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

Center For The Evaluation Of Risks To Human Reproduction Ê

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Butyl Phthalate (DBP) Ê

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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 website 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. DEHP has the greatest production volume of the selected phthalates (approximately 260 million pounds [1994]), followed by DIDP (approximately 240 million pounds [1994]), and DINP (approximately 215 million pounds [1994]). 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 DBP, a list of the expert panel members (Appendix I), the expert panel's report on DBP (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 DBP 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 DBP to cause adverse reproductive or developmental effects in people. It is based upon information about DBP 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 DBP 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.

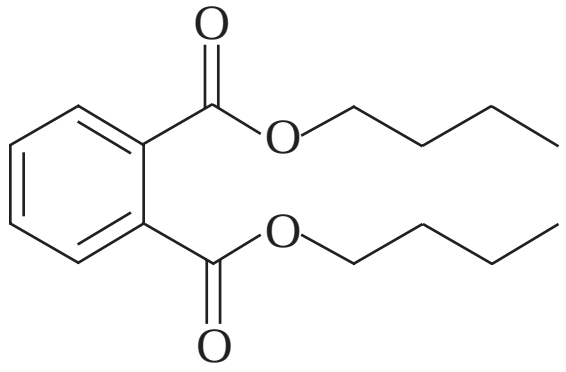
NTP Brief on Di-n-butyl Phthalate (DBP)

What is DBP?

DBP is a clear, oily liquid with the chemical formula $C_{16}H_{22}O_4$ and the structure shown in Figure 1. It is one of a group of industrially important chemicals known as phthalates. Phthalates are used primarily as plasticizers to add flexibility to plastics. Unlike many phthalates, DBP is not currently used as a plasticizer in polyvinyl chloride (PVC) plastics. Typically, DBP is used as a component of latex adhesives. It is also used in cosmetics and other personal care products, as a plasticizer in cellulose plastics, and as a solvent for dyes.

DBP is produced by reacting n-butanol with phthalic anhydride. The most recent information available indicates that approximately 7.7 million kilograms (17 million pounds) of DBP were produced in the United States in 1994 (ATSDR, 2001).

Figure 1. *Chemical structure of DBP*



Are People Exposed to DBP?

Yes. Blount et al. (2000) reported that more than 75% of the people they studied were exposed to DBP. There are several ways that people may be exposed to DBP at home or at work. Human exposure to DBP can occur during the manufacture of DBP, during the manufacture of DBP-containing products such as latex adhesives, cellulose-based plastics, during the use of such products, or through the presence of DBP in the environment.

Environmental exposures can occur through air, water, or food. Most people are exposed to DBP primarily through food. DBP migrates into foods, particularly fatty foods, from DBP-containing materials that are used to process and package food. Possible, sources of DBP found in food are latex adhesives used in food processing equipment and food wraps made of cellulose-based plastics. Cosmetics and other personal care products may be another important source of exposure through inhalation or contact with the skin. Studies to determine the extent of such exposures have not been conducted.

The expert panel estimated that the U.S. general population is exposed to approximately 2-10 μg/kg bw/day (micrograms per kilogram body weight per day). This reflects a total daily exposure of approximately 140-700 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 DBP 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 DBP. Kohn et al. estimated that 95% of people are exposed to less than 10 μg DBP/kg bw/day, consistent with the expert panel's estimate. However, they found that some women of reproductive age (20-40 years) are exposed to higher DBP levels than other age or sex groups. The majority of women in the age group were exposed to DBP levels well within the range of exposures estimated by the expert panel.

However, a small percentage was exposed to

30 μg/kg bw/day or greater and one individual was exposed to over 100 μg/kg bw/day. Neither the sources nor circumstances of these apparently higher exposures are known. It has been suggested that these higher exposures might be related to the use of DBP-containing personal care products such as perfumes, nail polishes, and hair spray (Blount et al., 2000).

Workers producing DBP can be exposed through skin contact or inhalation. The expert panel estimated that such exposures might be as high as 143 μg/kg bw/day, but are generally thought to be well below this level. Information is not available on exposure of workers who use DBP to manufacture other products.

Can DBP Affect Human Development or Reproduction?

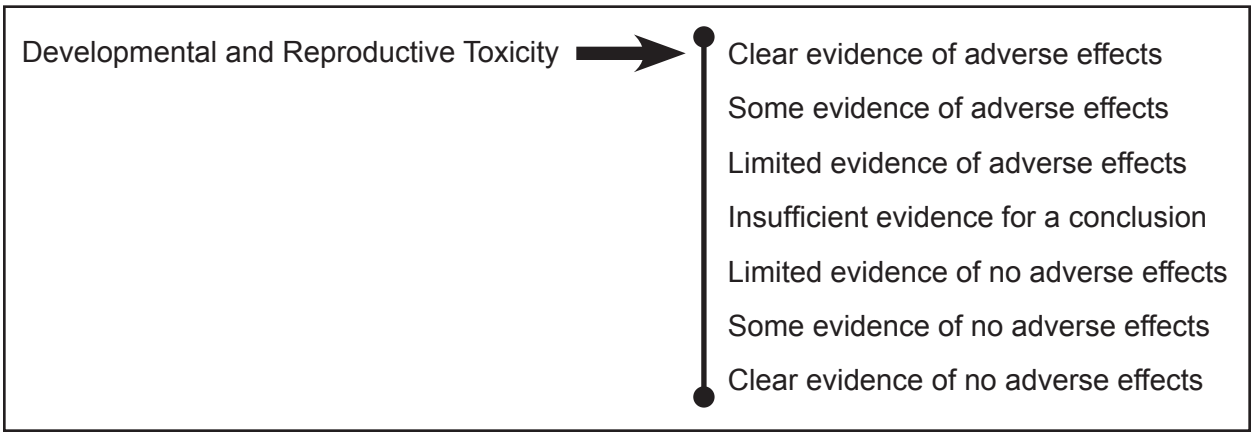
Probably. Although there is no direct evidence that exposure of people to DBP adversely affects reproduction or development, studies with laboratory rodents show that exposure to DBP can cause adverse effects (Fig. 2). Based on recent data on the extent to which humans absorb, metabolize and excrete DBP, 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 humans.

Scientific decisions concerning health risks are generally based on what is known as "weight-of-the-evidence." In this case, recognizing the lack of human data and the clear evidence of effects in laboratory animals (Fig. 2), the NTP judges the scientific evidence sufficient to conclude that DBP may adversely affect human reproduction or development if exposures are sufficiently high.

Summary of Supporting Evidence

As presented in the expert panel report (see report for details and literature citations), many of the DBP studies in rodents addressed both developmental and reproductive effects. These studies have reported that exposure of pregnant dams to high doses of DBP (greater than 500,000 μg/kg bw/day) causes reduced fetal survival and reduced birth weights among surviving offspring. In some instances, this exposure was also associated with skeletal malformations and abnormalities of the reproductive systems and organs in both male and female offspring. Exposure to DBP has also been shown to reduce fertility in female rats and mice. It is clear from studies with laboratory animals that rodents in prenatal and early postnatal stages of development are more sensitive to the reproductive effects of DBP than are adult animals. It is important to note that

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



DBP exposure levels that lead to these adverse effects in rodents are generally far higher than those experienced by people.

The developing male reproductive system of rodents appears particularly sensitive to the adverse effects of DBP exposure. There is growing evidence that this male sensitivity may result from a reduction in the level of the male sex hormone, testosterone, by DBP.

In a study published after the expert panel report was completed, Shono et al. (2000) showed that exposure to the monoester metabolite of DBP, monobutyl phthalate (MBP), is toxic to the male reproductive tract. Pregnant dams were given an oral dose of 300,000 μg/day of MBP on various days during pregnancy; fetuses were obtained by Caesarean section.

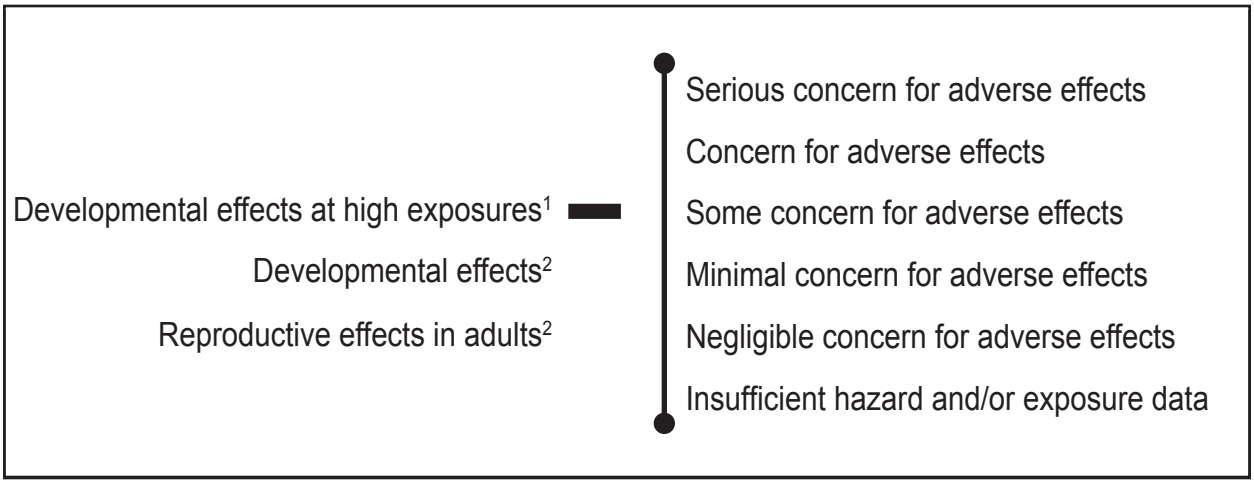
On gestation day 20, significant inhibition of testis migration was reported for male fetuses exposed to MBP on gestation days 11-14 and or 15-18, with the greatest inhibition observed in the latter group. There were also treatment-related effects on the male reproductive tract along with a reduction in testicular testosterone levels. This study supports the role of MBP in mediating DBP toxicity to the male reproductive tract.

Another recent report by Foster et al. (2000) proposes that the use of rat data to assess human risks for reproductive or developmental effects may be inappropriate because humans might be much less efficient at producing the active DBP metabolite, MBP. However, a recent study (Anderson et al., 2001) supports using DBP rodent data for evaluating potential effects in humans. It offers evidence that people efficiently absorb and metabolize DBP. The results show that human volunteers given an oral dose of 255 or 510 μg DBP excrete approximately 70% of it as MBP in urine after 24 hours.

Are Current Exposures to DBP High Enough to Cause Concern?

Possibly. More data are needed to better un-

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



[1] Based on Kohn et al. (2000) estimated exposure of some women of reproductive age to ~100 μg/kg bw/day (median 1.7; 95th percentile, 32; maximum, 113)

[2] Based on the experts panel's estimate of general population exposure from 2-10 μg/kg bw/day

derstand human DBP exposure levels and how these exposures vary across the population. The general U.S. population presently appears to be exposed to DBP at levels that are not of immediate concern for causing adverse reproductive or developmental effects. However, data are not available to permit conclusions regarding the possibility of effects in various age groups, occupations, or socioeconomic strata. Thus, the NTP offers the following conclusions (Fig. 3).

The NTP concurs with the CERHR Phthalates Expert Panel that there is minimal concern for developmental effects when pregnant women are exposed to DBP levels estimated by the Panel (2-10 µg/kg bw/day).

Based upon recent estimated DBP exposures among some women of reproductive age, the NTP has some concern for DBP causing adverse effects to human development, particularly development of the male reproductive system.

This level of concern is greater than that expressed by the Phthalates Expert Panel and is based on recent estimates that some women of childbearing age are exposed to levels of DBP that are approximately 10 times higher than general population exposures.

The NTP concurs with the CERHR Phthalates Expert Panel that there is negligible concern for reproductive toxicity in exposed adults.

However, further data and evaluation are needed to determine if the higher DBP exposure levels reported for some women of reproductive age justify a higher level of concern for effects on their reproductive system.

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.

References:

Anderson WAC, Castle L, Scotter MJ, Massey RC, Springall C. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Additives & Contaminants* **18**:1068-1074 (2001).

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David RM. Exposure to phthalate esters. *Environmental Health Perspectives* **108**: A440 (2000).

Foster PMD, Cattley RC, Mylchreest E. Effects of di-*n*-butyl phthalate (DBP) on male reproductive development in the rat: Implications for human risk assessment. *Food and Chemical Toxicology* **38**: S97-S99 (2000).

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).

Shono T, Kai H, Suita S, Nawata H. Time-specific effects of mono-*n*- butyl phthalate on the transabdominal descent of the testis in rat fetuses. *BJU International* **86**: 121-125 (2000).

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

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[2], 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 DBP 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).

[2]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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Appendix II

NTP-CERHR EXPERT PANEL REPORT

on

Di-*n*-Butyl Phthalate

October 2000 *NTP-CERHR-DBP-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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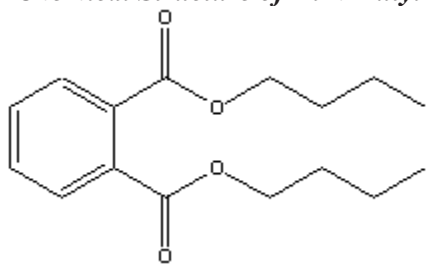
1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of Di-n-Butyl Phthalate



Di-n-butyl phthalate (DBP) (CAS RN 84-74-2) is produced through the reaction of n-butanol with phthalic anhydride (1).

Table 1: Physicochemical Properties of DBP

Property	Value
Chemical Formula	$C_{16}H_{22}O_4$
Molecular Weight	278.35
Vapor Pressure	2.7 x 10^{-5} mmHg at 25 °C
Melting Point	-35 °C
Boiling Point	340 °C
Specific Gravity	1.042
Solubility in Water	Slight: 11.2 mg/L
Log K_{ow}	4.45

(2)

1.2 Exposure and Usage

Overview

According to the American Chemistry Council (ACC, formerly CMA) (1), DBP is used mainly as a coalescing aid in latex adhesives. DBP is also used as a plasticizer in cellulose plastics and as a solvent for dyes. Although there was limited use of DBP in poly-vinyl chloride (PVC) plastics during the 1970's and 1980's, it is not currently used as a plasticizer in PVC. Release of DBP to the environment can occur during its production and also during the incorporation of the phthalate into plastics, adhesives, or dyes. Because DBP 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 intended for consumption by humans or livestock and can thus enter the food supply.

