR Expert Panel concludes that it has "minimal concern about effects to human development and development of the reproductive system from current estimated exposure to DBP." (p. 36) The Panel believes the data support an even stronger conclusion – there is essentially no risk or negligible risk from current estimated exposures. *See* comments on Section 5.3, below.

Specific Comments

Section 1.2 Exposure and Usage. The overview states, "Phthalates released to the environment can be deposited on or taken up by crops intended for human or livestock consumption, and thus, may enter the food supply." In the next paragraph, the monograph refers again to "environmental uptake during cultivation." Similar or identical language appears in each of the other monographs, giving the appearance that this language is boilerplate and not based on any phthalate-specific or DBP-specific data. The Panel is not aware of any evidence that environmental uptake by crops is significant for any of the phthalates, nor is any such evidence presented in this or any other monograph. Available evidence indicates the opposite:

- Kirchmann and Tengsved (1991)[2] investigated uptake of DBP and DEHP in barley grown on soil fertilized with sludge containing 37 mg/kg DBP and 116

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1 <http://cerhr.niehs.nih.gov/news/dbp-final-inprog.PDF>

2 Kirchmann, H., Astrum, G., and Jonsali, G. (1991). Organic pollutants in organic sewage sludge. 1. Effect of toluene, naphthalene, 2-methylnaphthalene, 4-nonylphenol, and di-2-ethylhexyl phthalate on soil biological processes and their decomposition in soil. *Swedish J. Agric. Res.* 21:107-113.

mg/kg DEHP. They concluded that only 0.1-0.2% of the phthalate added to the soil was taken up by grain.

- Overcash *et al.* (1986)[3] grew corn, soybean, wheat and fescue in soil containing 0.02 to 4 mg/kg of DBP and DEHP. Most plant bioconcentration values (plant concentration/soil concentration) were <0.1 and typical values were <0.01. These values were based on measurements of total [14]C and therefore overestimate the actual bioconcentration (*i.e.*, the total [14]C represents metabolites as well as parent compound).

- Aranda *et al.* (1989)[4] grew lettuce, carrots, chili peppers and tall fescue on soil amended with municipal sludge. Soil concentrations of DEHP were 2.6-14.1 mg/kg. No parent DEHP was detected in any of the plants.

- Schmitzer *et al.* (1988)[5] found no detectable DEHP in barley and potatoes grown in solids containing DEHP at concentrations of 0.2 to 3.3 mg/kg.

In addition, given the relatively low production volume and anticipated minimal releases to the environment of DBP (confirmed in EPA's 1997 Toxics Release Inventory which showed only 36,925 pounds released to air nationwide), crop uptake would appear to be an extremely remote concern. The reference to crops intended for consumption by livestock is scientifically inappropriate for the additional reason that metabolism data presented elsewhere in the monograph clearly show that this would not be expected to result in significant human exposure. The PE Panel therefore believes the statements quoted above should be deleted from the DBP monograph, as well as the monographs for the other phthalates. At the very least, the monograph should include the specific studies, summarized above, that indicate no significant crop uptake.

On page 9, the monograph describes an estimate of potential occupational exposures during phthalates production, prepared by the PE Panel and included in comments submitted on July 7, 1999. This calculation (143 ug/kg bw/day) was intended as an upper bound estimate only, based on an assumption, known to be unrealistic, that a given phthalate might be present continuously in the breathing zone of workers at a level of 1 mg/m^3. Additional data submitted to CERHR by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below the

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3 Overcash, M., Weber, J., and Tucker, W. (1986). *Toxic and priority organics in municipal sludge land treatment systems.* Water Engineering Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH (EPA/600-2-86/010).

4 Aranda, J., O'Connor, G., and Eiceman, G. (1989). Effects of sewage sludge on di-(2-ethylhexyl) phthalate uptake by plants. *J. Environ. Qual.* 18:45-50.

5 Schmitzer, J., Scheunert, I., and Korte, F. (1988). Fate of bis(2-Ethylhexyl) [^{14}C]phthalate in laboratory and outdoor soil-plant systems. *J. Agric. Food. Chem.* 36:210-215.

conservative estimate provided by the Panel. Thus, wherever this estimate is mentioned in the Expert Panel Report (*e.g.*, sections 5.1.1 and 5.3), the Panel believes the monograph should clearly indicate that this estimate is a theoretical upper bound calculation, and that "actual exposures are expected to be much lower."

Section 2.2 Toxicokinetics. The point of the discussion of the PBPK model (pp. 14-15) is unclear since the model is not used later in the monograph to estimate the dose of DBP (or MBuP) that reaches the fetus. It would be beneficial to provide that calculation or at least indicate what the model estimated.

Section 3.2.2 Postnatal Development. We have previously commented about the lack of relevance of including data for DEHP in the monograph on DBP. The detailed data presented for DEHP (p. 20, last paragraph, and Table 6) do not enhance the understanding of the mechanism for DBP. Instead, the discussion of DEHP only highlights the fact that these two esters produce similar effects. If that is the purpose, then other primate data for DEHP described in previous comments, also should be presented in the monograph.

Section 4.2. Reproductive Toxicity – Experimental Animal Toxicity – Mode of Action. The statement in the first paragraph (bottom of p. 24) that PPARα-knockout mice exposed to DEHP have failed to produce liver tumors should be deleted. To date, no study of the tumorigenic effects of long-term exposure to DEHP has been conducted using PPARα-knockout mice.

In the same paragraph (bottom p. 24), the monograph states, "Recently, an IARC review of the cancer issue led them to conclude that DEHP rat tumor data was of limited relevance to human risk." In fact, IARC went further and concluded, "Therefore, the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans." (Emphasis added.) IARC downgraded its DEHP cancer classification from Group 2B (possible human carcinogen) to Group 3 (not classifable as to human carcinogenicity).[6] Further, it is important to note that while IARC's Group 3 classification is used most commonly for substances "for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals," a substance will be placed in Group 3 despite sufficient evidence of carcinogenicity in experimental animals (as exists with DEHP), only "when there is strong evidence that the mechanism of carcinogencity in experimental animals does not operate in humans."[7] The Expert Panel Report should describe the IARC decision accurately and fully. The same correction is required when the IARC decision is discussed again on p. 33.

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6 IARC (2000). "Some Industrial Chemicals (Volume 77) (15-22 February 2000)", IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, (summary available at http://193.51.164.11/htdocs/accouncements/vol77.htm).

7 IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans, Preamble (available at http://193.51.164.11/monoeval/preamble.html).

The suggestion in the next paragraph (top p. 25) that activation of PPARγ is a possible mechanism for testicular toxicity is not supported by scientific evidence and therefore in our judgment is overly speculative. Maloney and Waxman (1999) (ref. #56)[8] measured a trans-activation of PPARγ and PPARα with MEHP. The authors did not investigate the levels of PPARγ in tissue. Instead, Maloney and Waxman incorrectly cite Greene *et al.*, (*Gene Expr.* **4**, 281-299, 1996) and Vidal-Puig *et al.*, (*J. Clin. Invest.* **99**, 2416-2422, 1997) as having demonstrated PPARγ levels in human testes. However, neither Greene *et al.* nor Vidal-Puig *et al.* investigated the levels of PPAR in testes. Therefore, to suggest that activation of PPARγ is a possible mechanism for testicular effects is not supported by any scientific evidence.

Section 5.11. Human Exposure Summary. The statement about potential exposure to DBP in infant formula (p. 26, last paragraph) needs to be clarified. On page 8, the monograph notes, "Infants in the US are likely exposed to lower levels of DBP through formula than are infants in the UK. In a survey of infant formulas conducted in 1996, DBP levels in the US were approximately 10-fold lower than concentrations measured in the UK and ranged from <5 to 11 ppb (<0.005 to 0.011 mg/kg) (*9*)." These statements should be repeated here to avoid leaving the reader with the impression that exposure might be as high in the U.S. as in the UK.

Section 5.13. Developmental Toxicity Summary. We disagree with the interpretation that the study by Ema *et al.* is appropriate only for prenatal endpoints and that the study by Mylchreest *et al.* is key for most sensitive endpoints at low doses (page 29, last paragraph, and page 30). First, the studies utilized the same exposure period. The differences between the studies are the route of administration (dietary admix versus oral gavage) and the strain of rat (Wistar versus Sprague-Dawley). If the major route of exposure is from food (Page 7, last paragraph), then the NOAEL from Ema should be the most appropriate value to use for comparison to human exposure levels. Second, there are no data to support the interpretation that Mylchreest *et al.* evaluated more sensitive endpoints. In fact, the monograph on DEHP indicates that for a similar study to that conducted by Ema, "that there are developmental effects that can be manifested postnatally, although these do not necessarily appear more sensitive than the reproductive effects in the current study" (page 95, last paragraph, last line, DEHP monograph).

Section 5.2. Integrated Evaluation. The first paragraph estimates that exposure to DBP for infants and young children is approximately 10 μg/kg/day, "with the possible exception of non-dietary intake through mouthing of phthalate-containing objects." The Panel believes mention of this "possible exception" is overly speculative, since the monograph already states that the use of DBP in toys is rare (Page 8, last paragraph). Indeed, on page 8, the monograph reports that DBP was detected in only 1 of 17 vinyl toys at 0.01% by weight. The PE Panel is not aware of any evidence that children receive significant exposure to DBP by mouthing objects.

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[8] If not provided in these comments, full citations to journal articles can be found in the Table of References in the Expert Panel's Final Report.

Section 5.3. Expert Panel Conclusions. We strongly disagree with the unqualified statement in the first paragraph that the mechanism is relevant for human reproduction. DBP has failed to demonstrate estrogenic or androgenic properties (page 33, last paragraph; Gray *et al.*, 1999), and the antiandrogenic mechanism occurs "via effects on testosterone biosynthesis and not androgen receptor antagonism" as stated in the monograph (page 36). The mechanism for reduced testosterone biosynthesis in unknown, but could be secondary to peroxisomal enzyme alteration of hormone-metabolizing enzymes (Corton *et al.*, 1997). Such a mechanism may not be relevant to humans because of significant species differences described in previous comments.

We also disagree with the overall conclusion that there is even "minimal" risk to human reproduction from exposure to DBP. Instead, we feel that the risk is negligible based on the vast difference between estimated human exposures and NOAEL values from laboratory animals. Even taking into account the most conservative studies, the difference between estimated exposures and animal NOAEL values is on the order of 5,000-25,000. Furthermore, recent data from the CDC reinforce the estimates for total exposure to DBP and support the conclusion that risk is negligible.[9] This conclusion does not take into account pharmacokinetics differences between rodents and primates that are alluded to in the monograph, which provide further evidence that reasonably anticipated exposures are unlikely to pose a risk to human reproduction or development.

Appendix III

[9] Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

ATTACHMENT 2

COMMENTS ON NTP CERHR
EVALUATION OF BUTYL BENZYL PHTHALATE (BBP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of BBP dated October, 2000.[1] We offer a general comment, followed by several specific comments.

General Comment

The PE Panel believes a potential risk to human development or reproduction from reasonably anticipated exposures to BBP is highly unlikely. General population exposures to BBP are estimated to be below 10 μg/kg bw/day. This value is more than 10,000-fold below NOAELs from existing reproductive and developmental toxicity studies, such that a risk to human reproduction for the general population is considered highly unlikely. Occupational exposures are estimated not to exceed 286 μg/kg bw/day (using worst case assumptions; actual exposures are expected to be much lower), which is approximately 1000-fold below reproductive and developmental toxicity NOAELs, indicating that an occupational risk also is unlikely. The results of the ongoing multigeneration study will provide important new information, but based on this scientific data that is currently available, the Panel believes current production and use of BBP is unlikely to pose any hazards or risks to human reproduction or development.

Specific Comments

Section 1.2 Exposure and Usage. The overview states (p. 6), "Phthalates that are released to the environment can be deposited on or taken up by crops intended for humans or livestock consumption, and thus can enter the food supply." On the next page, the monograph refers again to "environmental uptake during crop cultivation." Similar or identical language appears in each of the other monographs, giving the appearance that this language is boilerplate and not based on any phthalate-specific or BBP-specific data. The Panel is not aware of any evidence that environmental uptake by crops is significant for any of the phthalates, nor is any such evidence presented in this or any other monograph. Available evidence indicates the opposite:

- Kirchmann and Tengsved (1991)[2] investigated uptake of DBP and DEHP in barley grown on soil fertilized with sludge containing 37 mg/kg DBP and 116 mg/kg DEHP.

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1 <http://cerhr.niehs.nih.gov/news/BBP-final-inprog.PDF>

2 Kirchmann, H., Astrum, G., and Jonsali, G. (1991). Organic pollutants in organic sewage sludge. 1. Effect of toluene, naphthalene, 2-methylnaphthalene, 4-nonylphenol, and di-2-ethylhexyl phthalate on soil biological processes and their decomposition in soil. *Swedish J. Agric. Res.* 21:107-113.

They concluded that only 0.1-0.2% of the phthalate added to the soil was taken up by grain.

- Overcash et al (1986)[3] grew corn, soybean, wheat and fescue in soil containing 0.02 to 4 mg/kg of DBP and DEHP. Most plant bioconcentration values (plant concentration/soil concentration) were <0.1 and typical values were <0.01. These values were based on measurements of total [14]C and therefore overestimate the actual bioconcentration (*i.e.*, the total [14]C represents metabolites as well as parent compound).

- Aranda et al. (1989)[4] grew lettuce, carrots, chili peppers and tall fescue on soil amended with municipal sludge. Soil concentrations of DEHP were 2.6-14.1 mg/kg. No parent DEHP was detected in any of the plants.

- Schmitzer et al. (1988)[5] found no detectable DEHP in barley and potatoes grown in solids containing DEHP at concentrations of 0.2 to 3.3 mg/kg.

In addition, given the expected low releases of BBP to the environment, this would appear to be a very remote concern. The reference to crops intended for consumption by livestock is scientifically inappropriate because metabolism data presented elsewhere in the monograph clearly show that this would not be expected to result in significant human exposure. The PE Panel therefore believes the statements quoted earlier in this paragraph should be deleted from the BBP monograph, as well as the monographs for the other phthalates. At the very least, the monograph should include the specific studies, summarized above, that indicate no significant crop uptake.

The monograph on page 8 describes an estimate of potential occupational exposures during phthalates production, prepared by the PE Panel and included in comments submitted on July 7, 1999. This calculation (143 ug/kg bw/day) was intended as an upper bound estimate only, based on an assumption, known to be unrealistic, that a given phthalate might be present continuously in the breathing zone of workers at a level of 1 mg/m^3. Data submitted to CERHR by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below the conservative estimate provided by the Panel. Thus, wherever this estimate is mentioned in the manuscript (*e.g.*, sections 5.1.1), the Panel believes the monograph should clearly indicate that this is a theoretical upper bound calculation, and that "actual exposures are expected to be much lower."

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3 Overcash, M., Weber, J., and Tucker, W. (1986). *Toxic and priority organics in municipal sludge land treatment systems.* Water Engineering Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH (EPA/600-2-86/010).

4 Aranda, J., O'Connor, G., and Eiceman, G. (1989). Effects of sewage sludge on di-(2-ethylhexyl) phthalate uptake by plants. *J. Environ. Qual.* 18:45-50.

5 Schmitzer, J., Scheunert, I., and Korte, F. (1988). Fate of bis(2-Ethylhexyl) [^{14}C]phthalate in laboratory and outdoor soil-plant systems. *J. Agric. Food. Chem.* 36:210-215.

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the Panel's estimate for these potential exposures (286 ug/kg/day) also was based on an upper end and purposefully unrealistic assumption (that the phthalate would be continuously present in workplace air in these facilities at 2 mg/m^3, and that workers would be exposed to that level for their full shift every day). Data submitted to CERHR by Dr. McKee (see previous paragraph) show that exposures to phthalates in downstream facilities typically are very low (at or below the level of detection most of the time). Excursions toward the value assumed by the Panel may occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis. Thus, the estimate of 286 ug/kg/day is a theoretical worst-case value, and actual exposures are expected to be much lower.

Section 1.2 (Page 7). "Adult BBP intake was estimated at 2 micrograms/kg bw/day." It would be better to indicate a range of exposure, as IPCS did (2-6 micrograms/kg bw/day), than a single point estimate for dietary exposure. This occurs again in section 5.1.1. (page 23), and section 5.3 (page 31).

Section 1.2 (Page 7). Reference No. 7 should be to written comments submitted by the PE Panel on June 30, 2000, rather than to personal communication.

Section 1.2 (Page 7). "IPCS reported that median air levels of 0.034 - 0.035 ng/m^3 were measured in a survey of 125 California homes." The correct values and units should be 34-35 ng/m^3. This error also occurs in section 5.1.1, page 23, and section 5.3, page 32.

Section 2.1.1 Human Data. (Pages 8-9). No information is given regarding the quality of the epidemiology studies. The studies cited are of limited value, are in marked contrast with other epidemiological reports, and demonstrate no causal relationship. As such, a statement should be made to put the epidemiology data into context.

Section 3.2.1 Prenatal Development. (Page 14). In the discussion of Ema *et al.*, (28), the Expert Panel concludes that "The Expert Panel did not agree with the author's identification of developmental effect levels given that live litter size was reduced at 375 mg/kg/day (11.3 vs. control value of 13.9) and 654 mg/kg bw/day (12.3 vs. control value of 13.9); fetal body weights (by sex per litter) were significantly reduced at 654 mg/kg bw/day. The data did support a developmental NOAEL of 185 mg/kg bw /day." Although we agree with the conclusion on fetal body weight, we do not believe the data support the CERHR Expert Panel's conclusion based on litter size. The reduction observed at 375 mg/kg/day was not dose dependent. Further, the reduction observed was not associated with a significant increase in both pre- and post- implantation loss per litter. We do not recall this change of the author's conclusions being discussed publicly during the CERHR Expert Panel meetings, and we urge that it be reconsidered.

Section 4.2 Experimental Animal Toxicity. (Page 20). In discussion of Piersma et al. (48), it is noted that "F1 pup weight was reduced at birth in mid- and high-dose groups and a developmental NOAEL of 250 mg/kg bw/day was identified." The reduction of pup weight

was noted at 500 mg/kg bw/day on post natal day 1; however, pup weight had returned to control levels by post natal day 4.

Section 5.2, Integrated Evaluation, Last Paragraph. (Page 31). Data on urinary levels of BBP metabolites has been reported (Blount et al., 2000).[6] These data indicate that exposure to BBP is in line with the estimates in the CERHR report.[7] This comment applies also to Section 5.4 – Human Exposure.

Section 5.3 Expert Panel Conclusions. (Page 32). With regard to developmental toxicity, the Expert Panel states that the database supports a conclusion that BBP can cause developmental toxicity in rats and mice and reproductive toxicity in rats. The Expert Panel goes on to say that the current database is insufficient to fully characterize the potential hazard. The Expert Panel identifies developmental toxicity NOAELs of 182 mg/kg/day in CD-1 mice and 185 mg/kg/day in Wistar rats and concludes that, given the margin of human exposure, there is negligible concern for male reproductive effects from adult exposure. The Expert Panel goes on to say that there is not an adequate database to determine NOAELs/LOAELs for male or female reproductive effects from perinatal exposure nor could the Panel ascribe a level of concern for postnatal consequences from perinatal exposure to BBP. Given the appearance of papers by Gray et al., Nagao et al., and Piersma et al. (referenced below) the Expert Panel may want to revise its position on the utility of the BBP developmental and reproductive toxicity databases, especially with regard to perinatal/postnatal evaluations.