General Population Exposure

Exposure of the general population to DBP has been estimated by at least four authoritative sources: the International Program on Chemical Safety (3), the UK Ministry of Agriculture, Fisheries, and Food (MAFF) (4, 5), Health Canada (6), and the US Agency of Toxic Substances and Disease Registry (7). Levels of DBP in exposure media, assumptions used in exposure calculations, and estimated exposure levels are detailed in Table 2 (3), Table 3 (7), and Table 4 (6).

Table 2: IPCS Exposure Estimates for Adults

	Ambient Air	Indoor Air	Drinking Water	Food
DBP Concentration in Media	0.0045–0.0062 μg/m^3	0.420 μg/m^3	<1.0 μg/L	Various levels in a Canadian market basket survey. (See text)
Assumptions	22 m^3 inhaled/day; 64 kg bw; 4/24 hours outdoors	22 m^3 inhaled/day; 64 kg bw; 20/24 hours indoors	1.4 L/day intake; 64 kg bw	Various intake rates for different food types; 64 kg bw
Estimated Doses μg/kg bw/day	0.00026–0.00036	0.120	<0.02	7

(3)

Table 3: ATSDR Exposure Estimates for Adults

	Ambient Air	Drinking Water	Fish
DBP Concentration in Media	0.003–0.006 μg/m^3	0.2 μg/L	78–200 μg/kg
Assumed Intake Rate	20 m^3/day/70 kg adult	2 L/day/70 kg adult	6.5 g/day/70 kg adult
Assumed Absorption Fraction	0.5	0.9	0.9
Estimated Dose (μg/kg bw/day)	0.0005–0.0009	0.005	0.007–0.02

(7)

Table 4: Health Canada DBP Exposure Estimates

	ESTIMATED INTAKE DBP (μg/kg bw/day)				
Substrate/Medium	0.0–0.5 years old	0.5–4 years old	5–11 years old	12–19 years old	20–70 years old
Ambient Air*	0.00030	0.00040	0.00041	0.00038	0.00034
Indoor Air	0.68	0.91	0.1	0.87	0.78
Drinking Water	0.11	0.062	0.033	0.022	0.021
Food	1.6	4.1	3.2	1.4	1.1
Soil*	0.0070	0.0054	0.0018	0.00049	0.00040
Total Estimated Intake	2.4	5.0	4.3	2.3	1.9

* Value represents the upper range of the estimates. (6)

As noted in exposure estimates by the IPCS, Health Canada, and ATSDR, the largest source of DBP exposure to the general population is food. Sources of DBP in food include environmental uptake during crop cultivation or migration from processing equipment or packaging materials. IPCS (3) and Health Canada (6) conducted more comprehensive exposure estimates. Both exposure estimates were based on a 1986 Canadian market-basket survey of 98 different food types. Foods reported to contain DBP included butter (1.5 mg/kg), margarine (0.64 mg/kg), freshwater fish (0.5 mg/kg), cereal products (0–0.62 mg/kg), baked potatoes (0.63 mg/kg), bananas (0.12 mg/kg), coleslaw (0.11 mg/kg), gelatin (0.09 mg/kg), and white sugar (0.2 mg/kg). DBP exposure through food intake in adults was estimated at 7 μg/kg bw/day by IPCS (3) and at 1.9 μg/kg bw/day by Health Canada (6). DBP exposures in children were also estimated by Health Canada by applying appropriate assumptions such as intake rates of different food types per age group. Estimated DBP exposure levels from food ranged from 2.3 μg/kg bw/day in children aged 12–19 years to 5.0 μg/kg bw/day in children aged 6 months to 4 years.

MAFF (4) estimated adult DBP exposure through dietary intake based on a 1993 survey of fatty foods in the UK. DBP was detected in carcass meat (0.09 mg/kg), poultry (0.2 mg/kg), eggs (0.1 mg/kg), and milk (0.003 mg/kg). 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 DBP intakes were estimated at 13 μg DBP/person/day and 31 μg DBP/person/day, respectively. Specific details describing the calculations and assumptions used were not provided. Using the IPCS-assumed (3) adult body weight of 64 kg, the exposure values were converted to 0.20–0.48 μg/kg bw/day.

MAFF also addressed DBP exposure in infants resulting from the consumption of infant formula. A survey published in 1996 reported DBP levels of 0.08–0.4 mg/kg in infant formulas purchased in the UK, while a later survey reported DBP levels of <0.05–0.09 mg/kg (5, 8). It is speculated that the drop in DBP concentration occurred because infant formula manufacturers were urged to reduce phthalate levels after MAFF published the results of the 1996 survey. Exposure levels were estimated for infants based on the results from the 1998 survey using assumed body weights of 2.5–3.5 kg at birth and 7.5 kg at 6 months of age. Formula intake rates were determined from manufacturer instructions. Exposure levels for infants were estimated at 2.4 μg/kg bw/day at birth and 1.4 μg/kg bw/day at 6 months of age. 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). DBP has also been reported in baby food and breast milk samples collected from Germany and Japan; average values were within ranges reported by MAFF. DBP was measured in 7 German baby food samples (average 0.033 mg/kg), 8 baby formulas (<0.2–0.9 mg/kg; average 0.042 mg/kg), and in the breast milk of 5 mothers from Germany (average 0.035 mg/kg) and 3 from Japan (0.02–0.08 mg/kg). The time period when these samples were collected was not specified (1).

In their estimates of dietary exposure, ATSDR (7) only considered fish intake because at that time it was the only food source for which reliable data were available. The dietary estimate of 0.007–0.02 μg/kg bw/day was based on DBP levels of 78–200 μg/kg that were reported for fish in studies

published between 1973 and 1987.

Levels of DBP in drinking water were estimated to be minimal. DBP exposure to adults through drinking water was estimated at 0.02 μg/kg bw/day by IPCS (3) and Health Canada (6) based upon a survey of drinking water supplies in Ontario, Canada. Health Canada also estimated DBP exposures through drinking water intake in children and those values ranged from 0.022 μg/kg bw/day in children aged 12–19 years to 0.11 μg/kg bw/day in infants aged 0–6 months. Adult DBP exposure through drinking water was estimated by ATSDR (7) at 0.005 μg/kg bw/day. The value was based on a survey of drinking water in 10 unspecified cities prior to 1986.

Mouthing of toys is another potential source of oral phthalate exposure in children. However, use of DBP in toys appears to be rare. In an analysis of 17 plastic toys, DBP was only detected in 1 polyvinyl chloride doll's head at 0.01% by weight (10).

Although off-gassing from building materials has been reported as a potential source of DBP exposure through inhalation, exposure has been postulated to be minimal because of the low vapor pressure of DBP. The available data, though minimal, support this view. IPCS (3) estimated that adults are exposed to 0.120 μg/kg bw/day through inhalation of indoor air. The estimate was based on the mean air concentration of DBP measured within 125 homes in California in 1990. Health Canada also estimated indoor inhalation exposure to DBP based on a survey of DBP air levels in 9 homes in Montreal (reported in 1985). Exposure to adults was estimated at 0.78 μg/kg bw/day and exposures in children ranged from 0.68 μg/kg bw/day in 0–6 month-old infants to 1.1 μg/kg bw/day in 5–11 year-old children. Exposures to DBP through ambient air was also estimated by IPCS (3) and Health Canada (6); the values were roughly 2–3 orders of magnitude lower than the indoor air estimates.

Dermal contact with products containing DBP is possible, but absorption through skin is most likely minimal. Studies in rats have demonstrated that absorption of DBP through skin is fairly slow (11). An *in vitro* study conducted with rat and human skin has demonstrated that human skin is much less permeable to DBP than is rat skin (12).

Caution is required to interpret exposure data for the general population. IPCS has emphasized that dietary intake can vary widely depending on the types of food eaten and the types of material in which the foods are packaged. In addition, the majority of data used to estimate exposure levels was collected 15–20 years ago and may not reflect current exposure levels. Lastly, exposures in children may be higher due to non-dietary intake through mouthing of DBP-containing objects.

Medical Exposure

According to IPCS (3), a DBP level of 5 mg/g was measured in plastic tubing used for oral/nasal feeding. There are no other known uses of DBP in medical equipment.

Occupational Exposure

Exposure in occupational settings can occur through skin contact and by inhalation of vapors and dust. Phthalates are manufactured within closed systems, but workers can be exposed 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. In a limited number of surveys, DBP levels in US plants have ranged from concentrations below the detection limit (0.01–0.02 mg/m^3) to 0.08 mg/m^3 (3). OSHA established a permissible exposure limit of 5 mg/m^3 for DBP. Following a review of six studies, the ACC has estimated exposure to DBP in the workplace based upon an assumed level of 1 mg/m^3 during the production of phthalates (1). Exposure levels during the incorporation of DBP into plastics are not known. 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 estimate was 143 μg/kg bw/workday for workers employed in phthalate manufacturing. The maximum exposure, by regulation, would be five-fold greater. As stated in the General Exposure section, absorption of DBP through skin is expected to be minimal.

Conclusion

Exposure estimates varied between authoritative bodies. However, in all cases it was evident that food was the primary source of exposure to DBP. ATSDR only considered fish intake, and their exposure estimate therefore provides no information on total dietary exposure. The dietary exposure estimate by MAFF is approximately one order of magnitude lower than estimates by IPCS and Health Canada. 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 DBP levels in foods from different countries; and changing DBP levels in food over time. Table 5 lists the dietary DBP estimates calculated by the different agencies for infants and adults.

Table 5: Comparison of DBP Dietary Estimates

Agency	Exposure in Infants (0–6 months) (μg/kg bw/day)	Exposure in Adults (μg/kg bw/day)
IPCS (3)	N/A	7
MAFF (4, 5, 8)	1.4–2.4	0.2–0.48
ATSDR (7)	N/A	0.007–0.02
Health Canada (6)	1.6	1.1

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

There were no human data located for Expert Panel review.

2.1.2 Experimental Animal Data

Multiple evaluations are available for assessing the effects of oral exposure to DBP. A few inhalation and dermal evaluations have also been conducted; these studies are primarily in rats with a few assessments in mice, rabbits, hamsters, and guinea pigs.

Acute studies

The oral LD_{50} for DBP appears to be between 8,000 and 20,000 mg/kg bw in rats (3) and the 90-day dermal LD_{50} is 4,200 mg/kg bw in rabbits. Slight irritation was observed in rabbit dermal occlusion studies at 520 mg/kg bw.

Repeat-dose studies

In a 3-month sub-chronic study, 6-week old Wistar rats, 10 of each sex per dose, were fed a diet containing 0, 400, 2,000 or 10,000 ppm DBP (13) (Table 7-1). In addition to developing a toxicological profile of DBP, a stated purpose of the study was to evaluate possible neurological or testicular toxicity. A battery of standard hematological and clinical chemistry parameters (including thyroid function) was evaluated at points approximately halfway through and at the end of the study. Cyanide insensitive palmityl-CoA oxidation (PCoA) was also determined as a measure of peroxisome proliferation. Urinalyses were performed at the midpoint and at the end of the study. Neurological function, using the EPA functional observation battery, was assessed prior to DBP administration, and on days 34, 59, and 90 of the study.

Dietary consumption was not a factor in the study; nominal daily doses were calculated to be 27 (M) and 33 (F) mg/kg bw/day, 142 (M) and 162 (F) mg/kg bw/day, and 688 (M) and 816 (F) mg/kg bw/day for the three dose groups. Effects were observed only in the high-dose group, 688 (M), and 816 (F) mg/kg bw/day. Statistically significant increases in liver and kidney to body weight ratios were observed in the absence of body weight changes in females. Histologically, a decrease in lipid deposition was noted in hepatocytes; this effect was possibly due to peroxisome-related enzyme increases in the liver. An increase in PCoA activity was confirmed. Serum triglycerides and triiodothyronine were both decreased. RBC, hemoglobin, and hematocrit were transiently decreased in males. No histological effects on testes appropriately preserved in Bouin's fixative were observed.

Neurological function was assessed at three time points during the study and no effects were observed. A LOAEL was observed at 688 (M) and 816 (F) mg/kg bw/day based on multiple impacts and a NOAEL was determined at 142 (M) and 162 (F) mg/kg bw/day.

Marsman (14) reported two 13-week, sub-chronic NTP studies using male and female F344 rats. One of the studies was of traditional design; 5–6 week-old rats were exposed to either control or one of four test diets. In the second study, rats placed in a standard sub-chronic design were born and reared by mothers exposed to 10,000 ppm DBP during pregnancy and nursing; at weaning, they were further exposed to a 10,000 ppm DBP diet until 8 weeks of age.

In the standard study, 10 F344 rats/sex were exposed to DBP in their diet for 13 weeks starting at 5–6 weeks of age (Table 7-2). The dietary levels were 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm (M: 0, 176, 359, 720, 1,540, and 2,964 mg/kg bw/day; F: 0, 177, 356, 712, 1,413, and 2,943 mg/kg bw/day). At the end of the study the rats were killed and necropsied with extensive tissue examination (testes preserved in 10% neutral buffered formalin), hematology and clinical chemistry, sperm morphology, and vaginal cytology parameters were evaluated. Zinc and testosterone levels were measured in sera and testes of all males. An increase in serum albumin was observed in exposed males at 176 mg/kg bw/day, the lowest dose tested. No other effects were seen in either sex at this dose. Adverse effects in males seen at the next highest dose (359 mg/kg bw/day) were evidenced by a decrease in hemoglobin and erythrocyte counts. Severity of the hematological effects, seen only in males, progressed in a dose-response manner at all other doses. Platelets and serum albumin were increased, as were liver and kidney organ to body weight ratios. An increase in PCoA activity was seen in both sexes, and an increase in bile acid was seen in females. Decreases in body weight occurred in males at the 720 mg/kg bw/day dose, the third highest out of 5 treatment levels. Males exposed to 359 mg/kg bw/day and males and females exposed to 712–720 mg/kg bw/day had increased liver and kidney organ to body weight ratios. Hepatic lesions in males and females and testicular lesions were first noted at 712-720 mg/kg bw/day. Testicular lesions consisted of focal seminiferous tubule atrophy in 4/10 males. The chemistry changes noted at the next lower dose (356–359 mg/kg bw/day) continued at this dose (712–720 mg/kg bw/day) with the addition of increases in alkaline phosphatase activity. The histologic hepatic lesions persisted and testicular lesions increased in severity at the higher doses with all males of that dose group affected. Hypospermia of the epididymis was observed at the two highest doses. Decreases in testicular organ weight ratios, testicular zinc, and testosterone were not observed until the 1,540 (M) mg/kg bw/day exposure level. Peroxisome proliferation was noted histologically at the highest dose tested (2,964 [M] and 2,963 [F] mg/kg bw/day). Good dose-response data was available for almost all parameters in this study. A NOAEL of 176 mg/kg bw/day was identified by the Expert Panel.