Subsequent to the release of the October, 2000 CERHR draft monograph on BBP, Piersma et al., published results of an oral gavage developmental toxicity study in Harlan rats.[8] The study employed gavage dosing of BBP in corn oil to pregnant rats on days 6-15 or 6-20 of gestation. Ten dose groups of 10 dams each were used in the study and the authors point out that the total number of animals in the study (100) was equivalent to 4 test groups of 25 dams. This appears to be a suggestion that the statistical power of the study as it was performed is equivalent to a study with two and one-half times the number of animals per group, a suggestion with which the PE Panel disagrees. Piersma et al. found evidence for fetal and maternal toxicity: maternal deaths occurred at the two highest doses (1600 and 2100 mg/kg/day); the dams in the top three dose levels ate less food than controls for a substantial portion of the dosing/gestation period (one-half and one-third of the dosing period for the two exposure regimens, respectively) and all dosed groups gained less weight than controls. Systemic effects of BBP in pregnant dams included increased liver weight and increased serum liver enzyme concentrations (PCO and ALAT) in all but the lowest dose group (350 mg/kg/day and up); relative maternal kidney weights increased in all treated dose groups and extramedullary hematopoiesis was increased in all maternal dose groups. Fetal body weight was decreased in all dose groups; skeletal anomalies

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6 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982

7 Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

8 Piersma, A. (2000). Developmental toxicity of buytl benzyl phthalate in the rats using a multiple dose study design. *Reproductive Toxicology* 14:417-425,.

were reported for treatment groups but incidence data were not provided; supernumerary 13th lumbar ribs were reported to be increased in treated groups; soft tissue malformations were observed but not in a dose-related fashion. Diminished fetal testes weight and retarded fetal testicular descent were reported to be dose-related in treated groups. Data tables showing body or organ weights and malformation incidence were not included in the report. Statistical significance of findings relied on the authors' selection of Critical Effects Sizes (CES) and calculation of Critical Effects Doses (CED), all presented in a benchmark dose-type calculation.

The authors chose to establish critical effects criteria for fetal effects at 4-fold to 20-fold lower than critical effect criteria for maternal toxicity. Accordingly, even though there was evidence of maternal systemic toxicity at all dose levels where fetal effects were reported, the choice of critical effects sizes rendered these maternal effects nonsignificant in all but the highest dose levels. Using their choices for critical effects sizes, and therefore critical effects doses, the authors were able to claim that fetal effects occurred with significance at lower doses than maternal effects. In their paper the authors state, "...in any particular case, experts may deviate from these default values for CES (critical effect sizes) when they have good (biologic) reason for doing so." The PE Panel believes that there is no good biologic reason for dissimilar levels of significance within one study where the dose-response metric is the dosed pregnant dam and her litter. In analyzing their data, the authors calculate that the lowest benchmark dose (BMD) is 27 mg/kg/day for maternal extramedullary hematopoiesis and the next lowest BMD is 77 mg/kg/day for maternal peroxisome proliferation. The lowest BMD for fetal toxicity is 95 mg/kg/day (testes descent). The authors discard extramedullary hematopoiesis effects in the pregnant dams by stating that it is normal in pregnant rats but not in pregnant women, but did not show data to support this and did not account for the observation that the extramedullary hematopoiesis increased in a dose-related fashion in treated animals. The authors similarly dismissed any effect peroxisome proliferation may have had on a normal pregnancy in the Harlan rat and did not consider that hepatomegally and increased ALAT signal altered liver function. While there may be validity to the authors' claim that "PCO and extramedullary hematopoiesis are considered irrelevant for human risk assessment," the impact of these conditions on the gestation of the animals in which these conditions occurred in this study is not irrelevant.

Notwithstanding these flaws in the authors' analysis, the Expert Panel should note that the BMD of 95 mg/kg/day offered by Piersma et al. does not detract from the conclusion that estimated human exposure to BBP is so far below animal effect levels that the risk to humans is negligible.

As already noted, the Expert Panel in Section 5.3 states that there is not an adequate database to determine NOAELs/LOAELs for male or female reproductive effects from perinatal exposure nor could the Panel ascribe a level of concern for postnatal consequences from perinatal exposure to BBP. In drafting these statements, the CERHR Expert Panel was aware of information on BBP which reported that high oral gavage doses (750 mg/kg/day) administered to pregnant and lactating female Sprague-Dawley rats produced reproductive tract defects in male offspring. The work, then in press, is now published by Gray et al.[9] Gray's work

[9] Gray, E., et al. (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat, *Tox. Sci.* 58:350-365.

addresses the question of perinatal exposure/postnatal evaluation in Sprague-Dawley male rats. Female offspring were not evaluated by Gray. The PE Panel encourages the Expert Panel to examine the Gray publication, which reports effects at the very high dose of 750 mg/kg/day.

In addition, Nagao et al. have published the results of a two-generation reproduction study with BBP in Sprague-Dawley rats.[10] The study by Nagao et al. included evaluations of reproductive development, fertility, and reproductive system structures including endocrine sensitive parameters. Males and females were evaluated and animals in the study received oral gavage exposure to BBP prenatally, perinatally and postnatally for two generations. This study used the same test animal species and strain as that used in the Gray et al. study and dosed up to 500 mg/kg/day throughout all critical life phases. (Gray et al. dosed for two weeks at 750 mg/kg/day.) The Nagao et al. study did not produce evidence of an adverse effect on reproductive ability at any dose level. The effects reported by Nagao et al. were: reduced anogenital distance in high dose male pups on PND 0; delay in preputial separation in high-dose F1 males; intermittent increases and decreases in serum hormone levels in F0 and F1 males and females; absolute testes, epididymis, prostate and seminal vesicle weights decrease in high-dose F1 pups; absolute spleen and heart weight reduced in high-dose F1 female pups; atrophy of seminiferous tubules and decrease in sperm in F1 high-dose young adults. High- and mid-dose (500 and 100 mg/kg/day, respectively) F1 male and female pups were born at a statistically-significantly lower body weight. The authors of this paper did not report testing the effect of lower body weight on any of the parameters reported as affected by BBP treatment, i.e., covariance of the observed effect with body weight differences. With the possible exceptions of the seminiferous tubule changes and hormone levels, all of the changes reported as induced by BBP are subject to covariance with pup body weight and vary in the direction of the body weight change. That is, smaller pups have smaller AG distances and acquire secondary sex characteristics later than larger pups. These animals eventually all mature and have normal reproductive function. Whether the reported effects on sensitive indictors of endocrine disruption are primary or are secondary effects of high-dose BBP-induced reduced birth weight cannot be known from this paper.

In summary, the Gray et al. paper reports effects at 750 mg/kg/day. The study by Nagao et al. purports to find a NOAEL of 20 mg/kg/day, although the journal article leaves some questions unanswered. But even if a NOAEL of 20 mg/kg/day is accepted, this value is still approximately 1000-fold above the high end of estimated general population exposures, such that neither study is indicative of a likely risk to human reproduction or development.

Finally the last paragraph of the Expert Panel Conclusions refers to data for DBP. We believe it is not necessary to rely on DBP data to evaluate BBP, in light of the substantial BBP data that is available.

Critical Data Needs. Human Exposure. (Page 32). If "Occupationally-exposed cohorts... would be of limited utility if the major source of exposure is food," then why should "Priority be given to studies on occupational exposures"?

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[10] Nagao, T. (2000). Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study. *Reproductive Toxicology* 14:513-532.

ATTACHMENT 3

COMMENTS ON THE NTP CERHR
EVALUATION OF DI-n-HEXYL PHTHALATE (DnHP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DnHP dated October, 2000.[1] We offer a general comment, followed by several specific comments.

General Comment

Given that reproductive or developmental toxicity has been observed in animal studies only at very high doses, and that potential exposures to humans are very low, the PE Panel believes there is essentially no risk for reproductive or developmental toxicity from anticipated exposures to DnHP. The PE Panel agrees with the CERHR Expert Panel that, if any further testing is to be conducted, it should be conducted on the 6-10 mixture or DiHP. However, given the low potential for exposure and the results of existing studies, we believe DnHP should be considered a low priority for further research at this time. Accordingly, we agree with the Expert Panel's decision not to identify any specific data needs.

Specific Comments

Section 1.2 Exposure and Usage. The overview states (p. 6), "Phthalates that are released to the environment can be deposited on or taken up by crops intended for human or livestock consumption, and thus, can enter the food supply." The next paragraph refers again to "environmental uptake during cultivation." Similar or identical language appears in each of the other monographs, giving the appearance that this language is boilerplate and not based on any phthalate-specific or DnHP-specific data. The Panel is not aware of any evidence that environmental uptake by crops is significant for any of the phthalates, nor is any such evidence presented in this or any other monograph. Available evidence indicates the opposite:

- Kirchmann and Tengsved (1991)[2] investigated uptake of DBP and DEHP in barley grown on soil fertilized with sludge containing 37 mg/kg DBP and 116 mg/kg DEHP. They concluded that only 0.1-0.2% of the phthalate added to the soil was taken up by grain.

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1 <http://cerhr.niehs.nih.gov/news/DnHP-FINALinprog.PDF>

2 Kirchmann, H., Astrum, G., and Jonsali, G. (1991). Organic pollutants in organic sewage sludge. 1. Effect of toluene, naphthalene, 2-methylnaphthalene, 4-nonylphenol, and di-2-ethylhexyl phthalate on soil biological processes and their decomposition in soil. *Swedish J. Agric. Res.* 21:107-113.

- Overcash *et al* (1986)[3] grew corn, soybean, wheat and fescue in soil containing 0.02 to 4 mg/kg of DBP and DEHP. Most plant bioconcentration values (plant concentration/soil concentration) were <0.1 and typical values were <0.01. These values were based on measurements of total [14]C and therefore overestimate the actual bioconcentration (*i.e.*, the total [14]C represents metabolites as well as parent compound).

- Aranda *et al.* (1989)[4] grew lettuce, carrots, chili peppers and tall fescue on soil amended with municipal sludge. Soil concentrations of DEHP were 2.6-14.1 mg/kg. No parent DEHP was detected in any of the plants.

- Schmitzer *et al.* (1988)[5] found no detectable DEHP in barley and potatoes grown in solids containing DEHP at concentrations of 0.2 to 3.3 mg/kg.

In the case of DnHP, given the minimal potential releases to the environment, crop uptake would appear to be a very remote concern. The reference to crops intended for consumption by livestock is scientifically inappropriate, for the additional reason that metabolism data presented elsewhere in the monograph clearly show that this would not be expected to result in human exposure. The PE Panel therefore believes the statements quoted above should be deleted from the DnHP monograph, as well as the monographs for the other phthalates. At the very least, the monograph should include the specific studies, summarized above, that indicate no significant crop uptake.

On page 7, the monograph describes an estimate of potential occupational exposures during phthalates production, prepared by the PE Panel and included in comments submitted on July 7, 1999. This calculation (143 ug/kg bw/day) was intended as an upper bound estimate only, based on an assumption, known to be unrealistic, that a given phthalate might be present continuously in the breathing zone of workers at a level of 1 mg/m^3. Additional data submitted to CERHR by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below the conservative estimate provided by the Panel. Thus, wherever this estimate is mentioned in the manuscript, the Panel believes the monograph should clearly indicate that this is a theoretical upper bound calculation, and that "actual exposures are expected to be much lower."

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the Panel's estimate for these potential exposures (286 ug/kg/day) also was based on an upper end and purposefully

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3 Overcash, M., Weber, J., and Tucker, W. (1986). *Toxic and priority organics in municipal sludge land treatment systems.* Water Engineering Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH (EPA/600-2-86/010).

4 Aranda, J., O'Connor, G., and Eiceman, G. (1989). Effects of sewage sludge on di-(2-ethylhexyl) phthalate uptake by plants. *J. Environ. Qual.* 18:45-50.

5 Schmitzer, J., Scheunert, I., and Korte, F. (1988). Fate of bis(2-Ethylhexyl) [^{14}C]phthalate in laboratory and outdoor soil-plant systems. *J. Agric. Food. Chem.* 36:210-215.

unrealistic assumption (that the phthalate would be continuously present in workplace air in these facilities at 2 mg/m^3, and that workers would be exposed to that level for their full shift every day). Data submitted by Dr. McKee (see previous paragraph) show that exposures to phthalates in downstream facilities typically are very low (at or below the level of detection most of the time). Excursions toward the value assumed by the Panel are expected to occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis. Thus, the estimate of 286 ug/kg/day is a theoretical worst-case value, and actual exposures are expected to be much lower.

Section 5.3 Expert Panel Conclusions. The Expert Panel concluded that "there is insufficient information to ascertain the potential for risk to human reproduction." (p. 18) The Phthalate Esters Panel does not agree with this conclusion. Rather the Panel believes that the data available on DnHP along with data on other phthalates, provide sufficient information to support a determination of "minimal concern" (no likely risk) for adult human reproduction at ambient human exposures. The analysis by the Panel is described below.

The reproductive toxicity of DnHP was assessed by the National Toxicology Program as part of a comparative study involving phthalates of differing chain length (Lamb et al., 1986; Morrissey *et al.*, 1989; Chapin and Sloane, 1997). As demonstrated by these studies, exposure to DnHP reduced fertility in a dose-responsive manner. At the lowest dose (0.3% in the diet, or approximately 430 mg/kg/day as estimated by Morrissey *et al.*), fertility was reduced by about 18%. As noted by the Expert Panel, a no effect level was not experimentally defined; however, a NOAEL can be estimated from the dose-response curve. As shown below (pages 3-5 and 3-6), the NOAEL for loss of fertility, based on inspection, is approximately 300 mg/kg bw/day (based on extrapolation from linear portion of dose-response curve – see figure below). The maximum likelihood estimate of a 5% reduction is 364 mg/kg bw/day, and the lower 95% limit on that value is 219 mg/kg bw/day. As is also evident from the graph on page 3-6, DEHP, tested under the same circumstances, produced similar effects but at lower treatment levels. Thus, these data demonstrate that DnHP and DEHP produce similar effects but that DnHP is not as active as DEHP.

DnHP also produces testicular atrophy in juvenile rats when given at relatively high levels (Foster *et al.*, 1980). The effects of DnHP seem similar to those of DEHP (Gray *et al.*, 1977), but as these two substances have not been tested concurrently under identical protocols, a direct comparison is more difficult. Nevertheless, there is sufficient data to conclude that the effects of DnHP on fertility in rodents are similar to those of DEHP, and that DnHP seems similar to or less active than DEHP in studies conducted under the same protocol.

Exposure to DnHP has not been as well characterized as that of DEHP, but it is known that production volumes are much lower and uses are more restricted. When assessed, levels of DnHP are at or below detection limits in food and other media. DnHP is not used in medical devices and not reported in toys. The Expert Panel agreed that exposures to DnHP were likely to be lower than estimates of 3-30 ug/kg/day prepared for DEHP.

In its evaluation of DEHP, the Expert Panel expressed "minimal concern" that ambient human exposures could adversely affect human reproduction. The Expert Panel

expressed "concern" for reproductive development in human children if children's exposures were significantly higher than those of adults. As DnHP produces similar effects in rodents to those of DEHP, but is less active, and exposures to DnHP are believed to be lower than those to DEHP, it would be reasonable to assume that the conclusions for DEHP, i.e., that concerns are minimal unless exposures are substantially higher than estimated, also apply to DnHP.

Analysis of Fraction of Affected Pregnant Females
DnHP and DEHP

Data from a mating study indicated the following incidence data for pregnant/non-affected dams:

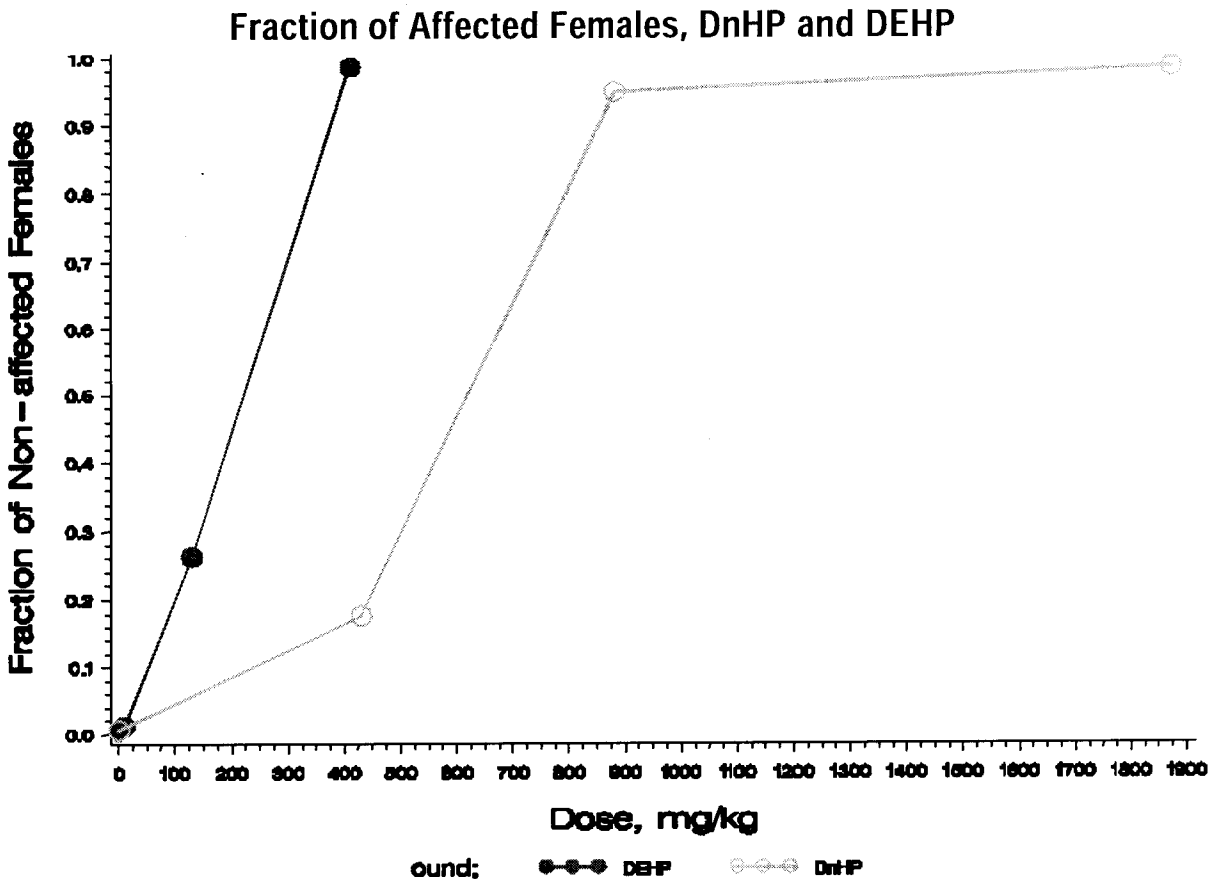
Compound	Dose (mg/kg)	Number Affected	Sample Size	Fraction Affected
DnHP	0	0	39	0.0
	430	3	17	0.18
	880	18	19	0.95
	1870	16	16	1.0
DEHP	0	0	40	0.0
	10	0	20	0.0
	130	5	19	0.26
	410	18	18	1.0

A probit regression analysis with compound and dose indicated a statistically significant difference in compounds ($p<0.001$). The model diagnostics indicated the statistical assumptions for the analysis were met.

Benchmark dose calculations were made using a quadratic model with a threshold. The estimated BMD10, BMD05 and lower 95% confidence intervals are:

	BMD10 (mg/kg)		BMD05 (mg/kg)	
	MLE	Lower 95% Limit	MLE	Lower 95% Limit
DnHP	393	269	364	219
DEHP	116	46	111	28

The figure below shows the data graphically and clearly demonstrates the difference between the two compounds based on these data. (**Note:** The labeling on the Y-axis contains a typographical error – it should say "Fraction of Affected Females." Unfortunately, correction of this error has eluded our computer skills. We apologize for the error – the title of the graph is correct.)



ATTACHMENT 4

COMMENTS ON NTP CERHR
EVALUATION OF DI-n-OCTYL PHTHALATE (DnOP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DnOP dated October, 2000.[1] We offer a general comment, followed by a few specific comments.

General Comment

Given that essentially no reproductive or developmental toxicity has been observed in animal studies using very high doses, and since potential exposures are very low, the PE Panel believes there is essentially no risk for reproductive or developmental toxicity from anticipated exposures to DnOP. The CERHR Expert Panel recognizes that general population exposure to DnOP is likely to be "well below" the exposure estimate for DEHP of 3 to 30 ug/kg/day. (p. 8) The high dose in the continuous breeding study for DnOP was 7,500 mg/kg/day, which is more than 200,000-fold above the high end of CERHR's range of general population exposure estimates for DEHP. Since DnOP exposure is "well below" that range, there probably is more than a million-fold margin between exposure and effect levels. Under these circumstances, notwithstanding any perceived limitations in the studies, we believe CERHR should offer a plain English conclusion along the following lines: "DnOP is highly unlikely to pose a reproductive or developmental toxicity hazard to the general population at expected exposure levels."