In the second NTP sub-chronic study, F344/N rats were born and reared by mothers exposed to 10,000 ppm DBP in diet throughout prenatal development and lactation; the weaned rats were then fed a 10,000 ppm diet until 8 weeks of age (14) (Table 7-3). At that time, the male rats, 10 per sex per group, were placed on 1 of 5 diets for an additional 13 weeks that contained 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm DBP (M: 0, 138, 279, 571, 1,262, or 2,495 mg/kg bw/day; F: 0, 147, 294, 593, 1,182, or 2,445 mg/kg bw/day) (14). The sub-chronic exposure doses and the protocols for histopathology, hematology, and chemistry were the same as the NTP sub-chronic study discussed above. The authors concluded that developmental exposure to DBP resulted in neither increased sensitivity nor resistance to DBP exposure during adulthood (compare results in Tables 2 and 3). The Expert Panel notes that there were significant increases in organ to body weight ratios for kidney and liver in females and in testes at the lowest exposure group, 138 (M) and 147 (F) mg/kg bw/day. Such findings were not observed at this dose level in the other sub-

chronic study.

The NTP also conducted a sub-chronic study in 6-week-old $B6C3F_1$ mice where 10 mice per sex were fed DBP in the diet for 13 weeks at levels of 0, 1250, 2,500, 5,000, 10,000, and 20,000 ppm (M: 0, 163, 353, 812, 1,601, and 3,689 mg/kg bw/day; F: 0, 238, 486, 971, 2,137, and 4,278 mg/kg bw/day (14) (Table 7-4). Experimental design in this study was similar to the 13-week sub-chronic study in rats. There were no clinical signs related to exposure and all mice survived until the end of the study. Decreases in body weight gain were observed in both sexes fed levels of 812 mg/kg bw/day or higher. Increases in absolute and relative kidney weight were seen in all treated female groups, but absolute kidney weight was decreased in high-dose males. There was no report of histological change in the kidney nor did weights increase with increasing dose. The liver was the only organ identified as a site of DBP toxicity by the study authors. Relative liver weights were increased at doses of 812 mg/kg bw/day and higher. Cytoplasmic alterations consisting of fine eosinophilic granules, more intensely-staining cytoplasm, and increased lipofuschin were observed at the 2 highest doses in males (1,601 and 3,869 mg/kg bw/day) and at the highest dose in females (4,278 mg/kg bw/day). A reduced hematocrit level was observed in high-dose females. Based on decreased body weight gain, the NOAEL is 353 mg/kg bw/day in males. A LOAEL based on increased kidney weight in females is 238 mg/kg bw/day, the lowest dose tested according to the Expert Panel.

In a series of three identical experiments, Walseth and Nilsen (15) examined lung and liver effects in groups of five male Sprague-Dawley rats. The rats were exposed for 6 hours/day for 5 days to DBP vapors at 0, 0.5, 2.5, or 7.0 ppm (0, 5.7, 28.4, and 79.5 mg/m^3 as calculated by authors). There were no effects on lung or liver weights. In the lung, there were dose-related decreases in microsomal cytochrome P-450 and cytochrome c-reductase levels in the two highest dose groups. There were no dose-related changes in liver cytochrome levels. A significant decrease in serum levels of alanine aminotranferase (ALAT) and significant increases in serum aspartate aminotranferase and albumin levels were observed, but the authors indicated that there was no evidence of liver cell damage. The authors concluded that the lung is the main target organ following inhalation exposure to DBP.

2.2 Toxicokinetics

Phthalate Moiety

Absorption

Humans: Dermal. In an in vitro study, human skin absorption rate was reported as 0.07 $\mu g/cm^2$/hour (12) which was considered "slow."

Humans: Oral. DBP was detected in blood from humans following ingestion of foodstuffs containing DBP (3). Background levels of DBP in human blood were much higher following exposure. Unfortunately, the authors measured only the parent compound so there is no estimate of

total DBP equivalents absorbed in this study. Similarly, levels of DBP in human adipose tissue were studied (16); again total DBP equivalents were not calculated.

Rodents: Dermal. Dermal absorption of DBP was studied in Fischer 344 rats by applying 30–40 mg/kg radiolabeled DBP to the skin (administration site occluded) and measuring the radioactivity in urine (11). Approximately 10–12% of the dose was excreted in urine per day with approximately 60% of the dose excreted within 1 week. Thirty-three percent of the dose was present at the application site 1 week following treatment.

Rodents: Oral. The extent of intestinal absorption of phthalate esters has been estimated by monitoring urinary excretion of the parent compounds or their metabolites after orally administering a known amount of the radiolabeled compound. Greater than 90% of radioactivity following an oral dose of DBP in rats is recovered in the urine within 2 days, indicating nearly complete intestinal absorption of this compound over a range of administered doses (17). This is consistent with the general observation that dialkyl phthalate esters are well absorbed following oral dosing. It is generally accepted that orally-ingested phthalate diesters are quantitatively hydrolyzed by gut lipases and absorbed almost entirely as the corresponding monoester.

Biotransformation

Humans. In a study comparing the relative rates of monohydrolysis of DBP by rat, baboon, and human gut preparations, Lake et al. (18) demonstrated that these species possess similar intrinsic lipase activity. Rates observed in human intestinal preparations were similar enough to the other species to expect that human intestinal metabolism of DBP would result in absorption of the monoester similarly to rats. The activity of pancreatic lipase was not assessed, so the quantitative relationships of this study to in vivo exposure cannot be accurately determined (18).

Rodents. Dialkyl phthalates including DBP were found to be metabolized to the monoesters by enzymes present in many tissues. It is generally accepted that orally-ingested phthalate diesters are quantitatively hydrolyzed by lipases in the wall of the small intestine and pancreatic lipases and not by gut flora. Absorption occurs almost entirely as the corresponding monoester (19).

Metabolites of DBP include monobutylphthalate, monobutylphthalate glucuronide, o-phthalic acid and oxidized monobutylphthalate glucuronide metabolites (17).

Distribution

Humans. No human data were located for Expert Panel review.

Rodents. DBP is rapidly cleared following oral or intravenous (IV) administration. There is little or no bioaccumulation observed. Radioactivity associated with DBP administration can be found in the GI tract and excretory organs of the liver and kidney, and in fat. Liver, kidney, and the GI tract probably accumulate the phthalate esters as a mechanism of excretion and not as depots (20). One week following dermal treatment of Fischer 344 rats with 30–40 mg/kg radiolabeled DBP, no tissues examined contained more than 2% of the administered dose (11).

Pregnant Rodents. Saillenfait et al. (21) studied metabolism and placental transfer of ^{14}C-DBP, administered by gavage on gestation day (gd) 14 at 500 or 1,500 mg/kg to Sprague-Dawley rats. Radioactivity peaked followed by a rapid decline in all tissues within 1–2 hours of administration. Maternal plasma had the highest peak concentration; all tissue levels were less than 7% of peak concentrations by 24 hours. Fifty-five percent and 29% of a 500 mg/kg ^{14}C dose were detected in urine and feces respectively in 24-hour samples; there was a slight increase to about 60% in urine at 48 hours, whereas fecal values did not change. Radioactivity in placenta, embryo, and amniotic fluid were 0.3, 0.15, and 0.2% of the administered dose, respectively. Concentrations in placenta and embryo did not exceed 30 and 21% of maternal plasma levels. The 1,500 mg/kg dose indicated slower absorption from the gastrointestinal tract; total fecal radioactivity was not affected, although there was lower excretion in urine over 48 hours. In maternal plasma, placental, and embryonic tissues, monobutyl phthalate (MBuP) and its glucuronide represented most of the DBP-derived activity. MBuP Levels ranged from 50 to 95%, dependent upon the time after administration when samples were taken. In contrast, unchanged DBP accounted for less than 1%. The authors speculate that the lower levels of MBuP glucuronide in embryonic tissues compared to those in maternal plasma could reflect limited placental transfer or limited ability to conjugate this substrate. Levels of radioactivity in placenta and embryos associated with DBP administration were approximately 65% of the levels found in maternal serum and there was no bioaccumulation of radioactivity observed in the embryonic tissues. DBP, MBuP, and MBuP-glucuronide were present in embryonic tissues at levels lower than were found in maternal plasma. MBuP accounted for most of the radioactivity recovered in maternal plasma, placenta, and embryos, which is consistent with the hypothesis that MBuP is the ultimate teratogenic species in vivo.

Distribution following IV exposure produces a different distribution pattern than that observed following oral administration. Since DBP is not in direct contact with gut esterases, metabolism to the monoester is slowed. This produces more DBP-associated radioactivity to distribute to lungs and blood in addition to liver and kidney. Radioactivity was detectable in adipose tissue 7 days after IV exposure (22). The difference between the oral and IV distribution probably reflects a higher concentration of parent DBP reaching adipose tissue following IV exposure, which would be expected to distribute to lipophilic tissues such as adipose tissue.

Excretion

Humans. No human data were located for Expert Panel review.

Rodents. The primary route of MBuP, the major DBP metabolite, elimination in rodents and humans is urinary excretion. The monobutylphthalate glucuronide appears to be the primary metabolite identified in rat urine (23). MBuP is excreted into the bile (about 45%), but only about 5% is eliminated in the feces, indicating that efficient enterohepatic recirculation occurs (17). Biliary metabolites of DBP include monobutylphthalate, monobutylphthalate glucuronide, and oxidized monobutylphthalate glucuronide metabolites (17). Following dermal exposure of rats to DBP, urine was the primary route of excretion with the excretion rate remaining nearly constant at 10–12% of the dose excreted per day (11).

Mice are known to excrete higher amounts of glucuronidated phthalate ester metabolites than rats and primates excrete higher levels of glucuronidated phthalate ester metabolites than mice (24). There appears to be little retention of DBP or MBuP in tissues of rats treated with DBP for 12 weeks (20).

Models

A physiologically-based pharmacokinetic (PBPK) model of the tissue distribution of DBP and its monoester metabolite, MBuP, in rats administered DBP by various routes has been developed by Keys et al. (25). The model is based on an earlier model developed by the same group for DEHP and its metabolite, MEHP (26). It includes a combined perfusion-limited and pH trapping mechanism for uptake of MBuP into tissues, and it provides a valuable tool for extrapolations of tissue doses among various routes and rates of exposure. With modification, the model can be used to extrapolate doses to target tissues among various species and ages and between genders and gravid vs non-gravid females. The model allows estimation of the internal dose to specific target tissues for the evaluation of risk, rather than using total exposure or total internal dose as a risk estimate.

Side Chain-associated Toxicokinetics (butanol)

Butanol, a metabolite of DBP, is a primary alcohol that is easily oxidized to butyric acid (n-butanoic acid) by alcohol dehydrogenase and aldehyde dehydrogenase. Further metabolism by oxidation pathways converts butyric acid into acetyl-CoA conjugates in intermediary metabolism pathways with no toxicological importance (27).

2.3 Genetic Toxicity

DBP has tested negative or marginally positive in gene mutation and chromosomal aberration studies. The ASTDR (7) concluded that DBP may be weakly mutagenic. The significance of these findings is not known because in vivo genotoxicity studies have not been conducted. The Woodward et al. (28) review concluded that the evidence indicates that DBP is not directly genotoxic, but noted it does cause increases in sister chromatid exchanges and small increases in the incidence of gaps and breaks. However, the effect does not appear to be dose-related (29). IPCS (3) reviewed a number of mutagenic and related endpoints for DBP and concluded that the weight of the evidence indicated that DBP is not genotoxic. DBP was positive in the L5178Y mouse lymphoma assay in the presence, but not in the absence, of an Aroclor-induced rat liver activation system (S9) (30). The authors conclude that the positive activity was likely the result of in vitro metabolism of the DBP to an aldehyde, and therefore, that the results may not represent any real potential for in vivo genotoxicity. DBP is not mutagenic in the Salmonella/mammalian microsome mutagenicity assay (31), and was negative in the Balb/3T3 cell transformation assay (30).

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

A number of studies have evaluated DBP for both prenatal and postnatal developmental toxicity; the vast majority of studies have been performed in the rat using the oral route of exposure. In most cases, the doses were high (> 0.5% in diet; > 500 mg/kg bw/day), and the number of animals per dose group was small (10–15).

3.2.1 Prenatal Development

DBP

Results from a set of investigations in mice have been reported by Shiota et al. (32) and Shiota and Nishimura (33) (Table 5). They evaluated the effects of oral exposure to DBP in concentrations of 0, 0.05, 0.1, 0.2, 0.4, and 1.0% in the diet. On the day a cervical plug was observed (gd 0), female ICR-JCL mice commenced eating the DBP diet until they were killed on gd 18. Using food consumption data, the authors calculated mean daily intake of DBP to be 0, 80, 180, 350, 660, and 2,100 mg/kg bw/day. Six-to-nine litters were examined per dose group, except that 15 litters were examined from the highest dose group. Food intake levels were not affected in pregnant dams. Maternal weight gain was significantly reduced at the high dose (2,100 mg/kg bw/day), but the effect may have been secondary to increased fetal loss. Resorptions (prenatal mortality) were significantly increased (98.4%) in the high-dose group. At this dose, malformations in 2/3 surviving fetuses (increase not statistically significant) were limited to neural tube defects (exencephaly and spina bifida, to which murine species are predisposed). Delayed ossification was observed at all dose levels as indicated by a reduction in the number of ossified coccygia in treated fetuses (n=9.4, 5.1, 4.5, 6.0, and 2.6 in the control to 660 mg/kg bw/day groups). Reduced fetal body weight was observed at the two highest doses. Because ossification was delayed at all dose levels, a developmental NOAEL could not be identified for this study and, therefore, a LOAEL of 80 mg/kg bw/day was selected by the Expert Panel. However, the authors stated that "the maximum non-embryotoxic dose" was 370 mg/kg bw/day. The maternal NOAEL and LOAEL were identified as 660 and 2,100 mg/kg bw/day, respectively.

Ema et al. (34-36) used Wistar rats to evaluate the developmental toxicity of DBP by exposure through gavage and feed. In all studies, dams were sacrificed on gd 20–21 and examined for implantation sites. Fetuses were weighed and examined for external, skeletal, and visceral malformations. In one Ema (34) study, 12 dams/group were gavaged with 0, 500, 630, 750, or 1,000 mg/kg bw/day (0, 1.80, 2.27, 2.70, or 3.60 mmol/kg bw/day) on gd 7–15 (Table 7-6). Gestational weight gain was reduced in dams of the 630 mg/kg bw/day group and adjusted weight gain (dam weight not including gravid uterus) was reduced in dams exposed to 750 mg/kg bw/day and higher.

Complete resorptions occurred in 2/12, 10/12, and 9/9 litters of the 630, 750, and 1,000 mg/kg bw/day dose groups, respectively, thus resulting in decreased live fetuses/litter. Fetal weight was reduced in groups exposed to 630 mg/kg bw/day and higher. External malformations, consisting entirely of cleft palate, were increased in the 750 mg/kg bw/day group. Maternal and developmental NOAELs and LOAELs of 500 and 630 mg/kg bw/day, respectively, were identified.

Another study conducted by Ema et al. (36) is of particular interest because it examines additional endpoints including anogenital distance and testicular descent (Table 7-7). In this study, 11 dams/group were fed diets containing 0, 0.5, 1.0, or 2.0% DBP on gd 11–21. Authors estimated daily intake rates of 0, 331, 555, and 661 mg/kg bw/day for the control to high-dose groups, respectively. Maternal gestational and corrected weight gain were reduced in dams exposed to 555 mg/kg bw/day and higher and were accompanied by a reduction in food intake. Fetal weight was reduced and the incidence of external malformations (cleft palate) and skeletal malformations (fused sternebrae) were increased in the 661 mg/kg bw/day dose group. Reduced anogenital distance and increased incidence of undescended testes were observed in male fetuses exposed to 555 and 661 mg/kg bw/day. The maternal and developmental NOAEL and LOAEL of 331 and 555 mg/kg bw/day, respectively, were identified for this study.

The two remaining studies by Ema et al. (35, 37) focused on the time- and dose-dependency of DBP developmental toxicity. In the studies, groups of 10–13 pregnant rats were gavaged with 0, 750, 1,000, 1,250, or 1,500 mg/kg bw/day on gd 7–9, 10–12, or 13–15. Resorptions were increased in all dose groups at all time points. All dams treated with 1,500 mg/kg bw/day experienced complete litter resorptions. However, the types and frequencies of malformations varied according to the exposure time course. Treatment on gd 10–12 did not result in an increased malformation rate. Treatment with doses of 750 mg/kg bw/day and higher on gd 7–9 resulted in increased skeletal malformations (fusion or absence of vertebral arches and ribs). Administration of 750 mg/kg bw/day and higher on gd 13–15 resulted in the greatest incidence of teratogenicity, including increased external malformations (cleft palate) and skeletal malformations (fusion of sternebrae).

Saillenfait et al. (21) exposed Sprague-Dawley rats (27 per group) to a single administration of DBP by gavage on gd 14 at 0, 500, 1,000, 1,500, or 2,000 mg/kg body weight. Increased resorptions at 1,500 and 2,000 mg/kg and reduced fetal body weights at 2,000 mg/kg were observed. Skeletal variations were also increased at these doses. Key aspects of the paper were studies on metabolism and placental transfer of ^{14}C-DBP, administered by gavage on gd 14 at 500 or 1,500 mg/kg. The toxicokinetic data are presented in Section 2.2. The authors concluded that their data support the view that MBuP may be the proximate toxicant.

Developmental effects were also noted in reproductive toxicity studies, which are discussed in detail under Section 4. In a continuous-breeding study, two generations of Sprague Dawley rats were exposed to 0, 80, 385, or 794 mg/kg bw/day through diet during a 98-day mating period (38). Maternal effects were only observed in the high-dose group and included a decrease in body weight for both generations and increased liver and kidney weights in F_0 dams. Developmental effects included a reduction in litter size in all dose groups and in live pup weight in the two highest doses of F_1 rats. F_2 pups in all treatment groups experienced a reduction in body weight. A developmental LOAEL of 80 mg/kg bw/day and a maternal NOAEL of 385 mg/kg bw/day were identified.

A similar continuous-breeding study was conducted in one generation of CD-1 mice treated with 0, 53, 525, and 1,750 mg/kg bw/day in diet (39, 40). Fetal effects that were observed only at the highest dose included reductions in litter size, live pups/litter, and pup weight. The developmental NOAEL was identified as 525 mg/kg bw/day, but a maternal NOAEL could not be identified because necropsies were only conducted in the high-dose group. In a multigeneration reproductive study, Long Evans Hooded rats were treated with 0, 250, or 500 mg/kg bw/day DBP by gavage from the time they were weanlings through the time that they nursed their own litters (41). Maternal toxicity was not reported. Developmental effects included malformations in reproductive organs, kidneys, and eyes in F_1 rats and reductions in F_2 litter size in all dose groups. The developmental LOAEL was identified as 250 mg/kg bw/day.

MBuP

The prenatal developmental effects of administering MBuP by gavage in the Wistar rat were reported (42, 43). The Expert Panel noted that some of the doses used in these studies were equimolar equivalents to doses used in earlier studies with DBP (described above). Ema et al. (42) studied doses of 0, 250, 500, and 625 mg/kg bw/day (0, 1.13, 2.25, or 2.80 mmol/kg bw/day) on gd 7–15. They observed maternal toxicity at the two highest doses expressed as reduced weight gain and feed consumption. Also, at these doses there were significant increases in post-implantation loss/litter and decreases in live fetuses/litter and fetal body weight/litter. Fetal malformations were increased, with cleft palate, deformed vertebral column, and dilated renal pelves the predominant findings. A maternal and developmental NOAEL and LOAEL of 250 and 500 mg MBuP/kg bw/day, respectively, were identified for this study.

Ema et al. (43) then followed up with evaluation of stage specificity studies by administering MBuP at doses of 0, 500, 625, or 750 mg/kg bw/day on gd 7–9, 10–12, or 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 at the 500 and 750 mg/kg bw/day doses on gd 7–9 and 13–15. Increased skeletal malformations were observed at 500, 625, and 750 mg/kg bw/day on gd 7–9 and at 625 and 750 mg/kg bw/day on gd 13–15 (deformed cervical vertebrae were predominant on gd 7–9). Cleft palate and fused sternebrae were observed on gd 13–15. These results are consistent with the findings for DBP and imply that MBuP (and/or subsequent metabolites) may account for the developmental toxicity (embryolethality and malformations) for DBP.

3.2.2 Postnatal Development

DBP

Marsman et al. (14) exposed F344/N rats and $B6C3F_1$ mice to high dietary concentrations of DBP during gestation and lactation. Both species were exposed to 0, 1,250, 2,500, 5,000, 7,500, 10,000, and 20,000 ppm. Dosages in mg/kg bw/day were estimated by using average values from 2 NTP studies that included a food intake rate of 14.8 g/day and a body weight of 203.71 g for rats and a food intake rate of 7.18 g/day and body weight of 39.63 g for mice (44-46). The dosages are listed in Tables 8 and 9. After weaning on pnd 21, up to 10 F_1 pups/group were fed a diet with a DBP

concentration identical to that fed to their dams fed for an additional 4 weeks. Author-calculated doses for pups were: 143, 284, 579, 879, and 1,165 mg/kg bw/day for male rats; 133, 275, 500, 836, and 1,104 mg/kg bw/day for female rats; 199, 437, 750, 1,286, and 3,804 mg/kg bw/day for male mice; and 170, 399, 714, and 1,060 mg/kg bw/day for female mice. Complete necropsies were performed on one rat and one mouse pup of each sex per litter at weaning and on all pups at the end of the 4-week post-weaning dietary exposure. Organ weights were obtained on major organs, including testis. Histopathological examination was performed on a broad array of tissues from all animals in the control and highest exposure group. In addition, the epididymis of rats from the 2,500, 5,000 and 7,500 ppm groups were studied.

For the rats (Table 7-8), gestational index was reduced (fewer live litters) at 5,000 and 20,000 ppm, and gestational length was reduced at 5,000 ppm. Litter size and postnatal survival were reduced at 10,000 and 20,000 ppm. All F_1 pups died by pnd 1 in the 20,000 ppm group. Male pup body weights were reduced during lactation in dose groups receiving 7,500 ppm and higher. In the post-weaning period, relative liver and kidney weights were increased in female offspring exposed to ≥2,500 and ≥5,000 ppm (275 and 500 mg/kg bw/day), respectively. Increased liver and kidney to body weight ratios were observed in males of all dose groups. Reduced relative testis weights were observed at the highest dose. Mild-to-marked hypospermia was seen in all males at the 879 and 1,165 mg/kg bw/day doses and in 4/10 males of the 579 mg/kg bw/day dose group. There were no histopathological lesions observed in liver or kidney. Acquisition of vaginal patency and preputial separation were not assessed. Based on increased liver and kidney to body weight ratios in all treated males, no NOAEL was identified.

For B6C3F_1 mice (Table 7-9), length of gestation was increased at 2,500 ppm and higher with 75 and 95% of litters lost at 10,000 and 20,000 ppm. Decreases were observed in litter size and pup body weights at 2,500, 7,500, and 10,000 ppm. In the F_1 post-weanling phase, males exhibited increased relative liver weights (one surviving male pup at 10,000 ppm exhibited hepatic lesions), and females exhibited increased relative kidney weights at 1,250 ppm (170–199 mg/kg bw/day) and higher. Except for liver lesions in the male at 10,000 ppm, no histpathological changes were observed, including in the testis. No NOAEL was identified.

Taking note of the Wine et al. (38) continuous-breeding study results (see Section 4), Mylchreest et al. (47) followed up the study using comparable dose levels (Table 7-10). However, three important changes in experimental design were introduced: 1) shortening the exposure period to include only gestation and lactation; 2) using gavage (with corn oil) to control exposure more closely; and 3) including more sensitive endpoints of reproductive development, such as markers of sexual maturation. Thus, pregnant CD rats (10 per group) were administered DBP by gavage at 0, 250, 500, or 750 mg/kg bw/day from gd 3 until pnd 20. At birth, pups were counted, sexed, weighed, and examined for signs of toxicity. Sexual maturity was assessed by observing age of vaginal opening and preputial separation in females and males, respectively. Estrous cycles were assessed in females for 2 weeks. The F_1 rats were sacrificed at 100–105 days of age. Necropsies were conducted on all males and up to three females per litter. A histological examination of sex organs was conducted on all rats with lesions and up to two unaffected rats per litter. Testes were preserved in Bouin's fixative.

Maternal body weight gain was comparable to controls throughout the dosing period. At 750 mg/kg bw/day, the number of live pups per litter at birth was decreased and maternal effects on pregnancy and postimplantation loss are likely to have occurred. Anogenital distance was decreased at birth in the male offspring at 500 and 750 mg/kg bw/day. The epididymis was absent or underdeveloped in 0, 9, 50, and 71% of adult offspring (100 days old) at 0, 250, 500, and 750 mg/kg bw/day, respectively, and was associated with testicular atrophy and widespread testicular germ cell loss. Hypospadias occurred in 0, 3, 21, and 43% of males, and ectopic or absent testes in 0, 3, 6, and 29% of males at 0, 250, 500, and 750 mg/kg bw/day, respectively. Absence of prostate gland and seminal vesicles as well as small testes and seminal vesicles were noted at low incidence in the 500 and 750 mg/kg bw/day dose groups. Dilated renal pelves, frequently involving the right kidney, were observed in all DBP dose groups. Vaginal opening and estrous cyclicity were not affected in the female offspring, although low incidences of reproductive tract malformations, mainly involving development of the uterus, were observed in 2 rats and 1 rat at the 500 and 750 mg/kg bw/day doses, respectively.

In the Mylchreest et al. 1998 study (47), all exposed groups showed adverse effects on male reproductive tract structure and indices of puberty. Based on this, the LOAEL in this study is 250 mg/kg bw/day/day. Based on the relationship between testis weight/histopathology and sperm production, the relationships between sperm numbers and fertility (48), and the number of major malformations of the reproductive tract, it is expected that at least the high- and mid-dose animals would be sub-fertile. The Panel's confidence in the quality of the study is high.

In a subsequent study, Mylchreest (49) reduced DBP exposure to just late gestation (gd 12–21) and compared the effects of DBP to the pharmacological androgen receptor antagonist, flutamide (Table 7-11). Pregnant CD rats received DBP at 0, 100, 250, or 500 mg/kg bw/day by gavage with corn oil (n =10) or flutamide at 100 mg/kg bw/day (n =5) on gd 12–21. Males were killed at approximately 100 days of age and females at 25–30 days of age. In F_1 males, DBP (500 mg/kg bw/day) and flutamide caused hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of the seminiferous epithelium, and interstitial cell hyperplasia of the testis. Agenesis of the epididymis was also observed at 250 mg/kg bw/day. Flutamide and DBP (250 and 500 mg/kg bw/day) also caused retained thoracic nipples and decreased anogenital distance. Interstitial cell adenoma occurred at 500 mg/kg bw/day in two males from the same litter. The only effect seen at 100 mg/kg bw/day was delayed preputial separation. The low incidence of DBP-induced intra-abdominal testes contrasted with the high incidence of inguinal testes seen with flutamide. Thus, the prenatal period is sensitive for the reproductive toxicity of DBP. Uterine and vaginal development in female offspring was not affected by DBP treatment. There were no signs of maternal toxicity with the exception of a 16% body weight loss at the time of birth and complete fetal mortality in 1 dam of the 500 mg/kg bw/day group. In addition, testicular focal interstitial cell hyperplasia and an adenoma (in 1 male) were observed in males at 500 mg/kg bw/day at 3 months of age. A LOAEL of 100 mg/kg bw/day was established in this study, based on delay in preputial separation at all dose levels. A NOAEL was not established.