Specific Comments

Section 1.2 Exposure and Usage. The overview states (p. 7), "Phthalates released to the environment can be deposited on or taken up by crops intended for human or livestock consumption, and thus, may enter the food supply." In the next paragraph, the monograph refers again to "environmental uptake during cultivation." Similar or identical language appears in each of the other monographs, giving the appearance that this language is boilerplate and not based on any phthalate-specific or DnOP-specific data. The Panel is not aware of any evidence that environmental uptake by crops is significant for any of the phthalates, nor is any such evidence presented in this or any other monograph. Available evidence indicates the opposite:

- Kirchmann and Tengsved (1991)[2] investigated uptake of DBP and DEHP in barley grown on soil fertilized with sludge containing 37 mg/kg DBP and 116 mg/kg DEHP.

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1 http://cerhr.niehs.nih.gov/news/DnOP-final-inprog.PDF

2 Kirchmann, H., Astrum, G., and Jonsali, G. (1991). Organic pollutants in organic sewage sludge. 1. Effect of toluene, naphthalene, 2-methylnaphthalene, 4-nonylphenol, and di-2-ethylhexyl

They concluded that only 0.1-0.2% of the phthalate added to the soil was taken up by grain.

- Overcash et al. (1986)[3] grew corn, soybean, wheat and fescue in soil containing 0.02 to 4 mg/kg of DBP and DEHP. Most plant bioconcentration values (plant concentration/soil concentration) were <0.1 and typical values were <0.01. These values were based on measurements of total [14]C and therefore overestimate the actual bioconcentration (*i.e.*, the total [14]C represents metabolites as well as parent compound).

- Aranda et al. (1989)[4] grew lettuce, carrots, chili peppers and tall fescue on soil amended with municipal sludge. Soil concentrations of DEHP were 2.6-14.1 mg/kg. No parent DEHP was detected in any of the plants.

- Schmitzer et al. (1988)[5] found no detectable DEHP in barley and potatoes grown in solids containing DEHP at concentrations of 0.2 to 3.3 mg/kg.

Given the relatively low production volume and anticipated minimal releases of DnOP to the environment, crop uptake would appear to be an extremely remote concern. The reference to crops intended for consumption by livestock is inappropriate for the additional reason that metabolism data for phthalates show that this would not be expected to result in significant human exposure. DnOP is detected in the environment, if at all, only at very low levels, as reflected by data summarized in the monograph at the bottom of p. 7. DnOP's low vapor pressure and low water solubility are obvious factors, but its ready degradation in the environment and rapid metabolism in biological species also are relevant. Given the statements on page 7 that recognize the "minimal" potential for exposure to DnOP through air, and for all of the above reasons, the Panel believes the references to "environmental uptake" should be deleted from the Expert Panel report. At the very least, the monograph should include the specific studies, summarized above, that indicate no significant crop uptake.

On page 8, the monograph describes an estimate of potential occupational exposures during phthalates production, prepared by the PE Panel and included in comments submitted on July 7, 1999. This calculation (143 ug/kg bw/day) was intended as an upper bound estimate only, based on an assumption, known to be unrealistic, that a given phthalate might be present continuously in the breathing zone of workers at a level of 1 mg/m^3. Additional data submitted by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and

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phthalate on soil biological processes and their decomposition in soil. *Swedish J. Agric. Res.* 21:107-113.

3 Overcash, M., Weber, J., and Tucker, W. (1986). *Toxic and priority organics in municipal sludge land treatment systems.* Water Engineering Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH (EPA/600-2-86/010).

4 Aranda, J., O'Connor, G., and Eiceman, G. (1989). Effects of sewage sludge on di-(2-ethylhexyl) phthalate uptake by plants. *J. Environ. Qual.* 18:45-50.

5 Schmitzer, J., Scheunert, I., and Korte, F. (1988). Fate of bis(2-Ethylhexyl) [^{14}C]phthalate in laboratory and outdoor soil-plant systems. *J. Agric. Food. Chem.* 36:210-215.

DIDP, clearly show that actual occupational exposures during phthalate production typically are far below the conservative estimate provided by the Panel. Thus, wherever this estimate is mentioned in the manuscript (*e.g.*, sections 5.1.1 and 5.3), the Panel believes the monograph should clearly indicate that this is a theoretical upper bound calculation, and that "actual exposures are expected to be much lower."

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the Panel's estimate for these potential exposures (286 ug/kg/day) also was based on an upper end and purposefully unrealistic assumption (that the phthalate would be continuously present in workplace air in these facilities at 2 mg/m^3, and that workers would be exposed to that level for their full shift every day). Data submitted by Dr. McKee (see previous paragraph) show that exposures to phthalates in downstream facilities typically are very low (at or below the level of detection most of the time). Excursions toward the value assumed by the Panel are expected to occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis. Thus, the estimate of 286 ug/kg/day is a theoretical worst-case value, and actual exposures are expected to be much lower.

Section 2.1.2: Poon *et al.* (1997) (Ref. 15) Evaluation of Tissue Levels. The PE Panel appreciates the Expert Panel's explicit recognition that the PE Panel has questioned the reliability of tissue levels reported by Poon *et al.* (1997) for DnOP and DEHP. The PE Panel believes the measurements of DEHP and DnOP in liver and fat reported in Poon *et al.* (1997) are unreliable and accordingly not appropriate for inclusion in the document. Limitations on the use of the data include: failure to use MS identification of what was detected; absence of analytical blanks; and internal inconsistency of the data with respect to dose and the biology of hydrolysis and absorption. (This is not a question of holding a 10-year old protocol to a year 2000 standard; these are deficiencies that should have been apparent when the study was conducted, and should have been raised when it was published.)

ATTACHMENT 5

COMMENTS ON NTP CERHR
EVALUATION OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DEHP dated October, 2000.[1] We offer one general and several specific comments.

General Comment

The CERHR Expert Panel concludes that general population exposures are in the range of 3-30 ug/kg/day, that the animal LOAEL is approximately 38 mg/kg/day, and the animal NOAEL is about 3.7-14 mg/kg/day. Given that the effect at the LOAEL (Sertoli cell vacuolization) was minimal, the PE Panel believes the monograph should conclude that the data indicate that general population exposures are approximately three orders of magnitude below the dose at which effects begin to appear in laboratory animals. Therefore, the PE Panel believes it is unlikely that humans exposed at such levels would experience reproductive or developmental effects.

Comments on Potential Occupational Exposures

Section 1.2 Exposure and Usage. On page 9, the monograph describes an estimate of potential occupational exposures during phthalates production, prepared by the PE Panel and included in comments submitted on July 7, 1999. This calculation (143 ug/kg bw/day) was intended as an upper bound estimate only, based on an assumption, known to be unrealistic, that a given phthalate might be present continuously in the breathing zone of workers at a level of 1 mg/m^3. Additional data submitted to CERHR by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below the conservative estimate provided by the Panel. Thus, wherever this estimate is mentioned in the manuscript (*e.g.*, section 5.1.1, p. 78), the Panel believes the monograph should clearly indicate that this is a theoretical upper bound calculation, and that "actual exposures are expected to be much lower." The information from Dr. McKee's submission also should be included.

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the Panel's estimate for these potential exposures (286 ug/kg/day) also was based on an upper end and purposefully unrealistic assumption (that the phthalate would be continuously present in workplace air in these facilities at 2 mg/m^3, and that workers would be exposed to that level for their full shift every day). Data submitted by Dr. McKee (see previous paragraph) show that exposures to

[1] <http://cerhr.niehs.nih.gov/news/FINALinprog.PDF>

phthalates in downstream facilities typically are very low (at or below the level of detection most of the time). Excursions toward the value assumed by the Panel are expected to occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis. Thus, the estimate of 286 ug/kg/day is a theoretical worst-case value, and actual exposures are expected to be much lower.

Additionally, the monograph should recognize that workers do not work 365 each year. Thus, a worst case exposure estimate for production workers of 143 ug/kg/day is equal to 86 ug/kg/day annualized over 365 days. For workers in the manufacture of articles, the corresponding figures would be 286 ug/kg/day (worst case estimate) and 172 ug/kg/day (worst case estimate annualized).

Additional Technical Comments

1. Page 11, line 5. In its comments submitted to the NTP CERHR on June 30, 2000, the PE Panel commented on the scientific soundness of estimating a cumulative annual dose following dialysis since this does not take into account metabolism or excretion of DEHP. We feel that the values presented are not scientifically sound or defensible, and may be inaccurate. Doull *et al.* (1999) considered dose levels from long-term dialysis and calculated daily dose levels to be 32 mg/person/day over the course of 1 year (over 1000 times lower than the estimates of the Expert Panel) assuming dialysis 3 times per week rather than the twice per week and double the amount of DEHP per treatment used by the Expert Panel. Even using the blood concentrations listed in Table 7, a 70 kg person being dialyzed twice weekly would likely be exposed to a dose of only 0.9 mg/day or a cumulative dose of 342 mg/year.

2. Page 19, 3rd paragraph. The findings of Dalgaard *et al.* (ref. #74) are only partially reported. Important information concerning the **lack** of adverse findings in the functional observational battery (FOB) or the hindlimb grip strength is missing, leaving the reader to believe that DEHP is neurotoxic. The full results of Dalgaard and coworkers should be reported as they support the earlier studies by Moser *et al.* (1995)[2] and MacPhail *et al.* (1995),[3] who failed to find evidence of neurotoxicity for DEHP.

3. Page 23, next to last paragraph. There is an incorrect statement indicating that the CPSC is conducting a review of DEHP. The CPSC has convened a CHAP to review DINP.

4. Page 34, "Humans: Inhalation." Although the data presented by Roth *et al.* suggest that exposure to DEHP resulted from plasticized-PVC tubing used in artificial ventilation, the monograph clearly indicates on page 13 that respiratory tubing used in North

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2 Moser V.C., Cheek B.M., MacPhail R.C. (1995). A Multidisciplinary Approach To Toxicological Screening III. Neurobehavioral Toxocity. J. Toxicol. Environ. Health 45, 173-210.

3 MacPhail R.C., Berman E., Elder J.A., Kavlock R.J., Moser V.C. (1995). A Multidisciplinary Approach To Toxicological Screening IV. Comparison of Results. J. Toxicol. Environ. Health 45, 211-220.

America (US and Canada) is made from polyethylene and "contains no DEHP." This fact is missing from page 34 and leaves the reader to assume that exposure to DEHP is possible during artificial ventilation.

5. Page 66, 1st full paragraph. The NOAEL as stated by the authors was 500 ppm (28-30 mg/kg), not 146 mg/kg. The authors selected that NOAEL because aspermia was not observed after 78 weeks of treatment (roughly three quarters of the animal's lifespan), but only at terminal sacrifice suggesting that the aging process made the animal more sensitive.

6. Page 72, "Female reproductive effects." The statement indicating that MEHP suppresses aromatase activity in the ovary is technically incorrect. The authors clearly indicate that the velocity and affinity of the microsomal aromatase were not altered by exposure to MEHP. However, the availability of aromatase was decreased which resulted in a suppression of the conversion of testosterone to estradiol.

7. Page 74, 3rd paragraph and Page 97, 4th paragraph. The suggestion that activation of PPARγ is a possible mechanism for testicular toxicity is not supported by scientific evidence and therefore in our judgment is overly speculative. Maloney and Waxman (1999) (ref. #190) measured a trans-activation of PPARγ and PPARα with MEHP. The authors did not investigate the levels of PPARγ in tissue. Instead, Maloney and Waxman incorrectly cite Greene *et al.*, (*Gene Expr.* **4**, 281-299, 1996) and Vidal-Puig *et al.*, (*J. Clin. Invest.* **99**, 2416-2422, 1997) as having demonstrated PPARγ levels in human testes. However, neither Greene *et al.* nor Vidal-Puig *et al.* investigated the levels of PPAR in testes. Therefore, to suggest that activation of PPARγ is a possible mechanism for testicular effects is not supported by any scientific evidence.

8. Page 77, "General Population Exposure." As is stated in the monograph for DBP, the Centers for Disease Control have recently published data on the urinary levels of various phthalate esters in a selected human population.[4] These data better define the actual exposures to DEHP, which are below the estimated levels cited in the monograph.[5] Acknowledgement of these new data should be indicated.

9. Page 78, "Medical Exposure." The last sentence of the 1st paragraph in this section suggests that exposure may occur from ventilators. This statement contradicts the earlier statement in the monograph on page 13 that clearly states that respiratory tubing used in North America (US and Canada) is made from polyethylene and "contains no DEHP." Therefore, inhalation exposure from medical equipment is not likely in North America.

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4 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982.

5 Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

10. Page 78, "Medical Exposure." The statement about exposure over a year of dialysis assumes a cumulative dose. We believe that this representation is misleading and cannot be used to compare to animal data. *See* comment No. 1, above.

11. Page 84, "Mode of Action." The IARC decision should be described more completely. IARC concluded, "Therefore, the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans." (Emphasis added.) IARC downgraded its DEHP cancer classification from Group 2B (possible human carcinogen) to Group 3 (not classifiable as to human carcinogenicity).[6] Further, it is important to note that while IARC's Group 3 classification is used most commonly for substances "for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals," IARC has determined a substance will be placed in Group 3 despite sufficient evidence of carcinogenicity in experimental animals (as exists with DEHP), only "when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans."[7]

12. Page 84, line 4. The statement that PPARα-knockout mice exposed to DEHP have failed to produce liver tumors is incorrect. To date, no study of the tumorigenic effects of long-term exposure to DEHP has occurred using PPARα-knockout mice.

13. Page 102, Expert Panel Conclusions. We disagree with the level of concern expressed for pregnant women exposed to DEHP. First, the NOAEL value used is not derived from a developmental toxicity study, but from exposure to peripubertal male rats. Based on the data reviewed by the Expert Panel, a NOAEL value of 14-40 mg/kg is most appropriate to describe adverse effects on the developing fetus. In addition, there is a 10-fold difference between the NOAEL and the LOAEL value suggesting that the 14-40 mg/kg dose level is very conservative (as stated in the monograph). Second, the differences in pharmacokinetics between rodents and primates as stated by the Expert Panel are ignored --- a factor that would reduce the level of concern, as indicated in the monograph. Thus, the difference between effects in laboratory animals and exposure levels for humans is a minimum of 1000. Furthermore, the latest exposure information from the CDC study indicates that exposure levels of DEHP are generally lower than the estimated 30 μg/kg/day.[8] For women aged 20-40 years, the 95th percentile exposure value was 3.8 ug/kg/day and the maximum was 10 ug/kg/day.[9] Based on

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6 IARC (2000). "Some Industrial Chemicals (Volume 77) (15-22 February 2000)" , IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, (summary available at http://193.51.164.11/htdocs/accouncements/vol77.htm) (emphasis added).

7 IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans, Preamble (available at http://193.51.164.11/monoeval/preamble.html).

8 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

9 Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence).

this information, the PE Panel believes there should be minimal or negligible concern for development of offspring from pregnant or lactating women exposed to DEHP.

ATTACHMENT 6

COMMENTS ON THE NTP CERHR
EVALUATION OF DI-ISONONYL PHTHALATE (DINP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemistry Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DINP dated October, 2000.[1] We offer the following comments on the draft document.

General Comment

During the DINP discussions the Expert Panel considered that data on male reproductive development were insufficient. Although the published information provided no evidence of such effects, the Panel took note of an abstract which reported an increased incidence in rats of malformations of the male reproductive system. In the absence of published data, the Expert Panel expressed only moderate confidence in the NOAEL for reproductive toxicity and expressed the desire that such studies be conducted along with a better assessment of human exposure. Recently a paper has been published (Gray *et al.*, 2000)[2] which did assess developmental indicators at 750 mg/kg/day. There was a statistically significant increase in areolas at PND 13, and, according to the authors, a small increase in malformations. None of the other parameters measured in the study were affected by treatment. The availability of these data should increase the confidence of the Expert Panel in the selection of NOAELs and should also obviate the need for any further tests of this type. Further, urinary metabolite studies indicate that human exposures are many orders of magnitude below the effect levels in rodent studies (Blount et al., 2000; David, 2000; Kohn *et al.*, 2000).[3] Accordingly, the Phthalate Esters Panel believes that current production and use of DINP pose no risks to human reproduction or development.

Specific Comments

Section 1.2 Exposure and Usage. On page 7, the monograph states that occupational exposures during phthalates production typically are below a level of 1 mg/m^3. The PE Panel used this figure to produce a worst case estimate of occupational exposures during

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1 <http://cerhr.niehs.nih.gov/news/DINP-final-inprog.PDF>

2 Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. *Toxicological Sciences* 58:350-365.

3 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

phthalates production. Data submitted to CERHR by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below that conservative estimate. Thus, wherever this estimate is mentioned in the manuscript (*e.g.*, section 5.3), the Panel believes the monograph should clearly indicate that "actual exposures are expected to be much lower."

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the data submitted to CERHR by Dr. McKee (see previous paragraph) show that exposures to phthalates in downstream facilities typically are very low (at or below the level of detection most of the time). Excursions toward the value cited in the monograph (2 mg/m^3) may occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis.

On page 8, paragraph 2, the monograph states: "Vapor pressure is also extremely low, so measured concentrations in air are not available." There are two studies of concentrations in air. Wechsler (1984) reported di-nonyl phthalate as present at 15 ng/m^3, and Tienpont *et al.* (2000) as < 20 ng/m^3.[4]

Page 8, paragraph 3: It should also be noted that dinonyl phthalate was not detected in a German study (Pfordt and Brunsweller, 1999) (detection limit of 0.01 mg/kg).[5]

Page 10, paragraph 2, line 4: It would be more accurate to say that "...the amount of DINP presented to a child **has not been** well characterized..." rather than that it cannot be characterized.

Page 10, paragraph 3: The statement about potential dermal exposure ["Dermal exposure to DINP from toys may also occur, but has not been studied specifically in children."] seems inconsistent with the first paragraph on page 7, where it is stated that "dermal exposure is not expected to result in significant absorption into the body," as well as the statement in the integrated summary that "...the Expert Panel is confident that dermal exposure would not result in significant absorption into the body." (p. 32.)

Page 10, paragraph 4, exposure estimate: The Expert Panel estimates exposures to DINP as lower than 3-30 ug/kg bw/day. The Centers for Disease Control and Prevention (CDC) have recently reported data which confirm that DINP exposures are very low (median

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4 Tienpont, B., *et al.* (2000). Evaluation of sorptive enrichment for the analysis of phthalates in air samples. J. Microcolumn Separations 12:194-203; Wechsler, C. (1984). Environmental Science and Technology 18:648-651.

5 Pfordt, J., and E. Bruns-Weller (1999). Phthalate esters as a group of environmental chemic als with an endocrine disruption potential. Report on an evaluation of the scientific literature and on measurements of the exposure to phthalate esters via food, textiles and house dust. Lower Saxony Ministry of Food, Agriculture and Forestry, Hannover, Germany. [Note: The PE Panel has provided both the original German and an English translation of this report to CERHR]

value below detection limits, 95th percentile 1.7 ug/kg/day, maximum 22 ug/kg/day).[6] *See also* section 5.1.1.1 on page 23, supporting the Expert Panel view that exposures were likely to be below the range of 3-30 ug/kg bw/day estimated for DEHP.

Section 2.1.2 Experimental Animal Data. Page 15, paragraph 1: The monograph states, "According to Short *et al.* (22), 500 mg/kg bw/day is the maximum dose that can be absorbed by the monkeys." However, as estimated by Rhodes *et al.* (1986),[7] absorption by marmosets is limited to approximately 150-200 mg/kg. Similar data can be derived from the results of a study in the cynomolgus monkey (Astill, 1989).[8] A similar correction should be made to page 31, last paragraph.

Page 15, paragraph 2: The second sentence under "Mode of Action ["However, an increased rate of nephropathy was seen in female mice exposed to 1888 mg/kg bw/day which would not be consistent with the alpha-2-microglobulin mechanism."] is true but misleading. As shown elsewhere (e.g., Ward et al., 1998), the kidney is also a target organ for effects associated with peroxisomal proliferation, so it is not surprising that there should be some renal effects unrelated to alpha-2-microglobulin induction.[9] However, this should not detract from the observations (Caldwell et al., 1998) that alpha 2u-globulin induction does occur in male rats and is the mechanism for male rat kidney tumor induction.[10] As noted by the U.S. EPA (1991),[11] kidney toxicity unrelated to an alpha 2u-G mechanism does not preclude a conclusion that the male rat kidney tumors were the consequence of an alpha 2u-G process; in fact renal toxicity in female rats and/or mice was noted in some of the reference compounds. What is required is a demonstration that an alpha 2u-G process is the most plausible mechanism for the male rat kidney tumors. The evidence that alpha 2u-G is the most plausible explanation for the findings

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6 Blount, B., *et al.* (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

7 Rhodes, C. *et al.* (1986). Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: Extrapolation of effects in rodents to man. *Environmental Health Perspectives* 65:299-308.