To identify a NOAEL for DBP-induced developmental toxicity, Mylchreest et al. (50) gavaged 19–20 Sprague-Dawley CD rats/group with 0, 0.5, 5, 50, or 100 mg/kg bw/day and 11 Sprague-Dawley CD rats with 500 mg/kg bw/day in corn oil on gd 12–21 (Table 7-12). Dams delivered

and pups were weighed and examined at birth. After the pups were weaned, dams were killed and implantation sites and organ weights were evaluated. Pups were weighed weekly and examined for sexual maturation. When pups reached puberty they were killed and organ weights were determined. The testes and epididymides were preserved in Bouin's solution and examined histologically.

There was no evidence of maternal toxicity at any dose. In male pups, the incidence of retained aereolas or nipples was increased at the 100 and 500 mg/kg doses (31% of rats in 16/20 litters and 90% of rats in 11/11 litters, respectively). Malformations observed in the highest dose group included: hypospadias (9% of rats in 4/11 litters); and agenesis of the epididymis (36% of rats in 9/11 litters), vas deferens (28% of rats in 9/11 litters), and prostate (1/58 rats). Reduced testis, epididymis, prostate, and levator muscle weight and reduced anogenital distance in males were also observed at the high dose. Histological effects in high-dose males included interstitial cell hyperplasia (35% of rats in 3/5 litters), adenoma (1/23 rats), and seminiferous tubule degeneration (56% of rats in 3/5 litters). The single case of seminiferous tubule degeneration in the 100 mg/kg bw/day group was considered equivocal because the lesion does occur spontaneously in a small number of Sprague-Dawley rats. In female offspring, the age of vaginal opening and reproductive organ weight and histology were unaffected. A developmental NOAEL and LOAEL of 50 and 100 mg/kg bw/day, respectively, and a maternal NOAEL of 500 mg/kg bw/day, were identified for this study.

The qualitative findings of Mylchreest et al. (47, 49, 50) were confirmed by Gray et al. (41) who gavaged 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3 with corn oil vehicle or DBP at 500 mg/kg bw/day, and groups of 4–6 Long Evans Hooded rats with 0 or 500 mg/kg bw/day on gd 16–19.

Gray et al. (41) also compared the effects of DBP at 500 mg/kg bw/day and an equimolar concentration of 750 mg/kg bw/day DEHP administered by gavage to 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3 (Table 13). The male F_1 pups were evaluated for sexual maturation and were then killed and necropsied at 5 months of age. Organ weights were measured and a histological examination of reproductive organs (preserved in Bouin's) was conducted. The presence or absence of maternal toxicity was not described. Effects in F_1 males are summarized in Table 6 and included reduced anogenital distance, and increases in percent areolas and nipples at birth, numbers of areolas and nipples at birth and adulthood, hypospadias, and testicular and epididymal atrophy or agenesis. A decrease in weight for prostates, epididymides, testes, penis, and the levator ani muscle was also observed in the treated rats. None of the control pups were found to have nipple development, malformations, or testicular degeneration. DEHP and DBP exposure resulted in effects that were qualitatively similar. Several males from DEHP-treated dams also had hemorrhagic testes. The authors stated that DEHP was considerably more toxic to the male reproductive system than DBP.

Table 6: Comparison of Reproductive Effects following in Utero Exposure to Equimolar Concentrations of DEHP (750 mg/kg bw) and DBP (500 mg/kg bw) in Sprague Dawley Rats

Effect	Control	Chemical	
		DEHP	DBP
Anogenital distance (mm)	3.7±0.09	2.45±0.11*	2.79±0.09*
Areolas at birth (%)	0	88±12	55±14
Number of areolas at birth	2.7±0.75	8.4±15	2.7±0.75
Retained nipples at birth	0	8.1±1.4*	2.2±0.8*
Number of nipples at necropsy	0	8.1±1.4*	2.2±0.8*
Hypospadias (%)	0	67±14	6.2±6.2
Vaginal pouch (%)	0	45±17	0
Ventral prostate agenesis (%)	0	14±14	0
Testicular & epididymal atrophy or agenesis (%)	0	90±10	45.8±12

*Statistically significant.

(41)

In an abstract, DBP was reported to have been evaluated for developmental toxicity in amphibian and non-rodent mammalian test systems (51). Xenopus laeris (African clawed toad) tadpoles were exposed to 0 (n=14) or 10 (n=52) ppm DBP beginning at 2 weeks of age (stage 52) through complete metamorphosis (stage 66), with mortality and time to complete metamorphosis monitored weekly. Mortality at 10 ppm was 85% in week 1 (0% in controls) and 92% in week 16 (28% in controls). Seventy-five percent of the controls were metamorphosed by week 12 with 100% by week 14; none of the treated tadpoles completed metamorphosis until week 16. The authors concluded that DBP or its metabolite(s) may disrupt thyroid hormone cascade, since metamorphosis, a thyroid hormone-dependent event, is affected at 10 ppm. The same group administered DBP in corn syrup at 0 or 400 ppm/kg body weight to pregnant Dutch belted rabbits, 6 does/group, on gd 15–30. Does were allowed to litter and male pups were monitored until 12 weeks of age. At 12 weeks of age, body, testes, and epididymides weights were unaffected, but accessory gland weights and anogenital distance were lower in treated male offspring. In addition, analogously to male rats effects, one treated rabbit had undescended testes, ambiguous external genitalia, hypospadias, and was missing (agenesis of) the prostate and bulbourethral glands. The authors concluded that DBP disrupts androgen-dependent developmental events and is consistent with anti-androgenic effects of DBP observed in rodents after perinatal exposure.

MBuP

Imajima et al. (52) 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 based on actual rat body weights) (Table 7-17). Male offspring were evaluated on gd 20 and on pnd 30–40 to determine the position of the testes. In control males, all the 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, all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent on gd 20. On pnd 30–40, MBuP-exposed

males exhibited cryptorchidism (22 of 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 trans-abdominal migration of the testis.

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

The relationship between either human sperm density or total number of sperm and DBP concentration in the cellular fraction of ejaculates was studied in a group of unselected college students (53). A negative correlation between DBP concentration and the studied sperm indices was found. The authors point out that there was no reason to believe that any of the students examined had been exposed to phthalate esters other than at ambient levels in the environment. However, the use of this study to support a causal relationship to DBP exposure is limited because subjects' characteristics and other potential risk factors that could confound or modify the observed association were not taken into account by the authors.

4.2 Experimental Animal Toxicity

Approximately 20 studies were reviewed in the evaluation of the reproductive toxicity of DBP. Collectively, these studies predominantly used rodents, and built on the original observation that DBP produced testicular atrophy in a sub-acute toxicity study (54). The literature contains numerous redundant studies, usually at high doses (e.g., 2 g/kg, usually in rats), all of which show similar effects on the testis. For example, Gray et al. (55) reported on the testicular effects of DBP in the adult rat, mouse, guinea pig, and hamster. In these studies, DBP was administered by gavage for 7 or 9 days at doses of 2,000 or 3,000 mg/kg bw/day. Severe effects were seen on testis weight with histopathological damage (reduction in spermatids and spermatogonia) affecting almost all tubules. Mouse testis was less severely affected and no effects were observed in hamsters. The monoester of DBP was also essentially without effect in the hamster. As discussed in Section 2.1.2 of this monograph, sub-chronic oral exposure of adult F344 rats resulted in testicular lesions at doses of 720 mg/kg bw/day and higher (14). A second study (14) demonstrated that exposure to DBP during gestation and lactation did not increase sensitivity in rats exposed to DBP for 3 months during adulthood. Sub-chronic studies in $B6C3F_1$ mice at doses up to 3,689 mg/kg bw/day did not cause histological or organ weight changes in the testes.

A number of more specific studies in the rat have attempted to investigate the mode of action of DBP using in vivo and in vitro protocols. The papers summarized here illustrate important facets of DBP-induced reproductive effects.

The key study for the quantitative assessment of the reproductive toxicity of DBP is reported by Wine et al. (38) (Table 7-14). CD Sprague Dawley rats, 10 weeks old at the start of exposure, were used for continuous-breeding phase and cross-over mating studies. There were 20 breeding pairs in each treated dose group, and 40 pairs in the control group. DBP was mixed with feed to levels of 0, 0.1, 0.5 and 1.0% (w/w); this yielded calculated doses of 0, 52, 256, and 509 mg/kg bw/day for males and 0, 80, 385, and 794 mg/kg bw/day for females. Following a 7-day premating period, the rats were housed as breeding pairs for 14 weeks. Litters were removed immediately after birth. Endpoints in-life included clinical signs, parental body weight and food consumption, fertility (numbers of pairs producing a litter/total number of breeding pairs), number of litters/pair, number

of live pups/litter, proportion of pups born alive, sex ratio, and pup body weights within 24 hours of birth.

In the F_0 generation there was no effect on the overall fertility of the breeding pairs (i.e., the ability to produce litters with at least one live pup); all produced approximately five litters. There was clear indication that DBP, when administered in the diet, affected total number of live pups per litter in all treated groups (reduced by ~ 8–17%) and live pup weights in the 256–385 and 509–794 mg/kg bw/day groups by 6–12 %.

A cross-over mating study was conducted between the high-dose treatment group and the controls. The percent of pairs mating, becoming pregnant, and delivering a litter was unaffected, as was litter size, although adjusted live pup weight was reduced in litters from treated females. At F_0 necropsy, there were no gross or histopathologic effects in the reproductive tracts of treated animals. Epididymal sperm count, testicular spermatid number, and estrous cycle length were not affected by DBP treatment in the F_0 animals. Systemic effects in the F_0 rats included decreased body weight in females and increased liver and kidney to body weight ratios in both sexes of the high-dose group.

The final F_1 litters following the continuous F_0 breeding phase were weaned and raised to sexual maturity (pnd 88) and received the same dose in feed as their parents. Upon reaching sexual maturity, 20 non-sibling F_1 males and females within the same treatment group were housed in pairs for 1 week and then housed individually until delivery of an F_2 litter.

F_1 pup weight was significantly reduced in the high-dose group on pnd 0, 14, and 21. During rearing, three high-dose males were found to have small and malformed prepuces and/or penises and were without palpable testes. Mating, pregnancy, and fertility were significantly lower in the high-dose F_1 group with only 1 of 20 pairings resulting in a litter. While litter size was unaffected, F_2 pup weight was reduced in all treatment groups. All dose groups were killed and necropsied, at which point the body weights of the high-dose animals were 8–14 % lower than controls, but unchanged at other dose levels. For males only, kidney to body weight ratio increased at the 256–509 mg/kg bw/day levels and liver to body weight ratio was increased at the highest level. The relative weights of the ventral prostate and seminal vesicles and the absolute weight of the right testis were decreased in the F_1 males from the high-dose group. There were no effects on the ovary of F_1 females. Epididymal sperm count and testicular spermatid count was significantly reduced in the high-dose F_1 males. Histologic analysis was only performed on selected males (n=10) from the control, mid-, and high-dose groups (the solution used to preserve testes is not clear). Widespread seminiferous tubular degeneration was noted in 1/10 controls, 3/10 in the mid-dose group, and 8/10 in the high-dose group. The high-dose group also exhibited interstitial cell hyperplasia. Five of ten high-dose males also had underdeveloped or defective epididymides. No ovarian or uterine lesions were noted in F_1 females and there was no effect on ante-mortem estrous cyclicity.

In Wine et al. (38), the F_1 high-dose group had a high rate of infertility, the middle dose had fewer (F_0 mating) and lighter pups (F_0 and F_1 matings), while the low-dose animals had fewer pups (F_0 mating) and lighter pups (F_1 mating). Thus, a NOAEL was not established. The LOAEL was 52–80 mg/kg bw/day based on reductions in F_0 litter size and F_2 pup weight. The Expert Panel's confidence in the quality of the study is high, and our confidence is also high that these doses

correctly represent the LOAEL.

A multigeneration reproductive study was conducted to assess effects of DBP exposure in Long Evans Hooded rats (41) (Table 15). Weanling male and female rats of the parental (F_0) generation (10–12/sex/group) were gavaged daily with DBP in corn oil through puberty, adulthood, mating, gestation, and lactation. Females received 0, 250, or 500 mg/kg bw/day; male rats received 0, 250, 500, or 1,000 mg/kg bw/day. Sexual maturation and estrous cycles of the F_0 were evaluated. Treated rats were mated with untreated controls. When the F_1 litters were weaned, the parental rats were killed and necropsied. Implantation sites, serum hormone levels, organ weights, and testicular histology were evaluated.

A delay in puberty was observed in all treated F_0 males based on the age of preputial separation (42.6, 43.4, and 44.4 days from low to high-dose group vs 39.6 days in control group). Fertility was reduced in F_0 males and females in the 500 mg/kg bw/day group. Infertility in F_0 males was apparently due to testicular atrophy and reduced sperm counts. F_0 females in the 500 mg/kg bw/day group cycled and mated successfully, but experienced an increased incidence of mid-term abortion. Malformations were significantly increased in F_1 pups from the 250 and 500 mg/kg bw/day groups. Types of malformations included low numbers of hypospadias, abdominal testes, anophthalmia, uterus unicornous, and renal agenesis.

The F_1 pups were not treated with DBP after weaning. Four to eighteen pairs of F_1 pups from treated dams were selected for continuous mating within dose groups for 11 cycles and the remaining F_1 pups were necropsied. The F_2 pups born during the continuous breeding phase were counted and discarded. Fecundity was reduced in F_1 rats from treated dams and the number of F_2 pups born was reduced in breeding pairs from the 250 and 500 mg/kg bw/day groups. At necropsy, a non-significant reduction in caudal sperm counts (19%) and a significant reduction in caudal sperm levels (34%) were noted in F_1 males from the 250 and 500 mg/kg bw/day groups, respectively.

The study by Gray et al. (41) is somewhat limited because many endpoints and details of their experimental methods were not reported.

In Lamb et al. (39) and Reel et al. (40) (Table 7-16), DBP was one of four phthalate esters compared using the Continuous Breeding protocol in CD-1 mice; the same basic protocol as reported in Wine et al. (38). Male and female CD-1 mice, 20 pairs/treatment group and 40 pairs in control, were fed a diet with DBP at 0 300, 3,000, or 10,000 ppm (doses of 53, 525, and 1,750 mg/kg bw/day as reported by Reel et al. (40)) for 7 days prior to and during a 98-day cohabitation period. Litters were removed immediately after birth. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair and of live pups per litter, pup weight, and offspring survival. Testes were fixed in Bouin's solution for histological evaluation. DBP exposure reduced litter size, numbers of litters per pair, number of fertile pairs, live pups per litter, and proportion of pups born alive in the high-dose group. These effect were not seen at lower dose levels. A crossover mating trial demonstrated that female, but not male, mice were affected by DBP, as shown by significant decreases in the percentage of fertile pairs, the number of live pups per litter, the proportion of pups born alive, and live pup weight. Only the control and high-dose F_0

DBP groups were necropsied. There were no effects on sperm parameters in the males, although body weight was significantly decreased (8%) and liver to body weight ratio significantly increased (11%). For females, liver to body weight ratio was significantly increased (19%) and relative uterine weight significantly decreased (28%), but there was no effect on estrous cycles. No treatment-related gross or histological lesions were noted. A second generation was not evaluated.

In Lamb et al. (39), the high-dose group was subfertile and the middle-dose and the low-dose groups were functionally unaffected. Thus, the NOAEL was calculated at 525 mg/kg bw/day, based on reductions in litter size and in proportions of pairs having litters. The mid- and low-dose groups were not necropsied or evaluated for reproductive development or performance. For these reasons, the Expert Panel has moderate-to-low confidence that these doses correctly represent the LOAEL and NOAEL. Confidence in the quality of the data reported is high.

Mode of Action

The Expert Panel believes that data from studies with DEHP are relevant to a consideration of the mechanism by which DBP causes adverse effects. It is well understood that DEHP produces a range of hepatic effects in rats (induction of peroxisomes; increased Cyp4A1; PCoA) including hepatic tumors. The induction of these effects in rats is believed due to activation of PPAR-alpha. In PPAR-knockout mice, administration of DEHP does not result in the induction of hepatic effects or tumors unlike the wild-type control animals. In humans, PPAR-alpha is activated upstream of different enzymes from those noted in the rat. 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 studies with DEHP, a genetically-modified strain of mouse (the PPAR-alpha knockout mouse) cannot activate PPAR-alpha, but is susceptible to phthalate-induced developmental toxicity and testicular toxicity. This mouse does express PPAR-gamma in the testis which can be activated by MEHP (56). PPAR-gamma may conceivably play a role in the reproductive toxicity of phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. Other members of the PPAR family (beta and gamma) have not been extensively studied with regard to activation by phthalates.

Finally, the guinea pig, a non-responding species to the peroxisomal proliferating effects of DBP, is susceptible to the testicular effects of this phthalate.

Gray et al. (55) investigated the reason for the lack of testicular lesions in hamsters orally administered DBP and MBuP at doses exceeding those that produced testicular lesions in rats. Using ^{14}C-labelled DBP and monobutyl ester (MBuP), it was determined that intestinal esterase activities were similar in the two species and that the principal metabolite in the rat and hamster was MBuP glucuronide (23) However, the levels of unconjugated MBuP in urine were 3–4 fold higher in the rat. Finding that the activity of testicular beta-glucuronidase was significantly higher in the rat than the hamster, the authors speculated that the testicular damage might be associated with greater concentrations of unconjugated MBuP, the putative toxicant.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (57-59). It is known that some Sertoli cell functions are

mediated by follicle stimulating hormone (FSH) interaction with membrane bound receptors. Lloyd and Foster (60) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (61).

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, di-n-hexyl phthalate (DnHP), and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (62) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young versus mature rats. Further, in vitro studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (63). Lloyd and Foster (60) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats, but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals.

The Panel was not able to reach agreement that interfering with FSH signaling function was the accepted mode or mechanism 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 (64), rainbow trout hepatic cytosol (65), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (66, 67) and rainbow trout ERs expressed in yeast . Triated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. DBP exhibited no or weak activity in in vitro assays that measured binding of phthalates to estrogen receptors (64, 65, 68). The assays did not include the addition of esterases or lipases to metabolize DBP to its monoester.

Selected phthalate esters have been examined in a number of in vitro gene expression assays systems. The assays have used stably transfected cells (64), transiently transfected cells (64, 65), yeast based assays (64, 68-70) and vitellogenin induction in rainbow trout hepatocyte cultures (68). DBP was weakly active in an assay of estrogen-induced gene expression, but its metabolite MBuP was inactive (70). There was no synergism in estrogenic response with DBP and other phthalates (70, 71).

In vivo assays demonstrated that DBP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (64) and prepubertal mice (69). Uterine permeability was not affected following the subcutaneous injection of DBP (71). Malformations in reproductive organs and effects on androgen-related endpoints of male rats exposed to DBP or MBuP during prenatal development suggest antiandrogenic activity by DBP and MBuP (41, 49, 50, 52).

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

The major use of DBP is as a coalescing aid in latex adhesive. It is also used as a plasticizer for cellulose plastics and as a solvent for dyes. DBP is not used as a plasticizer for PVC plastics (1).

Several authoritative estimates of human exposure, described in Section 1, have been published since 1990. All estimates place total DBP exposure in the general population at less than 10 μg/kg bw/day and were consistent in identifying food as the major exposure source. In addition to food, general human exposure occurs primarily through indoor air followed by drinking water, soil, and ambient air. Infants and young children may have higher exposures than adults, primarily because of dietary differences and possible mouthing of DBP-containing household articles (not limited to toys). Using reasonable assumptions and data from surveillance and food surveys, Health Canada (6) estimated total exposures of 2.4, 5.0, 4.3, 2.3, and 1.9 μg/kg bw/day for humans aged 0–0.5, 0.5–4, 5–11, 12–19, and 20–70 years, respectively. Discrepancies in food exposure estimates may be due to inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations.

DBP was found in infant formula, but amounts vary internationally and seem to be falling (5, 9). The most recent estimate of DBP exposure from infant formula to a newborn in the UK is 2.4 μg/kg bw/day (5) and is the same as the Health Canada total exposure estimate. DBP has been found in 1 of 17 European children's toys at a very low level (0.01% by weight) (10). Use of DBP in plastic nasogastric tubing has also been reported (3). Occupational exposure during phthalate manufacture is estimated at 143 μg/kg bw/day. Exposures in other occupational settings have not been estimated.

5.1.1.1 Utility of Data to the CERHR Evaluation

DBP exposures resulting from contact with various media (e.g., food, drinking water, and air) have been estimated by several authoritative sources. Limitations in the dataset include the fact that most of the data used in calculations were 15–20 years old and may not reflect current exposure. Further, the majority of data was collected in Europe and Canada and may not accurately reflect US patterns. Data from Health Canada were selected for use since they provide age-based exposure estimates.

5.1.2 General Biological and Toxicological Data

Toxicity. The Expert Panel had to rely on animal toxicity data in its evaluation of general biology and toxicity. DBP is not acutely toxic to rodents with the oral LD_{50} given in grams per kilogram (g/kg) quantities. There are sufficient data to establish that DBP in the diet is toxic to adult rats and mice at repeated daily doses of ~350 mg/kg bw/day and higher (14). The liver and testes are consistently found to be target organs with the hematopoetic system also affected in some strains of rats and at higher doses in mice. Testicular lesions were observed at doses of 720 mg/kg bw/day and higher in adult rats (14). DBP increases liver to body weight and kidney to body weight ratios. These effects are consistent with effects seen with other phthalates. Indications of peroxisome proliferation, such as elevated levels of PCoA oxidation, were consistently observed. The lowest repeated dose NOAEL in rats was observed in males exposed through diet to 142 mg/kg bw/day (13). The corresponding NOAEL in male mice was 353 mg/kg bw/day (14). Chronic carcinogenicity studies were not available for review.

Table 7: Summaries of NOAELs and LOAELs and Major Effects in General Toxicity Studies

Protocol and DBP Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major Effects at Higher Doses
3-month repeat dose dietary study in Wistar rats. 6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 27, 142, 688; F: 0, 33, 162, 816. (13)	M: 142 F: 162	M: 688; F: 816 ↑Liver and kidney weight (F). Peroxisomal proliferation. Histological liver changes. ↓Thyroid hormone. Anemia (M). No testicular lesions.	No higher doses in study.
13-week repeat-dose dietary study in F344 rats. 5–6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 176, 359, 720, 1,540, 2,964 F: 0, 177, 356, 712, 1,413, 2,943. (14)	M: 176 F: 177	M: 359; F: 356 ↑ Liver and kidney weights (M). Peroxisomal proliferation. Anemia (M).	↑ Liver and kidney weights. Hepatic lesions. Changes in liver enzyme activity. Peroxisomal proliferation. Testicular lesions. Hypospermia. ↓Testes weight. ↓ Testicular testosterone levels. Anemia (M).
13-week repeat-dose dietary study in $B6C3F_1$ mice. 6 weeks-old at start of study, 10 mice/sex/group. Doses – M: 0, 163, 353, 812, 1,601, 3,689 F: 0, 238, 486, 971, 2,137, 4,278. (14)	M: 353 F: None	M: 812 F: 238 ↑ Kidney weight (F) (No dose response or histological changes). ↑ Liver weight (M). ↓Body weight gain (M).	↑ Kidney weight (F) (No dose response or histological changes). ↑ Liver weight. ↓Body weight gain. Mild histological liver effects. No testicular lesions.

Toxicokinetics. There are no conclusive in vivo toxicokinetic data in humans. Orally-administered DBP in rodents is rapidly hydrolyzed to the monoester, MBuP, by pancreatic lipases secreted into the small intestine. The monoester is rapidly absorbed from the gut, widely distributed in tissues, and is rapidly excreted in urine, mainly as a glucuronide. No studies are available on the absorption of orally-administered DBP in primates. Thus, it is not known whether DBP is more poorly hydrolyzed and absorbed in the gut of primates compared to rats, as has been observed with other phthalates. Rodent studies indicate there is no bioaccumulation of absorbed DBP or its metabolites (including testes and prostate tissue). In vitro human and rat skin were compared for their absorption of DBP; and human skin was found to be much less permeable than rat skin (12). In rats, dermal absorption of DBP as identified by urinary excretion of metabolites is 10–12% of the 30–40 mg/kg dose per day (11).

Rats treated with ^{14}C-DBP on gd 14 showed concentrations of radioactivity in placenta and fetuses that were approximately 65% of the levels in maternal serum. MBuP was the major metabolite found in both maternal and embryonic tissues (21).

A PBPK model of the tissue distribution of DBP and MBuP in rats has been developed by Keys et al. (25); the model includes diffusion limitations and pH trapping as mechanisms of uptake of MBuP into tissue. A model has been derived to extrapolate rodent data to predicted values in humans. The model does not contain parameters for estimating fetal or pediatric values.

Genetic Toxicity. IPCS (3) reviewed a number of mutagenicity and related endpoints for DBP and concluded that the weight of the evidence indicated that DBP is not genotoxic.

5.1.2.1 Utility of Data to the CERHR Evaluation

The oral subchronic studies in rats and mice are adequate for the evaluation of general toxicity induced by DBP. Some studies were conducted according to GLP standards and relevant exposure routes were utilized. Small group numbers, used in some studies, are of limited concern considering the reproducibility of effects between studies. Adult rodents were tested for DBP-induced testicular lesions. Sections 3 and 4 of this document address studies where the male rodent reproductive tract was exposed to DBP during prenatal and postnatal development. The examination of hepatic effects was adequate and included an evaluation of peroxisome proliferation in rodents.

There are acceptable toxicokinetic data for DBP, consisting of absorption, distribution, metabolism, and excretion, following oral and dermal exposure in the rat. The human data available are of very limited utility. In vitro comparisons of DBP metabolism suggest that effects observed in rodents are relevant to humans.

5.1.3 Developmental Toxicity

The are no data on the developmental toxicity of DBP in humans. The most complete description of effects characterizing key aspects of the developmental toxicity of DBP is contained in a series of

publications by Ema et al. (34-36) and Mylchreest et al. (47, 49, 50). Ema et al. (42) characterized the prenatal developmental toxicity of DBP in Wistar rats and subsequently demonstrated that the metabolite MBuP caused developmental toxicity similar to DBP. These effects were produced at approximately equimolar concentrations. For example, a maternal and development NOAEL and LOAEL of 500 and 630 mg/kg bw/day (1.80 and 2.27 mmol/kg bw/day), respectively, were identified for DBP following gavage of Wistar rats on gd 7–15 (34). Using a similar experimental design, a maternal and developmental NOAEL and LOAEL for MBuP of 250 and 500 mg/kg bw/day (1.13 and 2.25 mmol/kg bw/day), respectively, were identified (42). Similar fetal effects in these studies included increased prenatal mortality, decreased fetal weight, and cleft palate. Dose and time dependency studies with DBP and MBuP resulted in similar findings and are described in Section 3.2.1.

The most complete prenatal exposure study by Ema et al. from the perspective of group size and development of the male reproductive tract established a maternal and fetal NOAEL and LOAEL of 331 and 555 mg/kg bw/day, respectively, in Wistar rats fed DBP-dosed diets on gd 11–21 (36). Developmental effects at higher doses (≥555 mg/kg bw/day) included decreased fetal weight, cleft palates, fused sternebrae, reduced anogenital distance in males, and cryptorchidism.