8 Astill, B. (1989). Metabolism of DEHP: Effects of prefeeding and dose variation, and comparative studies in rodents and the cynomolgus monkey (CMA studies). *Drug Metabolism Reviews* 21:35-53;

9 Ward, J. *et al.* (1998). Receptor and non-receptor-mediated organ specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicologic Pathology* 26:240-246.

10 Caldwell, D. et al. (1999). Retrospective evaluation of alpha 2u-globulin accumulation in male rat kidneys following high doses of diisononyl phthalate. Toxicological Sciences 51:153-160.

11 U.S. EPA (1991). Alpha 2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. EPA/625/3-91/01F.

is summarized in Caldwell *et al.* (1999) and supplemented by more recent findings (Schoonhoven *et al.*, 2001).[12] *See also* paragraph 2 on page 24 and paragraph 3 on page 31.

Page 15, paragraph 2, last line: The monograph states "Unfortunately, peroxisome proliferation was assayed in mice only at the highest dose, and liver tumors were also observed at lower doses." This statement was true in the context of the Moore (1998) study (ref. 19). However, since that time the effect of DINP dose on peroxisomal proliferation in the mouse has been further investigated. There is now evidence for peroxisomal proliferation at the tumorigenic doses in the mouse as well as the rat. These data were provided to the CPSC in September, 2000, and will be presented at the SOT in 2001 (Kaufman *et al.* 2001).[13] (A copy of the CPSC submission is being included with the copy of these comments submitted by mail in hard copy. See Attachment 6, Annex II). See also paragraphs 2 and 3 on page 24.

Section 2.2 Toxicokinetics. Page 16, first paragraph: The last sentence ["Absorption was decreased at the high single dose and at all doses following repeated exposures."] is not correct. The results of cumulative urinary excretion were:[14] Single low dose (50 mg/kg) = 47.28%. Single high dose (500 mg/kg) = 34.29%. Repeated low dose = 45.90%. Repeated high dose = 54.39%. Thus it would be more correct to say that "Absorption was decreased at the single high dose by comparison to the low dose, but in the repeat dose studies, absorption was approximately 50% at both high and low doses."

Section 2.3 Genetic Toxicity. Page 16, last paragraph: Some additional genetic toxicity data including Salmonella, in vitro cytogenetics assays, and a micronucleus test are now in press (McKee et al., 2000).[15] These data were included in the OECD evaluation and do not constitute additional information.

Section 3.0 Developmental Toxicity. Pages 17-20: The Expert Panel did not take note of comments previously submitted on the nature of the findings in the developmental toxicity studies. As indicated in the Annex to this attachment, the dilated renal pelves and increased cervical ribs are common variants of doubtful toxicological significance. Further, as documented in the attachment, in most cases the incidences of these various effects fell within the historical control range of the testing laboratory.

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12 Schoonhoven, R., E. Bodes, and J. Swenberg (2001). D(isononyl)phthalate binds reversibly to alpha 2u-globulin and induces cell proliferation in male rat kidneys. *The Toxicologist* (in press).

13 Kaufman, W., K. Deckardt, R. McKee J. Butala and R. Bahnemann (2001). Tumor induction in mouse liver – Di-isononyl phthalate (DINP) acts via peroxisome proliferation. *The Toxicologist* (in press).

14 The data are shown in Table 4 of "Single and repeated oral dose pharmacokinetics of 14C labelled di-isononyl phthalate." by M. El-hawari, E. Murrill, M. Stoltz and F. Pallas. Final Report. Contract number 81 MR 1656. MRI project no. 7282-8. December 19, 1983.

15 McKee, R., R. Przygoda, M. Chirdon, G. Engelhardt and M. Stanley (2000). Di(isononyl) phthalate (DINP) and di(isodecyl) phthalate (DIDP) are not mutagenic. *Journal of Applied Toxicology* 20: in press.

Page 19, paragraph 5: The penultimate sentence ["Postnatal sexual maturation was not examined."] is misleading. The potential for developmental delays was not examined, but data were provided which demonstrated that the rats did become sexually mature, were able to mate, and showed no evidence of abnormal sexual development.

Section 4.0 Reproductive Toxicity. Page 21, first paragraph, next to last sentence: The dams and litters were sacrificed on PND **21**, not "1" as listed in the monograph.

Page 22, paragraph 3: A study by Knudsen and Pottinger (1999) is relevant to the mode of action section. Dinonylphthalate did not displace ligand from the estrogen receptor.[16]

Section 5.1.2. General Biological and Toxicological Data. Page 24, paragraph 3: "There were no toxicity studies with inhalation exposure." However, as there is essentially no possibility of exposure by inhalation, why should there be such studies?

Section 5.1.3 Developmental Toxicity. Page 27, paragraph 4: The discussion of the offspring body weight effects in the Waterman (2000) study identify the LOAEL as "0.2% (143-285 mg/kg bw/day during gestation through lactation)...." It is not clear why maternal doses, particularly those during gestation, were considered relevant to this endpoint. Data in Waterman (2000) and summarized in the CERHR review demonstrate that offspring body weights were not dramatically affected at birth or early in the lactational period but rather became progressively more pronounced as the offspring aged and began to transition to solid food. The interpretation most consistent with the data is that the body weight effects were due to relatively high phthalate doses as a consequence of ingestion of solid food by offspring at the end of the lactational period. These differences then disappeared over time as the offspring grew larger and the doses (as mg/kg) were reduced as shown by the F1 body weight data in Waterman. Additionally, there was direct evidence from switch dosing and cross fostering experiments with DIDP (reviewed in the last two paragraphs on section 3.2 of the DIDP monograph) that the effects on weight were associated with exposures during the lactational period and not with prior exposure to phthalate. Thus, there is no apparent reason why maternal doses during the gestational period should be considered as relevant in the determination of the LOAEL. Further, it is also important to note that the animals recovered from the body weight effects despite continued exposure at the same dietary levels. Thus, the effects on offspring body weight were transient and without any apparent postnatal consequences.

Comments Based on Recently Published Data

The CERHR Expert Panel Review of DINP referred to data from Gray's laboratory, available only in abstract form during the deliberations (Ostby *et al.*, 2000).[17] Although the conclusions from the abstract were cited in several places (*e.g.*, last paragraphs of

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[16] Knudsen, F. and T. Pottinger (1999). Interaction of endocrine disrupting chemicals, singly and in combination, with estrogen-, androgen-, and corticosteroid-binding sites in rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicology* 44:159-170.

[17] Ostby, J. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP, DINP but not DEP, DMP or DOTP permanently alters androgen-dependent tissue development in Sprague-Dawley rats. Triangle Consortium on Reproductive Biology, January 29, 2000.

sections 3.2 and 4.2) as evidence that DINP has an effect on male reproductive development, the absence of such data in the published literature concerned the Expert Panel, diminishing their confidence in their overall confidence in NOAELs, and resulting in a recommendation for additional studies listed in the critical data needs section. As the data from Gray's laboratory have now been published (Gray *et al.*, 2000),[18] the Expert Panel should fully evaluate those data and incorporate them in the monograph as suggested below.

As reported by Gray, female Sprague-Dawley (SD) rats were given DINP (CAS # listed as 68515-48-0) by oral gavage from GD14 to PND 3 at a single treatment level, 750 mg/kg/day. The offspring were examined at various times until terminal sacrifice at times ranging from 3-7 months of age. The parameters which were examined included:

(a) Body weight and anogenital distance on PND 2 – These parameters were unaffected by DINP treatment.

(b) Testicular examination on PND 3 – Testes weights of DINP-treated male offspring were similar to control.

(c) Inguinal examination of male pups – It was reported that one DINP-treated male offspring had "suspected" "hemorrhagic testes", but this was not confirmed by histologic examination.

(d) Examination for areolas on day 13 – The incidence of areolas (22%) was reported as significantly different from control at $p < 0.01$.

(e) Examination of onset of puberty (preputial separation) – Not affected by treatment.

(f) Determination of serum testosterone levels at terminal sacrifice – Not affected by treatment.

(g) Examination for retained nipples, cleft phallus, vaginal pouch and hypospadias – Of 52 male offspring examined, 2 had retained nipples; none had cleft phallus, vaginal pouch or hypospadia.

(h) Internal examination for undescended testes, atrophic testes, epididymal agenesis, prostatic and vesicular agenesis, and abnormalities of the gubernacular cord – One of the male offspring was reported to have had bilateral testicular atrophy and another exhibited epididymal agenesis with hypospermia and fluid filled testes. None of the 52 male offspring examined had undescended testes, prostatic and vesicular agenesis or abnormalities of the gubernacular cord.

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[18] Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. Toxicological Sciences 58:350-365.

(i) Body weights and weights of organs including ventral prostate, levator ani plus bulbocaernosus muscles, seminal vesicles, and epdidymides – Weights of all organs, including all of the reproductive organs were similar to controls.

(j) Sperm counts – It was not clear from the report whether or not sperm counts of DINP-treated animals were examined. The paper was silent on the results of sperm analysis for all substances except for BBP and DEHP for which sperm counts were reported to be reduced, but the data were not provided.

The abstract which was cited by the CERHR (Ostby *et al.*, 2000) contains a statement that "males in the ... DINP (7.7%, $p < 0.04$) treatment group displayed malformations of the testis, epididymis, accessory reproductive organs and external genitalia." As now reported in the full publication, 4 (of 52) treated male offspring were considered by the authors to have been malformed. These included 2 with retained nipples, one with "small" testes, and one with testicular atrophy. The statistical analysis compared the total incidence of offspring considered malformed against the controls rather than making comparisons for each anomaly. The statistical evaluation indicated $p < 0.05$ when the data were compared on an individual basis and $p < 0.06$ for a litter-based comparison. No data on historical control incidences were provided. Given the low incidence of anomalies, it is difficult to determine whether these are spontaneous or treatment related. Further, the validity of pooling all affected individuals for statistical analysis seems questionable. Certainly, the effects evaluated individually would not be significantly different from control. We believe that these results are marginal and do not form a basis for strong conclusions of the effect of DINP on male reproductive development.

More important is the question of whether this publication provides any information on reproductive toxicity beyond that provided by the two generation reproduction study previously reported by Waterman et al. (2000). Gray's study utilized oral gavage in contrast to dietary administration in Waterman and at a somewhat higher dose level (in Waterman the estimated maternal dose on GD 14-21 was 543 mg/kg and that on PND 0-4 was 672 as compared to 750 mg/kg in Gray). Nevertheless, Gray confirmed one of the most important findings of Waterman, i.e., that DINP treatment during the period of male reproductive development has no effect on male reproductive organs. More specifically, Gray found no effects on weights of testes or accessory reproductive organs, and identified only 2 rats (of 52) with what he considered to be malformed testes. Waterman also found weights of testes and accessory organs to be unaffected. In addition, Waterman found that within the parental generation, one male, from the control group, had unilateral focal testicular atrophy. In the F1 generation there were two males with diffuse unilateral atrophy and testicular degeneration; one from the control group and one from the high dose group. As similar effects were found at the same incidence in the treated and control groups, these findings were judged by Waterman to be incidental.

The one clear difference between these two studies is that Gray found an increase in areolas in 13-day old male pups. However, the toxicological significance of this effect is questionable since it appeared to be substantially reversible. Among the 13 day old male offspring, 22% had areolas; at terminal sacrifice, 2 (of 52) or 4% of the males had retained nipples. Although the frequency of aerolas was increased, the demonstration that DINP had no effects on fertility, and minimal effects on male reproductive development should provide the

Expert Panel with the information that these minor effects have no bearing on human reproductive risk. That males with areolas can reproduce was shown by Schilling (1999)[19] in a study of the potential reproductive effects of DEHP.

The above having been said, these data seem more relevant to the overall assessment of developmental toxicity than reproduction. There was a significant increase in frequency of areolas at 750 mg/kg, but this appeared to have been substantially reversed by terminal sacrifice. Although no NOAEL was defined, the level associated with this effect was higher than other developmental effects considered by the Expert Panel, and, therefore, should not influence the overall evaluation of developmental toxicity. The reproductive NOAEL had previously been defined by the absence of effects on fertility and/or reproductive organs as reported by Waterman. Gray provided no new data on fertility and confirmed the absence of effects on reproductive organ weights. Although Gray reported a low incidence of testicular effects, the marginal nature of those findings along with the absence of effects in Waterman indicate that these data should not be used for NOAEL determination. That, in effect, would leave in place the existing LOAELs and NOAELs, but should increase the Expert Panel confidence. With more confidence in both the toxicity and exposure information, it would be more appropriate to change the concern level to negligible.

Section 5.4 Critical Data Needs. With respect to critical data needs, the Expert Panel noted that nipple retention data were lacking and expressed the view that uncertainties would be reduced if this additional information was gathered. As described above, the data are now available and should substantially satisfy the request for additional studies.

(a) The Expert Panel requested a study to address landmarks of sexual maturation such as nipple retention, anogenital distance, age at testes descent, age at prepuce separation, and structure of the developing reproductive system in pubertal or adult animals. As indicated above, following oral administration at 750 mg/kg/day during the period considered critical for male reproductive organ development, areola frequency was significantly increased at PND 13, but by terminal sacrifice only 2 of 52 males had retained nipples. The other parameters were unaffected. These data, along with the previously published data showing that dietary DINP treatment has no effects on fertility or male reproductive structure provide the necessary information to satisfy this request.

(b) The Expert Panel went on to say that if "the effective doses are of possible human health concern," additional studies would be required. The Expert Panel may now wish to consider the potential relevance of the findings to human health, but other recently published data directly address the issue of human exposure. A study of phthalate metabolites in urine was recently published (Blount *et al.*, 2000).[20] Exposure estimates based on these data indicate a 95th percentile value in the

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[19] Schilling, K. *et al.* (1999). Reproduction toxicity of di-2-ethylhexyl phthalate. *The Toxicologist* 48:147-148.

[20] Blount, B., *et al.* (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982.

range of 1-2 ug/kg/day (David, 2000;[21] Kohn *et al.*, 2000).[22] There is such a wide margin between the doses used in the animal studies and the human exposure levels, that there simply cannot be any public health concern attached to the results.

(c) Note also that the CDC data satisfy the Expert Panel request for exposure information. There may still be some questions relating to exposures in very specific situations, as noted in the CERHR report, but any uncertainty about exposures of the general population should now be put to rest.

In summary, it would be reasonable to conclude that the questions raised by the Expert Panel have been substantially addressed and that further studies of DINP in experimental animals are unnecessary.

Typographical Errors

Page 8, pp 6 – Note symbol between 8.2 and 9.83 ug/11 cm...
page 13, pp 1 – The text should read...among control and **treated** groups (55-59/sex/**group**
page 13, pp 3 – remove the "," after "standard".
page 14, pp 2 – "carinoma"
page 21, pp 1 – Dams were allowed to litter and raise young until pnd **21** , at which time...
page 31, pp 3 - ...in adult rats and mice but not in marmosets **or cynomolgus monkeys**.

Appendix III

[21] David, R. (2000). Exposure to phthalate esters. Environmental Health Perspectives.

[22] Kohn, M. *et al.* (2000). Human exposure estimates for phthalates. Environmental Health Perspectives.

ANNEX I to Attachment 6
Interpretation of Developmental Toxicity Data for DINP

Introduction A-1
I. The variants observed in DINP studies may have little biological significance A-3
A. Biological significance of dilated renal pelves A-3
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Introduction

For its evaluation of the developmental toxicity data for DINP, the CERHR Expert Panel reviewed the rat studies by Hellwig *et al.* (1997) and Waterman *et al.* (1999). The conclusions of the Expert Panel regarding the effect levels in these studies differed from those of the authors. Therefore, the Phthalate Esters Panel (PE Panel) has gathered historical control information and has researched the literature on the biological significance of effects seen at lower doses. The data show that dilated renal pelves and cervical rib variants are unlikely to be toxicologically important and were found at levels consistent with historical control experience.

Table 1. Summary of the Incidence of Developmental Variations in the Developmental Toxicity study by Waterman *et al.* (1999)

Parameter	Control	100 mg/kg	500 mg/kg	1000 mg/kg	Historical Control
% Litters with visceral variations	4.2	12.0	16.7	30.4*	0-72%, average = 25%
% Litters with dilated renal pelves	0.0	12.0	16.7	26.1**	4-38%, average = 24%
% Litters with skeletal variants	62.5	64.0	91.7*	87	36-100%, average = 76%
% Litters with rudimentary lumbar ribs	25.0	20.2	54.2	78.3**	13-81%, average = 37%
% Litters with supernumerary cervical ribs	12.5	12.0	8.3	30.4	4-17%, average = 5%

* Significant at $p < 0.05$

** Significant at $p < 0.01$

In reviewing the historical control data and the literature, the PE Panel has identified several issues which are relevant to an evaluation of the developmental toxicity data.

Section II reviews the literature on the biological significance of the developmental variants observed in these studies. This reveals that supernumerary lumbar ribs and dilated renal pelves are considered normal developmental variants and generally occur at high frequency in control populations.[23] Section I provides historical control information for the laboratories used by Hellwig and Waterman. Comparison of this data to the Waterman fetal data shows that the observed levels of developmental effects are within historical control ranges and that the apparent statistical significance of dilated renal pelves and other lesions apparently is a chance result of an unusually low incidence in the concurrent control group. The PE Panel believes that, when taken together, these considerations indicate that it may be inappropriate to consider doses below 1000 mg/kg/day as associated with toxicologically significant findings.

Table 2. Measurements of malformation, fetal survival and fetal weight in the DINP Developmental Toxicity Study by Waterman *et al.* (1999)

Parameter	Control	100 mg/kg	500 mg/kg	1000 mg/kg
Mean Viable Fetuses/Dam	16.04	15.04	16.33	15.26
Mean Fetal Body Weight – Males	5.38	5.58*	5.5	5.59*
Mean Fetal Body Weight – Females	5.12	5.39**	5.23	5.29
Mean Number of Fetuses with Malformations	0.33	0.04	0.13	0.13

* Significant at $p < 0.05$

** Significant at $p < 0.01$

Appendix III

[23] Although the Waterman study revealed an increase in cervical ribs which, in fact, may be biologically significant, this effect was found only in the high dose group.

I. The variants observed in DINP studies may have little biological significance

In assessing development toxicity, statistical significance is ultimately less important than biological significance.[24] Factors considered important to biological significance include: the types and patterns of effects, the toxicological relevance of the findings, and the historical control information (EPA, 1991, p. 63805).

Review of the literature indicates that the various fetal alterations reported by Waterman and Hellwig are normal variants which are found in most developmental toxicity studies, are considered to be a consequence of maternal toxicity, are often reversible, and have no long term consequences. Moreover, as noted above, fetal mortality was not increased, there was no increase in malformations, and no evidence of fetal toxicity. In fact, the frequency of malformations was below control values at all treatment levels and fetal weights were above control values. (*See* Table 2).

On a percentage-fetuses basis, the Waterman study showed a statistically significant increase at 500 mg/kg/day of visceral variations, dilated renal pelves, skeletal variations, and rudimentary lumbar ribs. However, the increase in visceral variations is almost entirely due to the increase in dilated renal pelves, and the increase in skeletal variations is due to the increase in rudimentary ribs. For the reasons discussed below, the biological significance of the dilated renal pelves and the rudimentary ribs is questionable. Consideration of this information, in conjunction with the historical control data and the lack of serious fetal effects, suggests that the developmental effects observed in the Waterman and Hellwig studies at doses below 1000 mg/kg/day are of little biological significance.

A. Biological Significance of Dilated Renal Pelves

The biological significance of hydronephrosis and dilated renal pelves was questioned by Khera (1981) who drew attention to two points: 1) that there is a wide physiological variation in size of the renal pelvis, and 2) that there is no clear division between physiological and pathological variations. It was further pointed out by Woo and Hoar (1972) that an apparently enlarged renal pelvis can be created during normal development as a consequence of different rates of development of the renal papilla and renal parenchema. This is a transient condition which normally disappears quickly after birth. They concluded that diagnosis of this condition as a pathological lesion could only be determined postnatally.