A group of studies from Mylchreest et al. looked at postnatal effects following in utero exposure to DBP (47, 49, 50). CD rats were gavaged with DBP from gd 3 to pnd 20 or gd 12–21. Delayed preputial separation and retained nipples were observed at doses as low as 100 mg/kg bw/day. Effects noted at doses of 250 mg/kg bw/day or higher were consistent between studies and included hypospadias, agenesis of epididymides or seminal vesicles, cryptorchidism, decreased anogenital distance in males, and/or a low incidence of interstitial adenomas. A NOAEL of 50 mg/kg bw/day was identified. The three Mylchreest studies (47, 49, 50) exposed animals during the appropriate window of development, analyzed the tissues appropriately, and combined them with other indices of puberty and reproductive development. The concordance in dose-response to the Wine et al. (38) study is good.

The role of the monoester metabolite of DBP in developmental toxicity was elucidated by Saillenfait et al. (21), who gavaged Sprague-Dawley rats with 500 or 1,500 mg/kg of radiolabeled DBP/kg bw/day on gd 14. They demonstrated radioactivity in placentas and embryos at levels of 21–30% of those measured in maternal plasma. The majority of the radioactivity was associated with MBuP and its glucuronide. Postnatal effects following in utero exposure to the DBP metabolite MBuP were studied in WKA rats that were gavaged with 300 mg MBuP/day (~1,000 mg/kg bw/day) on gd 15–18 (52). Testes descent was reduced on both gd 20 and pnd 30–40. Although only one dose was administered, the findings are consistent with those observed in DBP developmental toxicity studies conducted by Ema et al. (36) and Mylchreest et al. (47, 49, 50), thus supporting the hypothesis that MBuP is responsible for effects associated with DBP exposure.

The hallmark of developmental toxicity in the mouse following oral exposure to DBP appears to be primarily systemic toxicity and death. In a study with ICR mice exposed to diet containing DBP on gd 0–18, Shiota et al. (32, 33) reported a 98% incidence of fetal mortality at 2,100 mg/kg bw/day. Fetal body weight was reduced at 660 mg/kg bw/day. The authors stated that the maximum non-embryotoxic dose was 370 mg/kg bw/day. However, the Expert Panel noted that delayed ossification

occurred at all dose levels, and selected the lowest dose, 80 mg/kg bw/day, as a LOAEL. These data are from groups with small sample size and have not been replicated. In a continuous breeding protocol with CD-1 mice, Lamb et al. (39) observed a decrease in the number of pups, live pups per litter, and pup weight in dams that consumed a dose of 1,750 mg/kg bw/day in the diet. The developmental NOAEL was identified as 525 mg/kg bw/day. Effects of in utero and lactational exposure to DBP were studied in $B6C3F_1$ mice where Marsman et al. (14) reported that length of gestation was increased at 2,500 ppm (454 mg/kg bw/day) and higher. Seventy-five and ninety-five percent of litters were lost at 10,000 (1,816 mg/kg bw/day) and 20,000 (3,632 mg/kg bw/day) ppm. Decreases were observed in litter size and pup body weights at 2,500, 7,500, and 10,000 ppm. The Expert Panel is not confident that these three studies fully assessed DBP developmental toxicity, including reproductive function, due to limitations in study design that include small group size, failure to perform necropsies in critical dose groups, and failure to assess appropriate landmarks of maturation.

NOAELs and LOAELs for the key developmental toxicity studies for DBP are listed in Table 8. The Ema et al. (36) study examined the most sensitive prenatal endpoints and allows for a comparison between maternal and developmental toxicity. The Ema et al. (34) study of DBP was also included to allow comparison with the study of its metabolite, MBuP (42) that was evaluated according to the same protocol. The Mylchreest et al. (50) study is considered key because it examined the most sensitive endpoints at the lowest doses.

Table 8: Summaries of NOAELs and LOAELs and Major Effects in Key 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 studies in Wistar rats. 11–12/group received DBP (0, 500, 630, 750, or 1,000 mg/kg bw/day) or MBuP (0, 250, 500, or 625 mg/kg bw/day) on gd 7–15 by gavage. In a third study rats were treated by diet with 0, 331, 555, or 661 mg/kg bw/day on gd 11–21. Fetuses were evaluated late in gestation. (34, 36, 42)	***DBP Gavage:*** Maternal: 500 Fetal: 500 (1.80 mmol/kg bw/day) ***MBuP Gavage:*** Maternal: 250 Fetal: 250 (1.13 mmol/kg bw/day) ***DBP Diet:*** Maternal: 331 Fetal: 331	***DBP Gavage:*** 630 (2.27 mmol/kg bw/day) ↓ Weight gain. ***MBuP Gavage:*** 500 (2.25 mmol/kg bw/day) ↓ Weight gain. ***DBP Diet:*** 555 ↓ Weight gain.	***DBP Gavage:*** 630 (2.27 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ***MBuP Gavage:*** 500 (2.25 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations. ***DBP Diet:*** 555 ↓ Anogenital distance in males. ↑ Fetuses with undescended testes.	***DBP Gavage:*** ↑ Prenatal mortality. ↓ Fetal weight. ↑ External malformations ***MBuP Gavage:*** ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations. ***DBP Diet:*** ↓ Fetal weight. ↑ External and skeletal malformations. ↓ Anogenital distance in males. ↑ Fetuses with undescended testes.
Prenatal gavage study with postnatal evaluation in CD rats. 11–22 per group received 0, 0.5, 5, 50, 100 or 500 mg/kg bw/day on gd 12–21. Pups were evaluated until puberty. (50)	Maternal: 500 Developmental: 50	None	100 Retained aereolas and nipples in males.	Retained aereolas and nipples in males. Testicular lesions and adenoma. Malformations of reproductive organ. ↓ Reproductive organ weights. ↓ Anogenital distance in males.
Prenatal dietary study in ICR-JCL mice. 6–15 dams per treated group received 0, 80, 180, 350, 660, and 2,100 mg/kg bw/day on gd 0–18. Dams and pups examined late in gestation. (32, 33)	Maternal: 660 Developmental: None	2,100 ↓ Body weight gain.	80* Delayed ossification (number of ossified coccygia from control to 660 mg/kg bw/day group: 9.4, 5.1, 4.5, 6.0, 2.6).	Delayed ossification. ↑ Prenatal mortality. ↓ Fetal weight. ↑ Neural tube defects.

* Effect level selected by Expert Panel differs from that of the study authors

5.1.3.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of developmental toxicity. Studies examined effects following dosing of dams through portions of or the entire period of pregnancy. Fetuses were evaluated for prenatal malformations and postnatal effects. Evaluations included an examination of reproductive organs and androgen-regulated endpoints, which are thought to be the most sensitive indicators of phthalate-induced toxicity. Prenatal effects following prenatal exposure to MBuP were also examined. A second rodent species, the mouse, was examined in a prenatal exposure and effect study. Based on the limited parameters examined in the mouse it is not possible to compare sensitivity in rats and mice.

5.1.4 Reproductive Toxicity

Human Data

The relationship of human sperm density and total number of sperm to DBP concentration was studied in a group of unselected college students (53). A negative correlation was found between sperm density and DBP concentration in the cellular fraction of ejaculate of non-occupationally exposed subjects, but the data are of little value to the Expert Panel due to the insufficient evidence for a causal relationship of sperm characteristics to DBP levels.

Experimental Animal Studies

Reproductive studies have been performed primarily in the rat and, to a lesser extent, the mouse. There are single reports of studies in guinea pigs and hamsters (55). Collectively, the data are sufficient to show that oral exposure to DBP can cause reproductive toxicity in male rats, mice, and guinea pigs. In contrast, the hamster failed to show testicular effects. Data that characterize effects on female reproduction are not as complete and detailed interpretation is therefore less certain. The data do indicate a decrease in female fertility in mice and rats.

Females. The Lamb et al. (39) data from a continuous breeding study in mice clearly show adult female functional effects at 1,750 mg/kg bw/day. The limited examination of the lower dose groups (necropsies were not performed) precludes the setting of a reliable NOAEL. The continuous-breeding study by Wine et al. (38) in F344 rats did not show specific deficits in female parameters; however, the data do not rule out that decreases in litter size at all doses may have a female component. In contrast, Gray et al. (41) reported that fertility in female Long Evans rats was reduced following treatment with 500 mg/kg bw/day from weaning through puberty, gestation, and lactation. The effect was apparently due to an increase in mid-term abortions. The F_1 female pups in this study were also mated and experienced a reduction in fecundity at doses of 250 mg/kg bw/day and higher. Thus, clear effects on female reproduction are seen in rats at doses of 250 mg/kg bw/day (LOAEL) and in mice at higher doses. NOAELs can not be established with any confidence.

Males. Data from the Wine et al. (38) continuous breeding study clearly show functional and structural reproductive effects in male Sprague-Dawley rats. In the F_0 generation there was clear indication that DBP, when administered in the diet, affected total number of pups per litter in all treated groups. The F_1 high-dose group had malformations of the reproductive tract and a high rate

of infertility. Dose related increases in seminiferous tubular degeneration were seen at the 256 and 509 mg/kg bw/day doses. The LOAEL was 52–80 mg/kg bw/day based on reductions in F_0 litter size. Thus, a reproductive NOAEL was not established.

A delay in preputial separation was observed in Long Evans rats exposed at the lowest dose of 250 mg/kg bw/day by gavage from the time they were weaned until the litters they sired were weaned in the Gray et al. study (41). At higher doses (500–1,000 mg/kg bw/day), reductions in sperm counts and fertility and testicular lesions were also observed. The F_1 offspring exposed to DBP only during gestation and lactation experienced a reduction in sperm counts.

Three studies by Mylchreest et al. (47, 49, 50), presented in Sections 3.2.2 and 5.1.3 of this document, indicated that the range of male structural abnormalities in the Wine et al. (38) study could be reproduced with a much shorter dosing regime. Mylchreest et al. (49, 50) also detected a significant increase in testicular Leydig cell hyperplasia and a low incidence of Leydig cell adenomas in ~3-month-old animals following only a late gestational exposure (gd 12–21) of 500 mg/kg bw/day. Wine et al. (38) dosed for 14 weeks with DBP in the diet, whereas Mylchreest et al. (50) exposed pregnant rats by gavage during gd 12–21. A NOAEL was established at 50 mg/kg bw/day in the Mylchreest et al. (50) study.

The existing data show consistent effects (testicular pathology, reduced sperm numbers, effects on reproductive tract development), and are sufficient to conclude that DBP is a reproductive and developmental toxicant in male rats at doses of 100 mg/kg bw/day and higher. Treatment of rat weanlings with 250 mg/kg bw/day resulted in delayed puberty and doses of 500 mg/kg bw/day induced testicular lesions. In general toxicity studies (Section 2.1.2), testicular lesions were observed in adult rats (6 weeks old) treated with 720 mg/kg bw/day, but not in adult mice treated with up to 3,689 mg/kg bw/day for 3 months (14). Histological changes in testes of 4–6 week-old mice and guinea pigs of a similar nature have also been observed following administration of a single high dose (2,000 mg/kg bw/day) for 7–9 days, but hamsters were unaffected. The overall effects on the testes indicate an age sensitivity with fetal sensitivity >pubertal sensitivity> adult sensitivity in male rats to the action of DBP.

The responses that occur at the lowest doses appear to involve the development of the reproductive system. These responses were seen with some consistency in the studies by Mylchreest et al. (47, 49) and Wine et al. (38). The report by Reel et al. (40) and the paper by Lamb et al. (39) did not report on measures of reproductive system development. However, they are consistent with the Mylchreest et al. and Wine et al. papers in that they show reproductive toxicity under oral (dietary) exposure, and do so in a second species, the mouse.

Mode of Action

Gray et al. (55) investigated the reason for the lack of testicular lesions in hamsters administered DBP and MBuP orally at doses exceeding those that produced testicular lesions in rats. Using ^{14}C-labelled DBP and MBuP, it was determined that intestinal esterase activities were similar in the two species and that the principal metabolite in the rat and hamster was MBuP glucuronide (23). However, the levels of unconjugated MBuP in urine were 3–to 4-fold higher in the rat. Finding that the activity of testicular beta-glucuronidase was significantly higher in the rat than the hamster, the

authors speculated that the testicular damage might be associated with greater concentrations of MBuP, the putative toxicant.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (57-59). It is known that some Sertoli cell functions are mediated by FSH interaction with membrane bound receptors. Lloyd and Foster (60) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (61).

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, DnHP, and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (62) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young versus mature rats. Further, in vitro studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (63). Lloyd and Foster (60) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals.
The Panel was not able to reach agreement that interfering with the FSH-signaling function was the accepted mode or mechanism of action.

The Expert Panel believes that data from studies with DEHP are relevant to a consideration of mechanism for DBP-induced toxicity. It is well understood that DEHP produces a range of hepatic effects in rats (induction of peroxisomes; increased Cyp4A1; PCoA) including hepatic tumors. The induction of these effects in rats is believed due to activation of PPAR-alpha. In genetically altered mice who do not express PPAR, administration of DEHP does not result in the induction of hepatic effects or tumors unlike the wild-type control animals. In humans, PPAR-alpha is activated upstream of different enzymes from those noted in the rat. 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 studies with DEHP, a genetically modified strain of mouse (the PPAR-alpha knockout mouse) cannot activate PPAR-alpha, but is susceptible to phthalate-induced developmental toxicity and testicular toxicity. This mouse does express PPAR-gamma in the testis which can be activated by MEHP (56). PPAR-gamma may conceivably play a role in the reproductive toxicity of phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. Other members of the PPAR family (beta and gamma) have not been extensively studied with regard to activation by phthalates.

Finally, the guinea pig, a non-responding species to the peroxisomal-proliferating effects of DBP, is susceptible to the testicular effects of this phthalate.

Imajima et al. (52) suggests that the active metabolite for reproductive effects due to gestational exposure is MBuP. This pattern of effects induced in rodents by late gestational exposure (gd

12–21) is ‘anti-androgenic,’ in that flutamide mimics these effects (49); however, DBP/MBuP does not bind to the androgen receptor (72). In pubertal and adult rodents, the Sertoli cell is the likely cellular target for testicular injury mediated by the monoester (63, 73).