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[24] As noted in EPA's guidance, undue reliance on statistical data can cause problems in two ways: (1) such reliance may increase the possibility of overlooking serious findings which occur at low frequency and (2) there are situations where statistical significance can be achieved by chance. since either outcome is potentially misleading, the EPA guidelines indicate that evaluations of developmental studies must take biological significance into account. (EPA, 1991, p. 63809). Similarly, the article which is the basis for establishing the CERHR process states that "[a]lthough the evaluative process strongly endorses the use of appropriate and rigorous statistical methods, it must be clear that, when the study meets conventional statistical criteria, it must also yield data that reflect an effect that is both biologically plausible and considered adverse." (Moore *et al.*, 1995, p. 74).

For DINP, the results of the Waterman and Hellwig studies clearly suggest that the incidence of dilated renal pelves was not biologically significant. (*See* Table 3.) The Hellwig studies of DINP found that the incidence of dilated renal pelves was above control values at the highest level but did not reach statistical significance for any of the types of DINP tested. Waterman did not discuss the dilated renal pelves data in detail, because the study indicated a low incidence, a minor effect, and a lack of biological plausibility. In any event, the apparent treatment-related response observed in Waterman appears to be purely a consequence of statistical chance, as indicated by historical control data. The Waterman study represents the only time that a concurrent control incidence for dilated renal pelves was zero. The historical average was approximately 5.5%, which exceeds the highest value found in the DINP study at any treatment dose. (*See* Tables 3 and 7.) Considering this, it is reasonable to conclude that the results for this endpoint represent variations around the historical mean, and not treatment-related effects. Thus, it is the PE Panel's belief that any apparent statistically significant increase in the incidence of dilated renal pelves is likely the result of unusually low concurrent control levels and is not biologically significant.

Table 3. Data on Dilated Renal Pelves (% Fetuses Affected)

Waterman Data					
	Control	**100 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**Historical Control Data**
	0.0	3.7**	4.0**	5.1**	0-12.6%, average = 5.5
Hellwig Data [1]					
	Control	**40 mg/kg**	**200 mg/kg**	**1000 mg/kg**	**Historical Control Data**
DINP 1	9	9	7	17	0-54%, average = 20%
DINP 2	9	9	16	11	
DINP 3	9	11	10	17	

** significant at $p<0.01$

1 Source: Tables 10, 12, and 14 in Hellwig et al. (1997). The tabulated data give number of fetuses affected. They were converted to percentages to be consistent with the Waterman paper.

B. Biological Significance of Variant Lumbar (14th) and Cervical Ribs

The biological relevance of variant ribs has been considered questionable for many years. Variant ribs in the lumbar region are a common finding, most likely the consequence of maternal stress, and not considered to be biologically significant. This was first addressed by Kimmel and Wilson (1973) who noted that supernumerary 14th ribs were common variants which occurred quite frequently in untreated controls. They concluded that these could be indicators of effects at higher doses but should not be regarded as abnormalities when they were the only signs of embryotoxicity. They also concluded that the biological relevance of these variants could be best interpreted in the context of relevant historical control data.

A similar cautionary note was echoed by Khera (1981), who subsequently reviewed the available information and concluded that rib variants in rats were the consequence

of maternal toxicity (Khera, 1985). Khera's hypothesis was tested by Kavlock and co-workers who found that for a variety of unrelated substances, maternal weight gain during gestation was related to the incidence of rib variants in mice. They concluded that this was the consequence of nonspecific maternal toxicity (Kavlock *et al.*, 1985) or maternal stress (Chernoff *et al.*, 1987). Wickramaratne (1988) showed that supernumerary ribs were reversible and without discernable postnatal consequences, and this was confirmed by Chernoff *et al.* (1991). Schwetz *et al.* (1971) found that the increased lumbar ribs had no long-term effect on fetal or neonatal survival or development. Although the biological significance of supernumerary ribs may not be considered fully resolved by all authors (Chernoff *et al.*, 1991), it is remarkable that nearly 30 years of study has failed to provide any evidence that they are anything other than incidental findings.

Table 4 - Data on Variant Lumbar and Cervical Ribs
(% Fetuses Affected)

Waterman Data					
	Control	**100 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**Historical Control Data**
Rudimentary Lumbar Ribs	3.7	5.4	18.6**	34.5**	3.4-28%, average = 10%
Supernumerary Cervical Ribs	1.6	1.6	1.0	5.7*	0.6-4.0%, average = 1%
Hellwig Data[1]					
	Control	**40 mg/kg**	**200 mg/kg**	**1000 mg/kg**	**Historical Control Data**
Accessory 14th Ribs					
DINP 1	0	0	2	28	0-4.1%, average = 1.2%
DINP 2	0	1	3	7	
DINP 3	0	0	7	28	
Rudimentary Cervical Ribs					
DINP 1	0	2	1	8	0-6.5, average = 3%
DINP 2	0	0	1	3	
DINP 3	0	0	1	10	

* significant at $p<0.05$, ** significant at $p<0.01$

1 Source: Tables 10, 12, and 14 in Hellwig et al. (1997). The tabulated data give number of fetuses affected. They were converted to percentages to be consistent with the Waterman paper.

Variant ribs in the cervical region are not as common in control rat fetuses as variant lumbar ribs (MARTA, 1993), although they are relatively common in control groups in the Exxon Biomedical Sciences Laboratory at which the Waterman study was conducted (Table

7). The development of variant cervical ribs is of unknown biological significance as no studies have examined their potential for postnatal consequences and/or reversibility.

For DINP, the Hellwig study found an increase in variant cervical rib frequency at only the highest dose. Similarly, Waterman found no increase in the incidence of variant cervical ribs at either 100 or 500 mg/kg/day, but noted that the incidence of supernumerary cervical ribs was above the historical control range at the 1000 mg/kg/day level. Although this elevated incidence at the highest dose level was not significantly different from control when expressed on a litter basis, these findings were discussed in considerable detail in the Waterman study and weighed heavily in the authors' decision to characterize the 1000 mg/kg/day dose as being associated with adverse developmental effects. (*See* Table 4).

C. Biological Significance of Total Visceral and Skeletal Variants

Review of the data shows that the fetal-based increases in total visceral and skeletal variants were almost entirely a consequence of the increased incidence of dilated renal pelves and variant ribs discussed above. (*See* Tables 4). Thus, the significance of the increased visceral and skeletal variations is no greater than the significance of those underlying lesions. Once this is taken into account, the data as a whole suggest that no biologically significant effects are occurring at doses of less that 1000 mg/kg/day.

Table 5. Visceral Variants in the Waterman *et al.* Study

Type of Variant	**Control**	**100 mg/kg**	**500 mg/kg**	**1000 mg/kg**
number of fetuses affected (number of litters affected):				
Dilated renal pelves	0 (0)	7 (3)	8 (4)	8 (6)
Distended ureter	0 (0)	1 (1)	3 (3)	1 (1)
Dilated Ventricles (head)	1 (1)	1(1)	0(0)	0(0)
% fetuses affected/% litters affected:				
Dilated Renal Pelves	0.0/0.0	3.7/12.0	4.0/16.7	5.1/26.1
Total Visceral Variants	0.5/4.2	3.7/12.0	4.0/16.7	5.1/30.4

Appendix III

Table 6. Skeletal Variants in the Waterman *et al.* Study

Type of Variant	Control	100 mg/kg	500 mg/kg	1000 mg/kg
number of fetuses affected (number of litters affected):				
Rudimentary Lumbar Ribs	7 (6)	10 (5)	36 (13)	60 (18)
Supernumerary Cervical Ribs	3 (3)	3 (3)	2 (2)	10 (7)
% fetuses affected/% litters affected				
Rudimentary Lumbar Ribs	3.7/25.0	5.4/20.2	18.6/54.2	34.5/78.3
Supernumerary Cervical Ribs	1.6/12.5	1.6/12.0	1.0/8.3	5.7/30.4
Total Skeletal Variants	16.8/62.5	15.0/64.0	28.4/91.7	43.7/87.0

II. The study results should be interpreted in light of historical control information

Historical control data provides further perspective on the biological significance of Waterman and Hellwig developmental toxicity study results for DINP. The historical control data for the Exxon Biomedical Sciences, Inc. laboratory used by Waterman and the BASF Laboratory used by Hellwig are given in Table 7. Comparison of these data to the results shown in Tables 1-6 indicates that the effects seen at doses below 1000 mg/k/day are within historical control ranges and therefore may not be treatment-related. As discussed above, Waterman reported fetal-based elevations for five parameters: total visceral variations, dilated renal pelves, total skeletal variations, rudimentary lumbar ribs, and supernumerary cervical ribs. The following discusses these endpoints from both a litter-based and fetal-based standpoint in the context of historical controls.

Table 7. Historical Control Data for Developmental Toxicity Studies

at Exxon and BASF

Exxon Data

% total visceral variations	per fetus, range = 0 - 29% average = 7% per litter, range = 0 - 72%, average = 25%
% dilated renal pelves	per fetus, range = 0.6 - 12.6%, average = 5.5% per litter, range = 4.2 - 37.5%, average = 24%
% skeletal variations	per fetus, range = 9-58%, average = 13% per litter, range = 36 - 100%, average = 76%
% rudimentary lumbar ribs	per fetus, range = 3.4 - 28%, average = 10% per litter, range = 13 - 81%, average = 37%
% supernumerary cervical ribs	per fetus, range = 0.6 - 4%, average = 0.9% per litter, range = 4 - 17%, average = 5%

BASF Data

% dilated renal pelves	per fetus, range = 0 - 54%, average = 20% per litter, range = 0 - 100%, average = 61%
% hydroureter	per fetus, range = 0 - 18%, average = 5.2% per litter, range = 0 - 64%, average = 23%
% accessory 14th ribs	per fetus, range = 0 - 4.1%, average = 4.2 per litter, range = 0 - 16 %, average = 7%
% rudimentary cervical ribs	per fetus, range = 0 - 6.5%, average = 3.0% per litter, range = 0 - 33%, average = 17%

A. Litter Based Data

Considering the Waterman data on a litter basis (Table 1) reveals that, for doses under 1000 mg/kg/day, all five parameters (1) are not significantly elevated from the concurrent controls and/or (2) are within historical control ranges. For total visceral variations, dilated renal pelves and rudimentary lumbar ribs, statistically significant differences were found at 1000 mg/kg/day but not at lower levels. Total skeletal variations were significantly different from concurrent controls at 500 mg/kg/day, but were within the historical control range.[25] Incidence of supernumerary cervical ribs was elevated at 1000 mg/kg/day by comparison to concurrent controls, but was not significantly different.

Appendix III

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[25] There was not a significant increase for this parameter at 1000 mg/kg/day. This absence of a dose-response relationship contributed to the conclusion that the skeletal variations were not biologically important.

The only findings of effects occurring above the historical control range were for rudimentary lumbar ribs and supernumerary cervical ribs at the 1000 mg/kg/day level. The remaining effects levels were within the historical control range and even the highest values were not greatly different from the historical averages. A reasonable interpretation of the litter data is that the increases in rudimentary lumbar and cervical ribs at 1000 mg/kg/day were treatment related, but that the other differences were not.

B. Fetal Based Data

Considering the Waterman data on a fetal basis reveals that, for doses under 1000 mg/kg/day, all five parameters are well within historical control ranges. (*See* Table 8.) Although four of the parameters were above concurrent controls, it is critical to note that, at the time the Waterman study was conducted, the concurrent control incidences reported for visceral variations, dilated renal pelves, skeletal variations, and rudimentary lumbar ribs were lower than any previously observed control values. In fact, as indicated above, the DINP study was the first in which the concurrent control incidence of dilated renal pelves was zero. In the treated animals, the frequencies of visceral variations, dilated renal pelves and total skeletal variations reported were all well within the historical control range. Thus, the appearance of statistically significant increases for these developmental effects is most likely a consequence of the exceptionally low control values, rather than an indication of actual treatment-related effects.

Table 8. Variants in the Waterman *et al.* Study at Doses Below 1000 mg/kg/day (% fetuses affected)

	Control	100 mg/kg	500 mg/kg	Historical Control Data
Dilated renal pelves	0.0	3.7**	4.0**	0-12.6, average = 5.5
Total visceral variants	0.5	3.7*	4.0*	0-29, average = 7
Rudimentary Lumbar Ribs	3.7	5.4	18.6**	3.4-28, average = 10
Supernumerary Cervical Ribs	1.6	1.6	1.0	0.6-4.0, average = 1
Total skeletal variants	16.8	15.0	28.4**	9-58, 13

* significant at $p<0.05$, ** significant at $p<0.01$

At the 1000 mg/kg/day dose, the variant lumbar and cervical rib data were significantly different from the concurrent control and also were above the historical control range. The PE Panel views this as consistent with and supportive of the conclusion that 1000 mg/kg/day is a LOAEL and that the lower levels -- 200 mg/kg/day (Hellwig) and 500 mg/kg/day (Waterman) -- are NOAELs.

III. Conclusion

The PE Panel believes that the conclusion most consistent with the data is that repeat exposure to DINP at 1000 mg/kg is associated with an increase in the incidence of mild developmental effects, but that there are no biologically important findings at lower levels.

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ATTACHMENT 7

COMMENTS ON THE NTP CERHR
EVALUATION OF DI-ISODECYL PHTHALATE (DIDP)

Submitted by the
American Chemistry Council Phthalate Esters Panel
December 11, 2000

This document provides comments of the American Chemical Council Phthalate Esters Panel (PE Panel) on the NTP CERHR Expert Panel evaluation of DIDP dated October, 2000.[1] We offer the following comments on the document.

General Comment

The CERHR Expert Panel concludes that it has "minimal concern about DIDP resulting in reproductive toxicity to humans." (p. 27) The Panel believes the data support an even stronger conclusion – there is essentially no risk or negligible risk from current estimated exposures. *See* comments on Section 5.3, below.

Specific Comments

Section 1.2 Exposure and Usage. On page 6, the monograph states that exposure may occur "through food as a result of uptake by food animals, certain vegetables, and migration of DIDP from food packaging." The very next paragraph documents that exposure from food is negligible; DIDP was not detected at all in recent studies of fatty foods and infant formula. The issue of uptake by food animals and vegetables is addressed in comments on several of the other monographs. We are aware of no evidence to support this concern for DIDP or any other phthalate, and we believe the idea is too remote to mention in the monograph, given the low releases of DIDP and other phthalates to the environment. Data for DEHP and DBP, summarized in the comments on the DBP monograph, provide strong evidence that uptake by crops in fact is not significant.

On page 6, the monograph states that occupational exposures during phthalates production typically are below a level of 1 mg/m^3. The PE Panel used this figure to produce a worst case estimate of occupational exposures during phthalates production. Data submitted by Dr. Richard H. McKee on September 12, 2000, pertaining to DEHP, DINP and DIDP, clearly show that actual occupational exposures during phthalate production typically are far below that conservative estimate. Thus, wherever this estimate is mentioned in the manuscript (*e.g.*, section 5.3), the Panel believes the monograph should clearly indicate that "actual exposures are expected to be much lower."

Any discussion of potential occupational exposures during downstream use of phthalates also should be accompanied by similar qualifying statements, as the data submitted by Dr. McKee (see previous paragraph) show that exposures to phthalates in downstream facilities

[1] <http://cerhr.niehs.nih.gov/news/DIDP-final-inprog.PDF>

typically are very low (at or below the level of detection most of the time). Excursions toward the value cited in the monograph (2 mg/m^3) are expected to occur only infrequently in connection with specific tasks, such as some maintenance functions. No workers are expected to be exposed to that level on a continuous or regular basis.

In the concluding paragraph of the exposure section, the monograph states that exposures to DIDP are estimated as lower than 3-30 ug/kg bw/day, the same exposure estimate as for DINP. The Centers for Disease Control and Prevention have recently reported data which indicate that DINP exposures are very low (median value below detection limits, 95th percentile 1.7 ug/kg/day, maximum 22 ug/kg/day).[2] Although not reported, data were also collected for DIDP which indicate even lower exposures than those for DINP.[3]

The monograph also states, "it is reasonable to postulate exposures several-fold higher than the general population in infants and toddlers who mouth DIDP-containing products." However, DIDP has not been found in toys in a US survey or in other products intended for young children. Thus, while it is possible that children might mouth objects containing DIDP, as these are not intended for mouthing, any exposures of young children to DIDP are likely to be episodic and of short duration. Therefore, it is questionable whether this is a reasonable postulate. Any dose to children resulting from mouthing of DIDP objects is likely to be exceedingly small. This questionable postulate appears again on page 18 (section 5.1.1.1) and page 26 (Section 5.3).

Section 2.2 Toxicokinetics – Biotransformation. It should be noted that there was no bacterial degradation of DIDP **under anaerobic conditions**. DIDP does undergo bacterial degradation under aerobic conditions as documented by Staples *et al.* (1997).[4]

Section 2.3 – Genetic Toxicity. (Page 12, paragraph 1). The reference to the micronucleus test (27), a laboratory report, can be changed to a publication: R. McKee, R. Przygoda, M. Chirdon, G. Engelhardt and M. Stanley (2000). Di(isononyl) phthalate (DINP) and di(isodecyl) phthalate (DIDP) are not mutagenic. *Journal of Applied Toxicology* 20: in press.

Section 3.2 Developmental Toxicity – Experimental Animal Toxicity. (Page 14, paragraph 3) In the statement "Age at which. . . offspring," the unit is wrong. There were 2 rats/sex/**litter** (or approximately 50/dose group) rather than 2/sex/dose group as stated in text.

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2 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

3 J. Brock, CDC, Personal communication to R. McKee, ExxonMobil Biomedical Sciences (Dec. 1, 2000).

4 Staples, C. et al. (1997). The environmental fate of phthalate esters: A literature review. *Chemosphere* 35:667-749.

At the end of the paragraph, it is stated that "A developmental NOAEL of 0.06% (38-44 and 52-114 mg/kg bw/day during pregnancy and lactation, respectively) was identified by the study authors." This is misleading. The study authors did identify 0.06% as the NOAEL but then converted that to a dose of approximately 50 mg/kg/day on the basis that that was the dose to the dams at the time the effect occurred. Had there been an effect during development, there should have been an effect on live birth index, but that was unaffected. As there were no effects on offspring survival after PND 4, exposure after that time was not relevant (see also pages 22 and 26). Thus, the dose estimate of 50 mg/kg/day which corresponds to the maternal dose during the first 4 days of lactation is the most relevant to this endpoint.

(Page 22 pp 1) The next to last sentence should either be "Hormonally mediated **effects** such as . . ." or Hormonally mediated endpoints. . . were not **affected** at doses. . . "

Section 5.3 Exert Panel Conclusions. We disagree with the overall conclusion that there is even "minimal" risk to human reproduction from exposure to DIDP. Instead, we feel that the risk is negligible based on the difference between estimated exposure and NOAEL values from laboratory animals, which is on the order of 10,000-100,000. As indicated above, data collected by the CDC confirm that exposures are very low – even less than estimated by the Expert Panel, supporting the conclusion that risk is negligible. The conclusion of minimal, rather than negligible, concern may reflect the Expert Panel's uncertainty about exposure from toys or occupations; however, as discussed above, those exposures are expected to be minimal.

Section 5.4 – Critical Data Needs. (Page 27). The CDC study apparently covered DIDP, although results have not yet been published. Thus, some of the recommendations for additional exposure information may already have been addressed.

COURTNEY M. PRICE
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April 11, 2001

American Chemistry Council *Good Chemistry Makes It Possible*

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Subject: Supplemental Comments on the CERHR Expert Panel review of DINP

Dear Drs. Shelby and Moore:

In December 2000, the American Chemistry Council Phthalate Esters Panel (PE Panel) provided comments on the evaluations of seven phthalate esters made available by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP CERHR) on its website in October 2000. Among these comments, the PE Panel brought to your attention two publications (Gray et al., 2000; Blount et al., 2000) relating to male reproductive development and exposure to DINP, respectively. As these two issues had been identified by the Expert Panel as critical data needs for DINP, we believed that the papers would be of particular interest to the CERHR. We also expressed the view that, as the data contained within these papers substantially addressed the concerns raised by the Expert Panel, no further testing of DINP was warranted, and that the critical data needs section of that monograph should be modified.

More recently, the groups represented by the Gray and Blount papers have provided additional data which, in our view, further substantiates our request for modifications to the critical data needs section. Accordingly, we have prepared some supplemental comments which, we hope, will be taken into consideration as the NTP CERHR develops its summary report on DINP.

The paper by Blount et al. (2000) reported results of urinary levels of phthalate metabolites, and, in particular found that the levels of DINP metabolites were very low. In two accompanying letters to the editor (David, 2000; Kohn et al., 2000), the urinary metabolite levels were used to estimate external exposures. Both letters estimated that the 95th percentile exposures to DINP would be less than 2 ug/kg/day. This confirmed the CERHR estimate that exposures to DINP would be less than the 3-30 ug/kg/day estimate for DEHP exposure, and demonstrated that the exposures of the general population to DINP are very low. The data published by Blount et al. (2000) have been further substantiated by the CDC in its publication

of urinary metabolite data from more than 1000 individuals in its National Report on Human Exposure to Environmental Chemicals (CDC, 2001). Although the CDC report did not list a 95th percentile value, the urinary metabolite level at the 90th percentile (4.3 ug/l) is equivalent to an external exposure of 0.6 to 1.0 ug/kg/day for the general population.[1] Thus there is now solid documentation that exposures of the general population to DINP are very low.

Along the same lines, we had previously brought to your attention data on phthalate absorption in humans previously only available in abstract form (Anderson et al., 2000). These data, which demonstrate that absorption of phthalate monoesters by humans is well below that in rodents even at relatively low exposure levels, are now being published and provide additional evidence that internal levels of phthalates in humans are very low (Anderson et al., 2001). For example, Anderson et al. state: "For dioctylphthalate (sum of the 2-ethylhexyl and isooctyl species) the yield was 14 and 12% of the low and high dose excreted as monooctylphthalate." In contrast, in rodents urinary excretion would be approximately 50% (Rhodes et al., 1986; Astill et al., 1989). Thus, even at exposure levels which are low, approximating those encountered by the general population, the amount of phthalate absorbed by humans is much less than that absorbed by rodents.

The paper by Gray et al. (2000) provided some data relating to the effects of DINP on male reproductive development. Based on this study, conducted at a single dose level of 750 mg/kg/day, Gray et al. reported a significant increase in males with areolas (22% vs. 0% in controls, $p < 0.01$) and also an increase in males with malformations (7.7%, $p < 0.04$). In the latter case, of 52 males examined, 2 had retained nipples, one had small testes and one had testicular atrophy. There were no effects on offspring body weights, anogenital distance, testes weights, preputial separation, serum testosterone levels; no effects on reproductive organ weights; no evidence of undescended testes, prostatic or vesicular agenesis, abnormalities of the gubernacular cord; and no reports of cleft phallus, vaginal pouch, or hypospadia. (Further discussion of this paper, which was included in our previous comments, is attached as an appendix to this letter.)

At the recent Society of Toxicology meeting, Gray's group reported results of studies of the effects of DINP given orally at 1000 and 1500 mg/kg/day (Ostby et al., 2001). Female weight gain during gestation and lactation was reduced by approximately 10% at both treatment levels; offspring body weight was unaffected at 1000 mg/kg/day but reduced by 10% in the 1500 mg/kg/day group. There was a large increase in areolas (55% at 1000 and 70% at 1500 mg/kg/day), but also a relatively high level in the controls (14.7%). There were also small but statistically significant reductions in anogenital distance and age at preputial separation in the 1500 mg/kg/day group, but these parameters were not different from control at 1000 mg/kg/day.

The necropsy results revealed increased nipple retention in both groups, and small but statistically significant reductions in weights of seminal vesicles and levator ani plus

[1] The range reflects the slightly different values provided by the two methodologies reported by David et al. (2000) and Kohn et al. (2000).

bulbocavernosus muscles in the 1500 mg/kg/day group. Weights of testes, ventral prostate, epididymis and bulbourethral glands were unaffected.

The histological examination revealed a small number of individuals in each group with lesions in the testes or secondary sexual organs, but there was no strong evidence for dose-response. In particular, there was no confirmation that small testes or testicular atrophy were associated with treatment. When these data are compared to the previous publication (Gray et al., 2000), it becomes apparent that baseline values for those parameters under consideration as indicators of anti-androgenic effects and/or male reproductive development need to be established before the toxicological consequences of small changes in such parameters can be confidently interpreted. That is, the incidence in controls in the more recent data indicates that some previous observations in treated animals may have been due to normal variation.

It is our view that the critical data needs for DINP identified by the Expert Panel have now been substantially satisfied, and that section of the CERHR report should be modified. Further, these additional data bear on the conclusions of the Expert Panel that were determined at the meeting in August 2000. The Expert Panel expressed minimal concern for the potential for developmental and reproductive effects in the human population. However, this was tempered in part by the absence of studies of sensitive indicators of male reproductive development and by the "moderate" confidence in the NOAEL for reproductive toxicity. The results now available for Gray's studies are, in fact, quite consistent with the results of the previously published two generation study (Waterman et al., 2000), and should, therefore, resolve some or all of the uncertainty expressed by the Expert Panel. Although Gray has not established a no effect level for areola retention, the low level of effects at 750 mg/kg/day indicate that, if this is not the no effect level, it must be close. Further, these data demonstrate that the effects on male reproductive development were not the most sensitive effects produced by DINP and would have no influence on risk assessments. As the NOAEL for all effects is in the range of 100-200 mg/kg/day, and human exposure is in the range of 1-2 ug/kg/day, the level of concern is better described as "negligible" than "minimal."

Please let us know if we can provide additional information. You may call Marian K. Stanley, Manager of the Phthalate Esters Panel, at (703) 741-5623 or e-mail her at Marian_Stanley@americanchemistry.com.

Sincerely yours,

[redacted]

Courtney M. Price
Vice-President, CHEMSTAR

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Ostby, J. et al. (2001). Investigation of the ability of diisononyl phthalate (DINP) to alter androgen-dependent tissue development in Sprague-Dawley rats. The Toxicologist 60:225.

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Appendix
Extract from The Phthalates Esters Panel December 11, 2000
Comments to NTP CERHR, Concerning the Gray Study

General Comment

During the DINP discussions the Expert Panel considered that data on male reproductive development were insufficient. Although the published information provided no evidence of such effects, the Panel took note of an abstract which reported an increased incidence in rats of malformations of the male reproductive system. In the absence of published data, the Expert Panel expressed only moderate confidence in the NOAEL for reproductive toxicity and expressed the desire that such studies be conducted along with a better assessment of human exposure. Recently a paper has been published (Gray *et al.*, 2000)[1] which did assess developmental indicators at 750 mg/kg/day. There was a statistically significant increase in areolas at PND 13, and, according to the authors, a small increase in malformations. None of the other parameters measured in the study were affected by treatment. The availability of these data should increase the confidence of the Expert Panel in the selection of NOAELs and should also obviate the need for any further tests of this type. Further, urinary metabolite studies indicate that human exposures are many orders of magnitude below the effect levels in rodent studies (Blount et al., 2000; David, 2000; Kohn *et al.*, 2000).[2] Accordingly, the Phthalate Esters Panel believes that current production and use of DINP pose no risks to human reproduction or development.

. . .

Comments Based on Recently Published Data

The CERHR Expert Panel Review of DINP referred to data from Gray's laboratory, available only in abstract form during the deliberations (Ostby *et al.*, 2000).[3] Although the conclusions from the abstract were cited in several places (*e.g.*, last paragraphs of sections 3.2 and 4.2) as evidence that DINP has an effect on male reproductive development, the absence of such data in the published literature concerned the Expert Panel, diminishing their confidence in their overall confidence in NOAELs, and resulting in a recommendation for additional studies listed in the critical data needs section. As the data from Gray's laboratory have now been

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1 Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. *Toxicological Sciences* 58:350-365.

2 Blount, B., et al. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., et al. (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

3 Ostby, J. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP, DINP but not DEP, DMP or DOTP permanently alters androgen-dependent tissue development in Sprague-Dawley rats. Triangle Consortium on Reproductive Biology, January 29, 2000.

published (Gray *et al.*, 2000),[4] the Expert Panel should fully evaluate those data and incorporate them in the monograph as suggested below.

As reported by Gray, female Sprague-Dawley (SD) rats were given DINP (CAS # listed as 68515-48-0) by oral gavage from GD14 to PND 3 at a single treatment level, 750 mg/kg/day. The offspring were examined at various times until terminal sacrifice at times ranging from 3-7 months of age. The parameters which were examined included:

(a) Body weight and anogenital distance on PND 2 – These parameters were unaffected by DINP treatment.

(b) Testicular examination on PND 3 – Testes weights of DINP-treated male offspring were similar to control.

(c) Inguinal examination of male pups – It was reported that one DINP-treated male offspring had "suspected" "hemorrhagic testes", but this was not confirmed by histologic examination.

(d) Examination for areolas on day 13 – The incidence of areolas (22%) was reported as significantly different from control at $p < 0.01$.

(e) Examination of onset of puberty (preputial separation) – Not affected by treatment.

(f) Determination of serum testosterone levels at terminal sacrifice – Not affected by treatment.

(g) Examination for retained nipples, cleft phallus, vaginal pouch and hypospadias – Of 52 male offspring examined, 2 had retained nipples; none had cleft phallus, vaginal pouch or hypospadia.

(h) Internal examination for undescended testes, atrophic testes, epididymal agenesis, prostatic and vesicular agenesis, and abnormalities of the gubernacular cord – One of the male offspring was reported to have had bilateral testicular atrophy and another exhibited epididymal agenesis with hypospermia and fluid filled testes. None of the 52 male offspring examined had undescended testes, prostatic and vesicular agenesis or abnormalities of the gubernacular cord.

(i) Body weights and weights of organs including ventral prostate, levator ani plus bulbocavernosus muscles, seminal vesicles, and epdidymides – Weights of all organs, including all of the reproductive organs were similar to controls.

(j) Sperm counts – It was not clear from the report whether or not sperm counts of DINP-treated animals were examined. The paper was silent on the results of sperm analysis for all substances except for BBP and DEHP for which sperm counts were reported to be reduced, but the data were not provided.

[4] Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. Toxicological Sciences 58:350-365.

The abstract which was cited by the CERHR (Ostby *et al.*, 2000) contains a statement that "males in the ... DINP (7.7%, $p < 0.04$) treatment group displayed malformations of the testis, epididymis, accessory reproductive organs and external genitalia." As now reported in the full publication, 4 (of 52) treated male offspring were considered by the authors to have been malformed. These included 2 with retained nipples, one with "small" testes, and one with testicular atrophy. The statistical analysis compared the total incidence of offspring considered malformed against the controls rather than making comparisons for each anomaly. The statistical evaluation indicated $p < 0.05$ when the data were compared on an individual basis and $p < 0.06$ for a litter-based comparison. No data on historical control incidences were provided. Given the low incidence of anomalies, it is difficult to determine whether these are spontaneous or treatment related. Further, the validity of pooling all affected individuals for statistical analysis seems questionable. Certainly, the effects evaluated individually would not be significantly different from control. We believe that these results are marginal and do not form a basis for strong conclusions of the effect of DINP on male reproductive development.

More important is the question of whether this publication provides any information on reproductive toxicity beyond that provided by the two generation reproduction study previously reported by Waterman et al. (2000). Gray's study utilized oral gavage in contrast to dietary administration in Waterman and at a somewhat higher dose level (in Waterman the estimated maternal dose on GD 14-21 was 543 mg/kg and that on PND 0-4 was 672 as compared to 750 mg/kg in Gray). Nevertheless, Gray confirmed one of the most important findings of Waterman, i.e., that DINP treatment during the period of male reproductive development has no effect on male reproductive organs. More specifically, Gray found no effects on weights of testes or accessory reproductive organs, and identified only 2 rats (of 52) with what he considered to be malformed testes. Waterman also found weights of testes and accessory organs to be unaffected. In addition, Waterman found that within the parental generation, one male, from the control group, had unilateral focal testicular atrophy. In the F1 generation there were two males with diffuse unilateral atrophy and testicular degeneration; one from the control group and one from the high dose group. As similar effects were found at the same incidence in the treated and control groups, these findings were judged by Waterman to be incidental.

The one clear difference between these two studies is that Gray found an increase in areolas in 13-day old male pups. However, the toxicological significance of this effect is questionable since it appeared to be substantially reversible. Among the 13 day old male offspring, 22% had areolas; at terminal sacrifice, 2 (of 52) or 4% of the males had retained nipples. Although the frequency of aerolas was increased, the demonstration that DINP had no effects on fertility, and minimal effects on male reproductive development should provide the Expert Panel with the information that these minor effects have no bearing on human reproductive risk. That males with areolas can reproduce was shown by Schilling (1999)[5] in a study of the potential reproductive effects of DEHP.

The above having been said, these data seem more relevant to the overall assessment of developmental toxicity than reproduction. There was a significant increase in frequency of areolas at 750 mg/kg, but this appeared to have been substantially reversed by terminal sacrifice.

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[5] Schilling, K. *et al.* (1999). Reproduction toxicity of di-2-ethylhexyl phthalate. *The Toxicologist* 48:147-148.

Although no NOAEL was defined, the level associated with this effect was higher than other developmental effects considered by the Expert Panel, and, therefore, should not influence the overall evaluation of developmental toxicity. The reproductive NOAEL had previously been defined by the absence of effects on fertility and/or reproductive organs as reported by Waterman. Gray provided no new data on fertility and confirmed the absence of effects on reproductive organ weights. Although Gray reported a low incidence of testicular effects, the marginal nature of those findings along with the absence of effects in Waterman indicate that these data should not be used for NOAEL determination. That, in effect, would leave in place the existing LOAELs and NOAELs, but should increase the Expert Panel confidence. With more confidence in both the toxicity and exposure information, it would be more appropriate to change the concern level to negligible.

Michael D. Shelby, Ph.D.
Director, CERHR
NIEHS / NTP B3-09
P.O. Box 12233
Research Triangle Park, NC
27709-2233

JAN 0 9 2001

Dear Dr. Shelby,

I have just learned CERHR has had an open invitation for comment that was to close December 15, 2000 regarding the findings of your Expert Panel on Phthalates. I hope you will consider my late entry. My particular interest is with DEHP.

My limited research suggests much of the data that supports DEHP as a carcinogen appears to be based on high doses of the chemical orally ingested by rats and similar creatures. From these relatively extreme exposure conditions, it is being inferred that human safety is at risk.

In a ECPI Press Release dated February 28, 2000, DEHP was downgraded from Group 2B to Group 3, "not classified as to carcinogenicity to humans". The Press Release went on to state, "...the mechanism by which DEHP increases the incidence of hepatocellular tumours in rates and mice is not relevant to humans".

Discovery Medical, Inc. manufactures disposable gloves including vinyl gloves so this issue is of concern to us. In a separate report from the U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry dated April, 1993 (http://www.atsdr.cdc.gove/tfacts9.html), ATSDR stated "You should have no health effects from skin contact with products containing DEHP because it cannot be taken up easily through the skin."

We want to make sure we are interpreting the various data sources accurately regarding this topic. From these sources we are inclined to conclude that DEHP is not been substantially proven to be a human safety issue and definitely not a human safety issue for those wearing vinyl gloves.

If you have any information that is contraindicated to this conclusion, specifically regarding vinyl gloves, you comments would be greatly appreciated.

Sincerely,

[redacted]

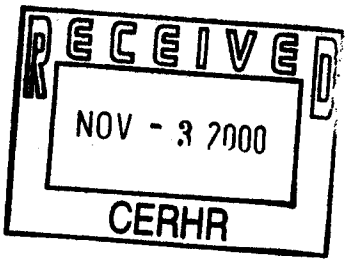
Doug Sallenbach
Director – Sales and Marketing
Discovery Medical, Inc.

Appendix III

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ENVIRONMENTAL WORKING GROUP™



October 30, 2000

Michael D. Shelby, Ph.D.
Director, Center for the Evaluation of Risks to Human Reproduction
The National Institute of Environmental Health Sciences
National Toxicology Program
B3-09
P.O. Box 12233
Research Triangle Park, NC 27709-2233

Dear Dr. Shelby:

We are writing to express our concern that key conclusions in CERHR's Expert Panel Report on Phthalates are fundamentally flawed in light of the recent revelation that human exposures to one of the phthalates reviewed by the panel, dibutyl phthalate (DBP), are higher than anticipated, particularly in those most vulnerable to its effects, women of childbearing age.

We commend the Expert Panel for its thorough analysis, but we are troubled that the report, as published, is missing new, critical exposure information on DBP. If not amended, the Expert Panel report will begin the formal public discussion of phthalate risk from a conclusion about exposure, particularly for women of childbearing age, that was known to be in error more than one month before the document was posted on the web for public comment.

The report, released for public comment on October 10, 2000, states "All estimates place total DBP exposure in the general population at less than 10 ug/kg bw/day." Data from CDC published more than one month before the Panel report was posted on the web showed the Panel's presumption of low exposures to be a substantial underestimate of the true high end of exposures, where risks are greatest. If more accurate data had been used, the Panel would have had difficulty concluding that high-end DBP exposures were essentially safe.

As noted, more than one month before the Panel report was posted for public comment, research published by the CDC, and a subsequent analysis by CDC and NIEHS, show that "the maximal value indicate that some individual exposures are substantially higher than previously estimated for the general population", and that high exposures in women of childbearing age are approximately five times greater than the highest exposures in the rest of the

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population. The NIEHS and CDC analysis, published in the October 2000 issue of Environmental Health Perspectives, now gives the high end of exposures for women of childbearing age, among a population of 289 people, as 113 ug/kg bw/day – an order of magnitude higher than the Panel assumed in forming their conclusion that DBP exposures are of minimal concern.

We ask that you amend the document as posted on the web, at a minimum to acknowledge the fact that women with high exposures to DBP were not considered, but optimally to provide a full consideration of this vulnerable, highly-exposed population. Without these changes, the public debate on phthalate risks will begin from a scientifically unsound starting point.

We appreciate the complexity of the task set before the Expert Panel as they attempted to categorize risk to human reproduction and development armed with only limited exposure data. But leaving the current Panel report as the point of departure for public comment of phthalate risks, unfairly biases the discussion in favor of lower exposure scenarios that we now know are wrong for perhaps millions of women of childbearing age.

Thank you very much for your attention to this matter.

Sincerely,

[redacted]

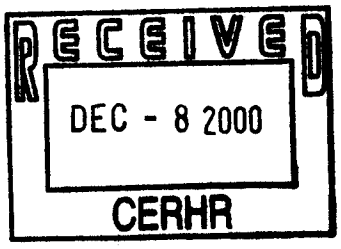
Richard Wiles
Vice President for Research

[redacted]

Jane Houlihan
Senior Analyst

Appendix III

ENVIRONMENTAL WORKING GROUP™



December 7, 2000

Michael D. Shelby, Ph.D.
Director, Center for the Evaluation of Risks to Human Reproduction
The National Institute of Environmental Health Sciences
National Toxicology Program
B3-09
P.O. Box 12233
Research Triangle Park, NC 27709-2233

Dear Dr. Shelby:

We write this letter to supplement our previous comments to you (dated October 30, 2000) regarding CERHR's Expert Panel Report on Phthalates. The concern we expressed previously stands, and is heightened based on our recent research on phthalates in cosmetics. We reiterate our request that you amend the document as posted on the web, at a minimum to acknowledge the fact that women with high exposures to DBP were not considered when CERHR concluded that DBP exposures were of minimal concern to human reproduction.

We reassert that the panel has failed to consider the reproductive risk faced by perhaps millions of women of childbearing age who are exposed to relatively high levels of dibutyl phthalate (DBP). If, as CDC scientists postulate (Bount et al 2000), the high exposures of DBP in women stem from cosmetics, our recent research shows that nail polish is likely a significant contributor. Far more than half of the nail enamels we studied contained DBP. Industry patents indicate that the chemical typically comprises about 5% of the product, by weight, and that DBP's purpose in the nail polish is to maintain the flexibility of the film on the nail. We conducted patent office and web-based label searches to reach this conclusion – the details of our study methods and results are presented in the attached report, *Beauty Secrets.*

In any assessment of effects of DBP to human reproduction, occupational exposures in nail salons must be considered. According to the 1997 U.S. Economic Census, the more than 81,000 beauty salons around the country employ 407,000 people. This workforce, many of whom are likely women of childbearing age, stands to have the highest levels of exposure to DBP of any other segment of the population. Since the Federal Food, Drug and Cosmetics Act specifically excludes from any labeling requirements all cosmetics used by professionals and not sold to the public, women who work in this industry are nearly powerless to take voluntary actions to reduce their DBP exposures while government assessments of the safety of DBP continue.

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We ask you to consider the potential effects of the high exposures in women of childbearing age found in CDC's recent biomonitoring study (Blount et al 2000). We also request that you address the DBP exposures that must be occurring in nail salons around the country.

Sincerely,

[redacted]

Richard Wiles
Vice President for Research

[redacted]

Jane Houlihan
Senior Analyst

Attachment

References

Blount BC, MJ Silva, SP Caudill, LL Needham, JL Pirkle, EJ Sampson, GW Lucier, RJ Jackson, JW Brock. 2000. Levels of seven urinary phthalate metabolites in a human reference population. Environmental Health Perspectives. 108(10):979-982. October 2000.

-----Original Message-----
From: Willem Faber [SMTP:wfaber@msn.com] <mailto:[SMTP:wfaber@msn.com]>
Sent: Monday, December 11, 2000 5:31 PM
To: jmoore@sciences.com <mailto:jmoore@sciences.com>
Subject: Comments on 2-EH and 2-EHA

Jack, please find attached my comments on the DEHP review as it pertains to 2-EH and 2-EHA. There is a Word document and an Excel file. I will follow this with an overnite mail of a hard copy tomorrow. Thanks for the opportunity to provide input. sincerely, Willem Faber <<final letter to CERHR.doc>> <<CERHR TABLE.xls>>

Section 2.1.2, Oral studies in rats with 2-EH – The 6% increase in relative (to body weight) testes weight corresponds perfectly with the 7% reduction in body weights observed in the male rats receiving 500 mg/kg/day 2-EH by gavage. The growth of the testes (and several other internal organs) would be spared under these test conditions and the decreased weight in rats of this age and strain is almost certainly due to reduced body fat when compared to matched control animals. In the absence of any histological lesions in the testes, to suggest there is evidence that "perhaps" the testes is a target organ is not supported by a close analysis of the data. Later in Section 4.2.3, the document suggests that because neutral buffered formalin (NBF) was used to fix the testes, significant fixation artifacts could have been caused. However, in both the experience of the laboratory and in the literature the use of NBF in causing fixation artifacts is very laboratory specific, and was not a problem in the laboratory this study was performed in. Furthermore, the pathologists that examined the slides from this study found them to be perfectly adequate for the purpose intended. Therefore, there were no fixation artifacts, no testicular lesions, and no evidence of testicular toxicity in this study.

Section 3.2.3, Dermal developmental toxicity studies with 2-EH – The CERHR review suggests there should be reduced confidence in this study due to the lack of a clearly maternally toxic dose. The authors reported a reduction in weight gain from gestational days 6-9 at the highest dose level and erythema and cellular exfoliation at the mid- and high-dose groups. The highest dose level is in excess of 2500 mg/kg/day, approximately 2.5-fold greater than the limit dose used in developmental toxicity by the oral route of exposure. Furthermore, red, injected, irritated, peeling skin at the site of application is very good evidence of dermal toxicity in the dams and to suggest a higher dose and/or to dismiss this finding would violate the humane treatment of these animals. The confidence in this study should be high and this study should be perfectly acceptable for risk assessment of 2-EH following a dermal exposure. It may not be of much use for evaluating oral or IV exposures to DEHP, but then none of the 2-EH or 2-EHA data is of much use for that anyway, since all of the low-dose DEHP effects (and those of any concern) are due to MEHP alone.

Section 3.2.4, Gavage administration of 2-EHA – For the rat study, the interpretation of this study in the CERHR review is in direct contradiction to the study authors and this discrepancy should be stated up front. Furthermore, the CERHR review should describe how a chemical treatment that reduces the incidence of seven fetal skeletal variations would qualify as "consistent evidence of fetotoxicity". The CERHR review does not state the level of confidence in the rat study. In this same section, the CERHR review describes the rabbit study and repeats the same absurd conclusion it did in the first draft of the document ("Confidence is limited due to the absence of a clearly maternally toxic dose.") The mid- and high-dose levels in this study killed some of the dams. How much more toxic would the CERHR reviewer like the material to be? This study is an excellent study that demonstrated no effects on development at maternally toxic levels in rabbits. The study was done by GLP and EPA Guidelines in very good laboratories by accomplished developmental toxicologists. The confidence level should be extremely high for use in risk assessment.

In the same section (3.2.4), the study by Ritter et al., (166) is reviewed. This study uses very high dose levels, levels that cause considerable maternal toxicity (convulsions, prostration, death,) in other comparable studies. This study does not examine the effects at lower doses, doses with minimal to no maternal toxicity. This study also fails to replicate the effects observed with DEHP observed in other developmental toxicity studies. The CERHR review also fails to assign a confidence rating for this study. In spite of all that, the CERHR review states "The results are compatible with the hypothesis that 2-EHA is the proximate teratogen." This is in direct contradiction to what is stated in the conclusion of the CERHR review, where it is clearly stated that MEHP is the proximate teratogen for DEHP.

Within this section, the CERHR review attempts to link the developmental toxicity of 2-EHA with that of valproic acid (VPA). As indicated in the earlier comments to CERHR, this review is about 5 years out of date. There does not appear to have been any attempts to upgrade this section from the previous draft and therefore the prior comments are still appropriate. The part of the review for the Chernoff-Kavlock assay (ref. 198) does not have a confidence rating. However, in light of the CERHR reviewers comments that death was not a clear indication of maternal toxicity in rabbits, it should be clearly stated as to whether this logic also hold for rats. The study (ref. 198) reports (to its credit) several signs of toxicity, including death to the dams; however, no conclusion is given as to whether the CERHR review considers this to be a clear indication of maternal toxicity. The review should be uniform in this respect and state that in rats, as was previously stated for rabbits, death to the dams is not considered a clear indication of toxicity. Also, the CERHR review should mention that the Chernoff-Kavlock assay is a screening assay and hardly appropriate to support a conclusion of a similarity of syndromes of developmental toxicity between VPA and 2-EHA, particularly since there are much better studies to use to prove or disprove that hypothesis. Also, in the last paragraph of that section, the word "neutralized" is supposed to be "ionized". The nonionized weak acids enter the conceptus and become ionized within the slightly alkaline environment and are trapped (ion trapping), or so the theory goes.

Section 3.2.4, Administration by Drinking Water - The problems with the drinking water studies using 2-EHA are well known, and were elucidated in the previous comments to CERHR. Again, nothing was changed in response to those comments and therefore the comments will not be repeated here (there are many problems and therefore many comments). This time the CERHR review assigned confidence ratings to these two studies, while failing to acknowledge the problems with study design, interpretation, etc. The confidence rating was assigned based upon the supposed replication of the NOAEL and LOAEL between the developmental toxicity study and the reproductive toxicity study for 2-EHA within the drinking water. However, the dose levels (and therefore the NOAELS and LOAELS) are the same since the same group performed both studies with the same concentrations in the drinking water, not because of any sort of concordance between the findings from the studies. The Panel should have little confidence in the data from these studies for all of the reasons in the comments previously submitted and reproduced again below.

The primary drawback with using the Pennanen et al. (1992) study is that there is no description as to how the chemical was administered in the drinking water and achieved target doses of 0, 100, 300, or 600 mg/kg/day of the test substance when the two highest exposure levels had significant decreases in rates of water consumption. Furthermore, the authors used the individual fetus as the unit of statistical analysis, not the dam. From close inspection of the data (mean and standard error), it is obvious that certain dams exhibited significant maternal toxicity, while others did not. We have tried to obtain the raw data from the study authors to do a statistical analysis based upon the dam as the unit, but the authors have refused to provide the data. The question of maternal toxicity in this study is particularly important in light of the work of Bui, et al., (1998) that demonstrated that maternal toxicity was critical to the subsequent developmental outcome of the fetuses.

Section 3.2.4, Mechanism – This part of the CERHR is greatly expanded, hopefully in response to the previous comments submitted. However, the review does not appear to reach a credible conclusion regarding the interpretation of the mechanistic studies available. First, they question as to whether chemical in the diet or drinking water can cause an acute phase response in the liver. The ability of the chemical to cause this response in the liver is determined by the dose reaching the liver and the residence time available to cause toxicity. The gavage route would theoretically provide higher concentrations for shorter periods of time while the diet/drinking water would provide lower concentrations but for much longer time periods. Either combination should be able to cause toxicity, whether it is the acute phase responses, systemic toxicity or developmental toxicity. All three routes have demonstrated to cause systemic and developmental toxicity with 2-EHA, as is reviewed in the CERHR document. In the interest of being conservative, the CERHR Panel should consider that drinking water and dietary exposure routes can cause toxicity (acute phase responses or developmental toxicity) just as gavage exposures can, until proven differently. There is no evidence to suggest that peak levels (as found following gavage) are required to cause the acute phase response in the maternal liver. In fact, dietary studies with 2-EHA examining systemic toxicity describe responses in the liver strikingly similar to what would be expected following an acute phase response.

The second point raised is that we do not know the zinc content of the rodent diet fed in the DEHP or 2-EHA studies and therefore cannot know whether they would correspond to inadequate, adequate, or supplemental levels such as were used in the Bui, et al., study. Actually, the zinc content within rodent diets is relatively constant and uniform throughout the USA and Europe. When this question was poised to Dr. Carl Keen, Head of Nutrition at UCal at Davis, (where the work of Bui, et al., was performed), Dr. Keen noted that they picked the adequate level for the experiment to simulate exactly the levels found in the diets fed the animals in the other 2-EHA studies. So it is possible to judge and know what the zinc content of the diets from the other 2-EHA studies was and to include them in the comparison.

Why DEHP is included in the discussion of the acute phase response mechanistic section is unclear. The mechanism of action of 2-EHA and DEHP are unlikely to be related since the molar amounts of 2-EHA formed from the lower teratogenic levels of DEHP are

not adequate to cause any developmental toxicity, while the molar amount of MEHP formed causes approximately the same incidence of developmental effects and of a similar spectrum. 2-EHA is not responsible for DEHP-induced teratogenicity; MEHP alone is responsible for the effects observed. This point is stated very clearly elsewhere in the document, it is only in the 2-EHA sections does the CERHR review seem to confuse this important point. In an attempt to provide this comparison for the CERHR Review, please find two tables in Excel that describe the amount of 2-EH and 2-EHA that would be formed following DEHP administration. It is very clear that the amount of 2-EH and 2-EHA formed from DEHP is so small that it cannot be responsible for the malformations. The amount of 2-EH and 2-EH that must be administered directly to cause similar incidences of defects (as found with DEHP) is approximately 20-fold higher for 2-EH and 10-fold higher for 2-EHA.

The last point the CERHR review raises, as a way to disregard the mechanistic work of Bui, et al., is to suggest that gavage dosing can alone induce the acute phase response. The supposed proof is the difference between the effects measured after a single dose versus after several doses. Of course, by this logic, all gavage developmental toxicity studies would have to be discarded since the method of dosing would be teratogenic. Therefore, the control groups should have higher rates of malformations from this route of exposure than from others, although this has never been observed in thousands of teratology studies conducted to date. What the reviewer is confusing is the degree of response of the measured variable (either liver MT levels, liver zinc levels, or serum zinc levels) to the dose administered. The manner in which an acute phase response in the liver causes a decrease in serum zinc level explains the difference. Following the first dose, the liver produces increased amounts of metallothionein, which sequesters zinc. The free zinc level in the liver falls, and serum zinc shifts into the liver compartment in response to this decrease. Therefore, the effect following the first dose can be quite dramatic. The continued dosing of the animal allows for continued MT synthesis and an altered equilibrium is attained between liver and serum zinc. At some point in time, the liver is saturated with MT and zinc and it cannot sequester any more, and serum zinc levels are reestablished. However, the damage to the embryo is done. The transient decrease in serum zinc at the critical time of development causes permanent defects because of a zinc deficiency in the embryo. The measure of liver MT levels, liver zinc levels, or serum zinc levels after repeated dosing may seem less pronounced but only because the serum zinc levels are starting to be re-established. The data do not support that single versus repetitive dosing/stress argument. Gavage dosing is done routinely without stress to the animals.

The last paragraph added to Section 3.2.4 since the last draft of the CERHR review attempting to correlate 2-EHA and VPA also underscores the previous point that this review is about five years out of date. The reviewers failed to include the most recent work regarding this topic (as was pointed out in the comments on the first draft) and have also failed to consider or mention work that establishes this hypothesis has little merit. The previous comments are repeated below.

. First, the work of Heinz Nau's group (**Reference**: Hauck, R.-S., Wegner, C., Blumtritt, P., Fuhrhop, J.-H., and Nau, H. (1990). Asymmetric Synthesis and Teratogenic Activity of (R)- and (S)-2-Ethylhexanoic Acid, A Metabolite of the Plasticizer Di-(2-ethylhexyl)phthalate. Life Sci. 46, 513-518.) regarding 2-EHA entantiomers is not even included. The results showed that a dose of 2000 mg/kg/day of the (R) enantiomer or racemic mixture produced ~10% embryolethality and 16% lower fetal weight. Of the total fetuses examined in these groups, 32 and 59% had exencephaly (racemic mixture and (R) enantiomer, respectively). There is no indication of the number of litters affected. The same dose of the (S) enantiomer (2000 mg/kg/day) and 500 mg/kg/day of the racemic mixture were not fetotoxic or teratogenic since embryolethality and fetal weight were at control levels. It is interesting that the reviewer has not considered the difference in dose-response relationship or potency between valproic acid and 2-EHA. In the paper of Nau et al., (1991), intraperitoneal administration of 3 mmol/kg (498 mg/kg)of 2-EHA causes a 5% incidence in exencephaly, while a comparable dose of valproic acid causes a 44% incidence. This roughly translates into a 9-fold difference in potency, assuming the two materials are acting via a similar mechanism. Even when the more potent enantiomer of 2-EHA is used [R(-)-EHA], a dose of 3 mmol/kg (498 mg/kg) four times (total dose of 1992 mg/kg) over two days is required to cause a 59% incidence of exencephaly. With such a dramatic difference in potency, it may be that 2-EHA and valproic acid are causing exencephaly by two different mechanisms and therefore structure activity relationships based upon the fact that 2-EHA and valproic acid are isomers is not valid.

Furthermore, the most recent work of Dr. Nau (Tox. And Applied Pharm. 160, 238-249, 1999. *New Molecular Bioassays for the Estimation of the Teratogenic Potency of Valproic Acid Derivatives In Vitro: Activation of the Peroxisomal Proliferator-Activated Receptor (PPARδ).* A. Lampen, S. Siehler, U. Ellerbeck, M.Gottlicher, and H. Nau) suggests a very specific structural requirement for neural tube defects to occur. The chemical of the series tested by Nau in this recent publication that most closely resembles 2-EHA is labeled "ethyl-4-yn-VPA" in Figure 1 of the paper. This chemical has a structural formula of CH_3-CH_2-CH(COOH)-CH_2-C=CH. For comparison, 2-EHA has the structural formula CH_3-CH_2-CH(COOH)-CH_2-CH_2-CH_2- CH_3. At 1.85 mmol/kg (276 mg/kg), ethyl-4-yn-VPA caused 0% exencephaly and 5% embryolethality in the 73 fetuses examined. In fact, it was used as a "negative control" in the remainder of the paper that deals with determining the mechanism of action. In contrast, valproic acid in the same test system caused 42% exencephaly and 49% embryolethality in the 60 fetuses examined, albeit at a higher dose level. Valproic acid also activated the specific genes in the test system Dr. Nau is using to elucidate the mechanism of neural tube defect induction while ethyl-4-yn-VPA did not. Clearly, much more than "*2-Ethylhexanoic acid and VPA are structural isomers; they are both carboxylic acids with eight-carbon alkyl chains*" is required to assign causality and commonality for these two materials.

Section 3.2.4, Embryo culture – Again, this review underscores a fundamental lack of understanding of the work of Bui, et al. The amount of 2-EHA in the culture medium prepared with serum from male rats treated with 2-EHA was measured and was found to be below detection. However, the zinc level was very low (as was expected from the

acute phase response) and thus was responsible for the altered development in vitro. The addition of supplemental zinc to the culture media prevented the altered development in vitro. If 2-EHA (or a metabolite) were responsible for the altered development, the presence of low zinc and the supplementation of additional zinc should have had no effect on the in vitro development of the embryos. The in vitro data proved the causation implied from the in vivo data. What this has to do with DEHP is anyone's guess and again underscores the point that the 2-EHA reviews should not have even been included in the first place.

Section 4.2.3, 2-EH – This section suffers from the same problems that the first draft did. The subject of fixation artifacts that the review is trying to conjure up is addressed above. The second paragraph states, "Relative testes weight was increased at the high dose." The increase was 6% and the decrease in body weight at that dose was 7%. The next paragraph states, "No histopathology was reported for the testes." Of course this is not true, it is included when the statement "All other tissues examined were normal." is used. Then it says (in the same paragraph) "The reproductive LOAEL is not calculable, because no adverse reproductive effects were seen. The NOAEL is 500 mg/kg/day, based on lack of effect on testes weight." Both sentences are correct; however, the second one directly contradicts (without explanation) the last sentence of the previous paragraph.

Section 4.2.4, 2-EHA – The CERHR review assigns a "moderate-to-high" rating to the Pennanen studies all the while understanding that these studies used a method of data analysis specifically discouraged by the EPA Developmental Toxicity, Reproductive Toxicity, and Risk Assessment Guidelines and had significant methodological problems (dose administration, dose calculation, sperm analysis, to name a few). Then the same review gives a moderate rating to the study reported by Juberg at al., (97) that was done and evaluated according to the EPA Guidelines, not even understanding that histology was conducted on reproductive organs (as per those same Guidelines).

Section 5.1.2.4, Utility of Data for the CERHR Evaluation – In general, this section is well written. However, the sentence (3rd paragraph) "Peroxisomal proliferation was not examined for 2-EHA" remains incorrect as pointed out in our first set of comments. The ability 2-EHA to cause of peroxisome proliferation has been examined (**Reference**: Moody, D.E., and Reddy, J.K. (1978). Hepatic Peroxisome (Microbody) Proliferation in Rats Fed Plasticizers and Related Compounds. Toxicol. Appl. Pharmacol. 45, 497-504, and Moody, D.E., and Reddy, J.K. (1982). Serum Triglyceride and Cholesterol Contents in Male Rats Receiving Diets Containing Plasticizers and Analogues of the Ester 2-Ethylhexanol. Toxicol. Lett. 10, 379-383.) 2-EHA is considered a weak agent for causing peroxisome proliferation.

Section 5.1.2.4, 2-EH and 2-EHA - The last paragraph reiterates the previous discussion attempting to link 2-EHA and VPA. This suffers the same problem as the previous discussion in terms of being up-to-date and ignoring information that contradicts the hypothesis.

Section 5.1, Discussion of data sufficiency for 2-EH (top of page 96) – The Panel brings up an argument that is not discussed previously in the review. The Panel states, "Based on the rapid in vivo conversion to the acid, the Panel believes that it is unlikely that 2-EH will act directly. Because it is rapidly converted to 2-EHA, exposure in vivo is to 2-EHA." The question of rapid conversion of 2-EH to 2-EHA was not addressed by the CERHR review. The only data available to directly address this question are two papers from Xenobiotica (24(5):429-440 and 28(7):699-714). Both of these papers used female F344 rats and the studies were conducted in the same laboratories. The earlier paper addressed 2-EH and the second paper investigated 2-EHA. 2-EHA is eliminated in a triphasic manner with T1/2's of 0.19, 6.6, and 117 hours after iv administration. Following an oral dose of 100 mg/kg 2-EHA, 50% of the radioactivity is eliminated into the urine within 8 hours, with 76% eliminated by 24 hours. Evidence of saturation of elimination pathways at higher dose levels is evident at 1000 mg/kg 2-EHA, with 20% of the radioactivity eliminated into the urine within 8 hours, and 73% eliminated by 24 hours. 2-EH is eliminated slower and all through the 2-EHA metabolic pathway; with 36% eliminated at 8 hours and 54% eliminated by 24 hours (50 mg/kg). Again, a higher oral dose of 2-EH (500 mg/kg) results in less elimination at the 8 hours time point (24.5%), and 54% eliminated at 24 hours. The important point from this comparison is that the elimination of 2-EHA is faster than the conversion of 2-EH to 2-EHA. This makes perfect sense when the in vivo data is considered, since approximately twice as large a dose of 2-EH is required to cause effects similar to 2-EHA.

Therefore, to simply interchange the two data sets (and assume what is true for 2-EHA is true for 2-EH) would not recognize the significant differences that exist between these two materials (would you interchange the data sets for ethanol and acetic acid?). Then to use a study fraught with problems (Pennanen; as discussed previously ad nauseum) to evaluate reproductive toxicity for 2-EH makes little, if any sense. The overwhelming data suggest that 2-EH is not a reproductive toxicant.

Section 5.2, Integrated Evaluation – For the most part, this portion of the document seems well written and evenhanded. It does suffer from a moderate schizophrenia, as it seems to suggest (correctly) that the effects of DEHP, at reasonable doses, are due to MEHP (by the way, 2-EHA is not formed from 2-EH by lipases, in the GI tract or elsewhere). The paragraph that addresses species differences in terms of sensitivity to agents causing peroxisome proliferation, fails to recognize that the developmental toxicity of DEHP is due to MEHP. The question of potency between metabolites is addressed only by considering a study that studied all the materials at once, which limits that analysis to one study, conducted as a screen with very high dose levels. The overwhelming evidence suggests that MEHP is much more potent than 2-EHA and simply because they were not studied all at once is no reason to ignore the evidence. Again, the VPA/2-EHA argument is brought up and again it is simply not up to date.

Section 5.3 Expert Panel Conclusions – Again, here the Panel refers to MEHP as the active metabolite and does not mention 2-EH/2-EHA at all. Perhaps the previous discussions within the review were not pertinent to DEHP.

Section 5.3, Critical Data Needs – No mention of 2-EH/2-EHA. Must not be important or relevant to the DEHP discussion.

COMPARISON OF DEHP, MEHP, 2-EH AND 2-EHA ON A MOLAR BASIS - MOUSE DT STUDIES

DEHP STUDIES - MOLAR COMPARISON FOR DOWNSTREAM METABOLITES

	DEHP	DEHP	MEHP	MEHP	2-EH	2-EH	2-EHA	2-EHA
Tyl, et al.,	mg/kg	mmol/kg	mmol/kg	mg/kg	mmol/kg	mg/kg	mmol/kg	mg/kg
in feed	0	0	0	0	0	0	0	0
NOAEL	44	0.113	0.113	31.5	0.113	14.7	0.113	16.3
LOAEL	91	0.223	0.223	64.9	0.223	29	0.223	33.6
	191	0.489	0.489	136.2	0.489	63.6	0.489	70.4
	293	0.75	0.75	209	0.75	97.5	0.75	108

MEHP and 2-EH STUDIES - w/MOLAR COMPARISON FOR 2-EHA

	MEHP	MEHP		2-EH	2-EH	2-EHA	2-EHA
Price, et al.,	mg/kg	mmol/kg	Tyl, et al.,	mg/kg	mmol/kg	mmol/kg	mg/kg
gavage	0	0	1991, in feed	0	0	0	0
LOAEL	35	0.126		17	0.13	0.13	18.7
incr. Resorp.	73	0.26		59	0.45	0.45	64.8
malformations	134	0.48	NOAEL	191	1.47	1.47	211.7
	269	0.965					

There are no mouse DT studies with 2-EHA directly administered

COMPARISON OF DEHP, MEHP, 2-EH AND 2-EHA ON A MOLAR BASIS - RAT GAVAGE DT STUDIES

DEHP STUDIES - MOLAR COMPARISON FOR DOWNSTREAM METABOLITES

		DEHP	DEHP	MEHP	MEHP	2-EH	2-EH	2-EHA	2-EHA
Wistar									
Hellwig, et al.,		mg/kg	mmol/kg	mmol/kg	mg/kg	mmol/kg	mg/kg	mmol/kg	mg/kg
	1997	0	0	0	0	0	0	0	0
		40	0.102	0.102	28.4	0.102	13.3	0.102	14.7
NOAEL		200	0.512	0.512	142.7	0.512	66.6	0.512	73.7
SEVERE EFF.		1,000	2.56	2.56	713.3	2.56	332.8	2.56	369

MEHP and 2-EH STUDIES - w/MOLAR COMPARISON FOR 2-EHA

		MEHP	MEHP		2-EH	2-EH	2-EHA	2-EHA
Wistar				Wistar				
Ruddick, et al.,		mg/kg	mmol/kg	Hellwig, et al	mg/kg	mmol/kg	mmol/kg	mg/kg
	1981	0	0	1997	0	0	0	0
		50	0.18	NOAEL	130	1	1	144
		100	0.36	LOAEL	650	5	5	720
		200	0.72		1300	10	10	1440
Mat. Lethal, dev NOAEL		225	0.8					
Litter loss		450	1.6	F344	2-EHA	2-EHA		
killed dams		900	3.23	Tyl, 1988	mg/kg	mmol/kg		
					0	0		
					100	0.69		
				NOAEL	250	1.74		
				LOAEL	500	3.5		

Appendix III

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Response to NTP-CERHR Report on Di-isononyl Phthalate (DINP)

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We wish to respond to the NTP-CERHR Expert Panel report on di-isononyl phthalate (DINP). The Panel report focused on reproductive effects of DINP, however, it also reviewed other effects such as systemic, long-term and carcinogenic. While we are in general agreement with the Expert Panel's assessment on the reproductive effects of DINP, we have derived a no observed-effect-level (NOEL) for systemic effects, which is different from that adopted by the Panel.

Two chronic studies were available for DINP (Lington et al.,1997; Moore,1998). The Expert Panel report reviewed the systemic effects of the two studies and adopted the conclusions of their authors, including the NOEL of 1,500 ppm

In the first study (Lington et al., 1997), groups of 110 Fischer 344 rats of each sex were exposed to 0, 0.03, 0.3 and 0.6% DINP1 diet up to two years. Expressed as mg of DINP1 ingested, the dose levels are 0, 15, 152, and 307 mg/ kg bw/day in male rats and 0, 18, 184, and 375 mg/ kg bw/day in females. Groups of animals were killed after 6, 12, 18 and 24 months of study. A significant reduction in body weight gain, increased relative liver and kidney weights, and elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed at 0.3 % (3,000 ppm) DINP and higher. A no-observed-effect level was demonstrated at a dietary level of 0.03 wt% (300 ppm, approximately17 mg/kg bw/day).

In the second two-year study (Moore, 1998), groups of 70- 85 Fischer 344 rats were fed 0, 500, 1,500, 6,000 and 12,000 ppm DINP1 diets (males: 0, 29.2, 88.3, 359 and 733 mg/kg bw/day; females: 0, 36.4, 109, 442, and 885 mg/kg bw/day) up to 104 weeks. Subsets of animals were killed after 26, 52, 78 and 104 weeks of exposure. While more severe effects were observed in the groups given 6,000 and 12,000 ppm DINP1, hematological (decreased erythrocytes and hematocrit) and biochemical (elevated serum ALT and AST) effects were also noted in female rats exposed to 1,500 ppm, and killed at weeks 26, 52 and 78. The author did not consider these hematological and biochemical effects treatment-related on the grounds that they were not observed at week 104, and were not seen in male rats. A NOEL of 1,500 ppm was reported for DINP 1 (male: 88 mg; female: 109 mg/kg bw/day).

After a review of Moore's study, we derived a NOEL of 500 ppm (males: 29.2 mg/ kg bw/day; females:36.4 mg/kg bw/day). An examination of the Moore's report (1998) revealed that the actual dose of DINP1 (mg/kg bw/day) ingested by the 1,500 ppm male rats is lower than that of the corresponding females. While both sexes consumed diets of the same concentration, female rats that were killed at weeks 24, 52 and 80 ingested 28-42% more DINP1 (mg/kg bw/day) than males (Table 1). Further, the female rats killed in weeks 24, 52 and 80 ingested 20- 28% more of the test substance (mg/kg bw /day) than those terminated at week 104.

In our opinion, the higher dose of DINP ingested by the female rats offers a reasonable explanation for the discrepancies in the biochemical and hematological effects observed in the two sexes. This observation is typical of a dose-dependent effect, and elevated serum

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transaminases suggest a liver injury in the female rats exposed to the 1,500 ppm DINP1. At week 104, both sexes consumed a substantially lower dose of DINP and hence did not exhibit these effects. This observation is consistent with those reported by Lington et al.(1997) who demonstrated that rats exposed to 0.3% dietary DINP (males:152 mg/kg bw/day, females: 184 mg/kg bw/day) had increased relative liver and kidney weights, and elevated serum transaminases.

Table 1. Amount of DINP ingested in different time periods in Moore's (1998) two-year study

Time (week of study)	Male Rats (mg/kg bw/day)	Female Rats (mg/kg bw/day)
24[a]	69	97.6
52	71	100.9
80[a]	74	94.9
104	73.9	79

a

No food consumption data were reported for 26 or 78 week and the consumption data of the nearest weeks were presented.

Based on the above analysis we conclude the NOEL for the systemic effects of DINP1 in the Moore study to be 500 ppm in diet (males: 29.2 mg/kg bw/day; females: 36.4 mg/kg bw/day).

References

Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala R a (1997) Chronic toxicity and carcinogenic evaluation of di isononyl phthalate in rats. *Fund. Appl. Toxicol.* **36**:79-89 .

Moore MR (1998) Oncogenecity study in rats with di isononyl phthalate including ancillary hepatocellular proliferation and biochemical analyses. Volume I, Covance Laboratories Incorporated, Vienna, VA 22182, May 13, 1998. Covance 2598-104. EPA/OTS Doc # 89-980000308/0556283-2.

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HEALTH CARE WITHOUT HARM

THE CAMPAIGN FOR ENVIRONMENTALLY RESPONSIBLE HEALTH CARE

December 8, 2000

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Comments on the NTP-CERHR Expert Panel Report on di(2-ethylhexyl) phthalate, October, 2000.

These comments are prepared by Ted Schettler MD, MPH on behalf of Health Care Without Harm (HCWH).

Exposure:

HCWH is aware that detailed human DEHP exposure data are limited. On pg. 8 of their report, the Expert Panel cites estimated daily intake by the population of Canada in Table 3. Here, indoor air exposures to DEHP are estimated to range from 0.85-1.2 micrograms/kg/day. However, Huber et. al note that indoor (or in car) inhalation exposures may exceed these estimates by as much as two orders of magnitude.[1 2] Highest indoor air exposures to DEHP are noted in rooms with flooring or wall-covering made of PVC plasticized with DEHP. Inhalation exposures to DEHP on the inside of cars may also be considerable, depending on temperature and construction materials. These observations imply that there may be a significant portion of the population exposed to DEHP in excess of the 3-30 micrograms/kg/day estimated by the panel.

The Panel also discusses DEHP inhalation exposures from PVC endotracheal tubes on page 13. As noted, Latini measured the DEHP content of endotracheal tubes before and after use and from that, was able to calculate the DEHP lost.[3] The Panel then says that the DEHP measurements involved overnight extraction in chloroform:methanol, and since that these conditions are much harsher than those present in vivo, the study can not be used to estimate exposures. This reasoning is unclear. Latini used that extraction technique in order to determine the amount of DEHP left in the endotracheal tube after varying periods of use. He was not suggesting that DEHP extraction with organic solvents somehow simulated in vivo conditions. Rather, he was simply asking how much DEHP was left in the tubes after their use and used the solvent extraction as a method for answering that question. He found an inverse relationship between the length of time that a tube had been used and the amount of DEHP that was later extractable.

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Of course, the extent to which DEHP from the tube is actually absorbed systemically is another question and was not examined in this study. Latini was prompted to study this question because of a hypothesized connection between DEHP exposure and bronchopulmonary dysplasia.

Animal models:

The Panel reviews a large body of animal data throughout their report and notes age- and species-dependent differences in the toxicity, absorption, metabolism, and kinetics of DEHP. Age-dependent differences are undoubtedly extremely important, in terms of risks to humans. Therefore, it is important that there be consistency and precision throughout the Panel report.

The reasons for age-dependent differences in testicular toxicity of DEHP are not fully understood. As the Panel notes, differences in tissue susceptibility are undoubtedly important. Metabolism of DEHP is also likely to be age-dependent, particularly in primates, where glucuronidation pathways are not mature at birth. Tissue susceptibility may be age-dependent for several reasons. Immature, dividing cells may be inherently more susceptible. But, it may also be the case that, in the immature testis, where the blood-testis barrier is not yet formed, circulating DEHP or MEHP may have greater access to the Sertoli cells and other components of the seminiferous tubules than in adults. That is, the tissue distribution of MEHP may differ in the immature and adult organism.

In humans and non-human primates, prepubertal Sertoli cells are scattered randomly throughout the seminiferous tubules.[4,5] Testosterone secretion early in puberty initiates migration of Sertoli cells toward the basement membrane, and nuclei show qualitative changes in size and shape. Realignment of the Sertoli cells along the basement membrane, along with other peritubular changes, form the blood-testis barrier. MEHP is >99% ionized at physiologic pH, based on a predicted pKa of 3.76.[6] Consequently, the presence or absence of an intact blood-testis barrier, along with the degree of development of metabolic and excretion pathways, are likely to be important determinants of exposure of the entire population of Sertoli cells and germ cells to circulating MEHP. Gray et al have shown that MEHP does not quickly cross the blood-testis barrier.[7] Dixon et al have shown the importance of pKa as a determinant of access to the tubular lumen.[8]

For these reasons, it is important to accurately characterize the age of animals used for experimental purposes. For example, in the study of cynomolgus monkeys by Pugh et al, the authors say that the animals were "young adult (~2 year old) male cynomolgus monkeys." The age of these animals is important but not precisely known. Lee, et al

report that cynomolgus monkeys at age 2.1 +/- 0.2 years already show evidence of testosterone rise and testicular volume.[9] It is, therefore, likely that these animals were studied when the blood-testis barrier was already somewhat adult-like and when tissue distribution of MEHP may vary from that expected in younger animals.

The Panel cites the study by Pugh et al and Kurata et al in a number of places in their report. As noted, the marmosets studied by Kurata et al are all also beyond the age of initial testosterone surge associated with puberty.[10] HCWH believes that it is important that the Panel report make it clear, whenever these studies are cited, that in each case, the animals were at least old enough to be in early puberty and that the observations can not be used to predict effects in younger animals. It would help if the Panel were to define what they mean by "prepubertal" (pg 25, 67). It would also be helpful for the Panel to make it clear on pg 72 that the marmosets were pubertal.

On page 94, the Panel says that "peripubertal" dosing is believed to be the most sensitive period for causing adverse effects. However, the Panel does not explain why they believe that to be true nor do they provide a reference.

Age-related sensitivity to DEHP exposure may be very important for estimating risks to humans. In humans, the blood-testis barrier is not intact until puberty and Sertoli cell proliferation occurs both in the neonatal period and again during puberty.[11] Therefore, human susceptibility to testicular toxicity from DEHP/MEHP exposure may be prolonged. Toxicological data from human studies will always be difficult, if not impossible, to obtain. Therefore, it is important that the animal data be carefully considered and accurately described.

Biotransformation:

In the discussion of biotransformation (pg 34-36) it would be helpful if the Panel were to make it clear that in the study of Albro, et al., humans and monkeys excrete glucuronides of MEHP to a significant degree (18% and 29% respectively) after IV dosing. This becomes important when estimating exposures to MEHP after dosing with DEHP via various routes.

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[3] Latini G, Avery GB. Materials degradation in endotrachael tubes: A potential contributor to bronchopulmonary dysplasia (letter). Acta Pediatr 88:1174-75, 1999.

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[7] Gray TJB, Gangolli SD. Aspects of the testicular toxicity of phthalate esters. Environ Health Perspect 65:229-235, 1986.
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[10] Abbott D, Hearn J. Physical, hormonal, and behavioral aspects of sexual development in the marmoset monkey, Callithrix jacchus. J Reprod Fertil 53(1):155-166, 1978.
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DEC 1 9 2000

Sept. 15, 2000

To;

National Institute of Environmental Health Sciences

P.O. Box 12233

Research Triangle Park, N.C. 27709

FROM: [redacted]

ECE
OCT 19 2000
BOF

RE: 60 day public comment period on phthalates.

I read the article in Science News of Sept. 2, 2000 - page 152-154 on phthalates with much interest. [redacted]

[redacted]

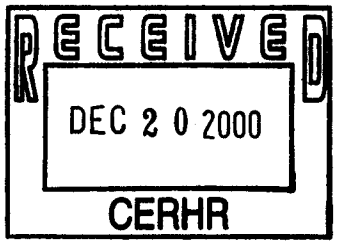
Appendix III

[redacted]

Sincerely,

[redacted]

Kemikalieinspektionen



20th December 2000

Comments on NTP-CERHR Expert Panel Report on Di(2-ethylhexyl)phthalate and Dibutyl phthalte.

Dear Dr. Shelby,

Thank you for allowing us an extended period to comment the NTP-CERHR Expert Panel Report on Di(2-ethylhexyl)phthalate.

Firstly we would like to congratulate you on your thorough and excellent presentation of information in your report on DEHP.

In overall we agree with the conclusions reached in the NTP-CERHR report on DEHP, with the exception for the conclusion that was reached with regards to the general adult population i.e. "minimal concern that ambient human exposures adversely affect adult human reproduction". We differ in our selection and emphasis placed on the Kurata et al. and Arcadi et al studies. Our assessment is found in detail in our EU Risk Assessment Report on DEHP (see attachment). For instance, considering the available information on the adverse testicular effects of DEHP and MEHP observed both in rodents and non-rodents we consider that exposure to DEHP is of concern also for adult humans. Although DEHP did not induce any adverse effects in the testes of sexually mature marmosets at both kinetically relevant (≥200 mg/kg/d) and irrelevant doses (e.g. 2500 mg/kg/d), there is at present no evidence that adult marmosets are the most relevant species regarding extrapolating testes effects to man. It is acknowledged that a recent publication (Sharpe et al) has demonstrated that the development of Sertoli cells in prepubertal marmosets are more similar to man than in the prepubertal rat, however, there is to our knowledge, limited toxicokinetic data (including biotransformation information) available for DEHP in the man and marmoset, neither is there any data available that support that the adult marmoset should be a more relevant species for man than other species from a dynamic point of view. . Furthermore, the effects of MEHP on marmoset apes is not known.

In our report we have accepted the results of the Arcadi et al to identify an LOAEL. We note from your report that you have not used the study to identify an NOAEL/LOAEL because you have concerns about the "exposure conditions" and this problem was not resolved by contacting the authors. We feel that it would be of benefit if you would more transparently detail your concerns in the report. Based on the physical-chemical properties of DEHP (lower density than water) and feeding practices normally used, we would, however, expect that the animals would have possibly received a lower dose of DEHP than document. In addition, that the recent study of Li et al., demonstrating effects on cell proliferation with a single dose of DEHP in three 3-day old rat pups further indicates that low doses of DEHP can cause adverse effects in very young rodents.

Exposure
We would also welcome a discussion of life time exposure and the possible consequences for a given population when considering a specific exposure scenario as a "snap-shot" in time. Although adults may be considered to be less sensitive to the effects of DEHP than young individuals, the young have previously been exposed to DEHP *via* other pathways of exposure. Because DEHP is ubiquitously present in our environment, persistent exposure, at a steady-state level, would be expected to occur both *in utero* and be life-long. It would be interesting if you would consider in your report the overall life time exposure with regard to the conclusion concerning adults.

The presence of DEHP in dental products intended for use by children is an area of potential concern. We know that this type of exposure occurs and we are endeavouring to collect further information – perhaps you have better access to this type of information in the US and, therefore, would consider including such information in your report.

We have detailed additional exposure situations in our EU Risk Assessment Report that may be relevant for your report:

- Car interiors
- Plastic gloves both in the residential setting and occupationally
- Occupational dermal exposure
- Dermal exposure of children to toys and child equipment

DBP
Concerning DBP, it is used in the coatings of pharmaceutical preparations (see attachment). For additional information, contact Kerstin Bergman at the Swedish Medical Protection Agency <Kerstin.Bergman@mpa.se>

Attachments:
- EU Risk Assessment Report on Di(2-ethylhexyl) phthalate – December 2000
- Exposure information on DBP in pharmaceuticals

New studies:
Loff et al., Polyvinylchloride Infusion Lines Expose Infants to Large Amounts of Toxic Plasticizers. Journal of Pediatric Surgery, Vol 35, 1775-1781, 2000

Li LH, Jester WF, Laslett AL, and Orth Jm. (2000). A single dose of di-(2-ethylhexyl) phthalate in neonatal rats alters gonocytes, reduces Sertoli cell proliferation, and decreases cyclin D2 expression. Toxicol. Appl. Pharmacol. 166, 222-229

Sharpe RM, Walker M, Millar MR, Atanassova, Morris K, McKinnell C, Saundrs PTK and Fraser HM. (2000). Effect of neonatal gonadotropin-releasing hormone antagonist administration on Sertoli cell number and testicular development in the marmoset: comparison with the rat. Biology of Reproduction 62, 1685-1693, 2000