DBP exhibited no or weak activity in in vitro assays that assess estrogenicity (64, 65, 68, 70). The assays did not include the addition of esterases or lipases to metabolize DBP to its monoester. However, the DBP metabolite MBuP was determined to be inactive in one assay (70). There was no synergism in estrogenic response with DBP and other phthalates (70, 71). DBP was inactive in rodent in vivo assays that measure endpoints such as increases in uterine wet weight, vaginal epithelial cell cornification, or uterine permeability (64, 69, 71). Malformations in reproductive organs and effects on androgen-mediated endpoints in male rats exposed to DBP or MBuP during prenatal development suggest antiandrogenic activity by DBP and MBuP (41, 49, 50, 52).

Table 9: Summaries of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects		Reproductive Effects Observed at Higher Dose Levels
		Reproductive	Systemic	
Dietary continuous breeding protocol with crossover breeding and evaluation of second generation in Sprague-Dawley rats. 20 pairs per group were treated at doses of M: 0, 52, 256, or 509 mg/kg bw/day; F: 0, 80, 385, or 794 mg/kg bw/day during a 14 week mating period. (38)	Reproductive: None Systemic: 256 (M); 385 (F)	M: 52; F: 80 ↓ F_1 live litter size. ↓ F_2 pup weight.	M: 509; F: 794 ↓Body weight gain in F_0 females and F_1 males and females. ↑ Liver and kidney weight in F_0 males and females and F_1 males.	↑ Malformed reproductive organs in F_1 males. ↓ Mating, pregnancy, and fertility in F_1. ↓ Reproductive organ weights in F_1 males. ↑ Testicular lesions in F_1 males. ↓ Sperm counts in F_1. ↓ F_1 litter size. ↑ F_1 pup mortality. ↓ F_1 and F_2 pup weight.
Dietary continuous-breeding protocol with crossover mating in CD-1 mice. 20 pairs per group were treated with 0, 53, 525, and 1,750 mg/kg bw/day during a 14-week mating period. (39, 40)	Reproductive: (M): 525 (F): 525 Systemic: Not known because only high-dose group was necropsied.	M: Can't determine F: 1,750 ↓ Fertility in F_0 females. ↓ Uterine weight in F_0. ↓ Live pups/litter. No effects on sperm in F_0.	1,750 ↓ Bodyweight in males. ↑ Liver weight.	No higher doses.
Multigeneration-reproductive study in Long Evans Hooded rats. 10–12 pairs per group were treated by gavage from weaning throughout puberty, adulthood, mating, and lactation with 0, 250 or 500 mg/kg bw/day. Males were also dosed with 1,000 mg/kg bw/day. F_1 rats were not treated following weaning. (41)	Reproductive: None Systemic: Not reported	250 Delayed puberty in F_0 males. ↓ Sperm production in F_1 males (non-significant). ↓ Fecundity in F_1. ↑ Malformations in F_1 reproductive organs. ↓ F_2 litter size.	Not reported.	Delayed puberty in F_0 males. ↓ Fertility in F_0 males and females. ↑ Midterm abortion in F_0 females. ↑ Testicular lesions in F_0 males. ↓ Sperm production in F_0 and F_1 males. ↓ Fecundity in F_1. ↑ Malformations in F_1 reproductive organs. ↓ F_2 litter size.

5.1.4.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of reproductive toxicity as several studies are available that evaluate both structure and reproductive function. Transgenerational effects were examined in many of the studies. Animals were treated during gestational development, during lactation, and at weaning, thus ensuring that the most sensitive age for reproductive effects was assessed. The evaluation included androgen-regulated endpoints that are believed to be the most sensitive indicators of DBP effects. Reproductive organs were preserved in Bouin's fixative, a method that reduces histological artifacts. Although studies in other species are not as detailed, they do allow for limited comparisons of interspecies sensitivity.

5.2 Integrated Evaluation

DBP is used as a coalescing aid in latex adhesives, as a plasticizer in cellulose plastics, and as a solvent for dyes. General human exposure occurs primarily through food. All estimates place total DBP exposure in the general population at less than 10 μg/kg bw/day. Although infants and young children may have higher exposures than adults, primarily because of different dietary patterns, estimates of their exposure remain at approximately 10 μg/kg bw/day, with the possible exception of non-dietary intake through mouthing of phthalate-containing objects. Workplace exposure at phthalate production facilities is estimated to be below 143 μg/kg bw/day. Exposure levels during incorporation of DBP in plastics are not known.

Following oral exposure to rodents and humans, DBP is quickly metabolized in the small intestine to mono-n-butyl phthalate, MbuP, and n-butyl alcohol. Several investigators have postulated that it is the monoester that is of toxicological interest. The Panel finds logic and data to support this view. Absorption of the monoester into blood occurs in both rats and humans. Although data for DBP is not available for humans or primates, it is reasonable to assume that MBuP would be rapidly glucuronidated and excreted in the urine in a manner analogous to DEHP in humans. The toxicokinetic data indicate that no tissue bioaccumulation would be expected via the oral or dermal route.

There are no data on the developmental or reproductive toxicity of DBP in humans. There are data in rats and mice that show oral exposure to DBP causes developmental toxicity. The developing male reproductive system is most sensitive to the formation of structural and functional abnormalities, with effects seen in rats whose mothers were exposed to 100 mg/kg bw/day during pregnancy. The NOAEL for male reproductive system developmental effects in rats is 50 mg/kg bw/day. Breeding studies provide a good indication of the potential for adverse functional reproductive effects from DBP exposure. Moreover, it is apparent that DBP testicular exposure late in gestation can induce Leydig cell hyperplasia and a low incidence of Leydig cell adenoma. Traditional teratogenicity protocols that evaluate fetuses just prior to birth were not effective in detecting these effects on the developing male reproductive system. While a series of three recent studies have replicated and characterized the male reproductive system effects in rats, studies of similar design have not been performed in other species. The Panel is confident that these studies in rats correctly characterize the effects based on replication, good dose response, and full reporting

of study results. As a default assumption, these data in rats are assumed relevant to a prediction of hazard to humans.

The Expert Panel notes that the male reproductive system is a sensitive target organ for effects in rodent studies where exposure is confined to the adult phase of life. Data in several species, including rat, mouse, and guinea pig, show such effects. The Panel also notes that studies in the hamster, although limited, do not show effects on the testes.

There are indications that oral exposure of females during the adult phase of life impairs functional reproductive performance in rats at doses of 250 mg/kg bw/day and higher. There is also a report that exposure to similar doses during gestation and nursing may impair fertility in female offspring. However, the data are not of the scope and quality for the Expert Panel to confidently characterize these effects.

Data indicate that the monoester of DBP, MBuP, is the principal toxicant. Studies suggest that an antiandrogenic mechanism appears to be responsible for the most sensitive endpoints observed in developing males rats (e.g., anogenital distance, nipple retention, preputial separation). It is not currently known whether the target for DBP is similar or different for gestational versus postnatal exposures.

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 populations.

5.3 Expert Panel Conclusions

DBP is used as a coalescing aid in latex adhesives, as a plasticizer in cellulose plastics, and as a solvent for dyes. The best estimate for exposures from all sources to the general population is 2–10 μg/kg bw/day. There is significant uncertainty in the exposure database based on the age of many of the values/studies. The Expert Panel has high confidence in the available studies to characterize reproductive and developmental toxicity based upon a strong database containing studies in multiple species using conventional and investigative study designs. When administered via the oral route, DBP elicits malformations of the male rat reproductive tract via a disturbance of the androgen status: a mode of action relevant for human reproductive development. This antiandrogenic mechanism occurs via effects on testosterone biosynthesis and not androgen receptor antagonism. DBP is a testicular toxicant in three species of young adult laboratory animals in high dose (>1,000 mg/kg bw/day), sub-acute oral exposure studies. In the rat, there is a life-stage sensitivity for testicular toxicity with the fetus most sensitive, pubertal less sensitive, and adult least sensitive. Adult female functional reproductive toxicity (decreases in fertility) has been noted in rats; however, the data do not permit confident characterization of dose-effects below 250 mg/kg bw/day. The Expert Panel has negligible concern for adult reproductive toxicity.

DBP is developmentally toxic to both rats and mice by the oral routes; it induces structural

malformations. A confident NOAEL of 50 mg/kg bw/day by the oral route has been established in the rat. Data from which to confidently establish a LOAEL/NOAEL in the mouse are uncertain. The Expert Panel has minimal concern about effects to human development and development of the reproductive system from current estimated exposure to DBP. A modified dietary multigeneration study is available, but did not establish a NOAEL. The LOAEL (M:52 F:80 mg/kg bw/day) is based on decreases in litter size and pup weight.

5.4 Critical Data Needs

The multigeneration study for DBP in rodents, with support from other studies that incorporated more modern endpoints including developmental landmarks, indicates no immediate data gaps. The potential effects of DBP on female rats warrant further investigation.

Although there are no critical data needs, studies in the following areas would increase understanding about reproductive and developmental effects that occur following DBP exposures.

There is a need to extend the current PBPK model for DBP to include parameters for pregnant women and their fetuses.

There is a need to find out how broad or narrow the window of prenatal exposure is that results in postnatal male effects. The known current window in rats, 12–20 days, is still quite wide from a rodent ontogenesis perspective. Greater precision as to size of the window of sensitivity may be relevant to estimating the temporal bounds of human sensitivity.

Much of the recent focus on reproductive toxicity of phthalate esters has focused on the ability of certain esters to induce effects on reproductive development. Significant primate data exist to support the view that the high blood levels of monoester necessary to achieve adult testicular toxicity in rodents will not occur in humans. Appropriate exposure to monoester in blood from diester exposure could be achieved such that experiments could be conducted in primates to elucidate species sensitivity for equivalent exposures. This would require exposure of pregnant animals during the critical window of development of the reproductive system for the species studied, followed by an examination of reproductive development in the resulting offspring. Such a study would indicate if there is a species sensitivity. In the absence of such a study, the rodent data must be considered relevant and critical for human risk examinations

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Table 7.1: DBP General Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Animal Number /Sex	Dose*	Body Weight	Organ/Body Weight Ratio	Histopathology	Hematology	Chemistry	Other
Wistar Rats BASF 1992 (1)	3-month sub-chronic study. Forty-two day-old rats of both sexes were exposed to DBP in the diet at concentrations of 0, 400, 2,000, or 10,000 ppm and then killed and necropsied. 26 tissues collected, histopathology of control and high dose liver, kidney, and testes examined at all doses. Hematology, clinical chemistry, urinalysis at mid- and end of study. Neurobehavior assessed 3x during study.	10	0						
		10	27(M)/ 33(F)	NE	NE	NE	NE	NE	
		10	141(M)/ 162(F)	NE	NE	NE	NE	NE	NOAEL
		10	688(M)/ 816(F)	NE	↑Li, Ki(F)	↓Lipid in hepatocytes. No testicular effects.	Transient ↓ RBC, Hb, Hct (M).	↑Glu, Alb (M). ↓Trigl, T3.	↑ PCoA No neurological effects.

*Dose in mg/kg bw/day.

NA=Not Analyzed
NE=No Effect
↑= Statistically Significant Increase
↓=Statistically Significant Decrease
PCoA=Palmitoyl-CoA Oxidase

M = Male
F = Female
Li = Liver
Ki = Kidney
T3 = Triiodothyronine

Trigl = Triglycerides
Alb = Albumin
RBC = Red Blood Cell
Hb = Hemoglobin
Hct = Hematocrit

Glu = Glucose

Table 7.2 : DBP General Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Animal/ Number/ Sex	Dose*	Body Weight Gain	Organ/Body Weight Ratio	Histopathology	Hematology	Chemistry	Other
F344/N Rats Marsman 1995 (2)	Sub-chronic study (13 weeks), 5–6-week-old rats were fed DBP and then killed and necropsied. Lowest dose was 2,500 ppm, doses then doubled until highest dose of 40,000 ppm achieved. Extensive tissue exam, hematology, clinical chemistry, semen, peroxisome proliferation enzyme evaluation at term.	10	0						
		10	176(M)/ 177(F)	NE	NE	NE	NE	↑Alb (M)	NOAEL
		10	359(M)/ 356(F)	↓(M)	↑Li and Ki (M)	NE	↓Hb (M) ↓RBC (M) ↑Pl (M)	↑Alb (M) ↓Trigl (M) ↑Bile Ac (F)	↑PCoA
		10	720(M)/ 712(F)	↓(M)	↑Li and Ki	Hepatic lesions. Testicular lesions.	↓Hb, Hct (M) ↓RBC (M) ↑MCV ↑Pl (M)	↑Alb (M) ↓Trigl (M) ↑Bile Ac (F) ↑AP (F)	↑PCoA
		10	1,540(M)/ 1,413(F)	↓	↑Li and Ki ↓Te	Hepatic lesions. Testicular lesions, marked hypospermia. ↓ Sperm motility and concentration.	↓Hb, Hct (M) ↓RBC (M) ↑MCV ↑Pl (M)	↑Alb (M) ↓Chol, Trigl ↑Bile Ac ↑AP ↓TP (F)	↑PCoA ↓Testic. Zn ↓Testost.
		10	2,964(M)/ 2,943(F)	↓[a]	↑Li and Ki ↓Te	Hepatic lesions and peroxisomal proliferation. Testicular lesions and hypospermia.	↓Hb, Hct (M) ↓ RBC (M) ↑ MCV (M) ↑Pl (M)	↑Alb(M) ↓TP ↓Chol, Trigl ↑Bile Ac ↑AP	↑PCoA ↓Testic. Zn & serum Zn ↓Testost.

*Dose in mg/kg bw/day.
[a]Food consumption only 58% (M) and 83% (F) of control.

NE= No Effect
↑= Statistically Significant Increase
↓=Statistically Significant Decrease
Hb = Hemoglobin
M= Male
F= Female
Li = Liver
Ki = Kidney
RBC= Red Blood Cell Count
Te =Testes
Tp = Total Protein
Alb = Albumin
Chol = Cholesterol
Trigl = TriglyceridesZn = Zinc
AP = Alkaline Phosphatase
Bile Ac = Bile Acids
PCoA = Palmitoyl-CoA Oxidase
MCV= Mixed Cell Volume
Pl = Platelets
Testost = Testosterone
HCT= Hematocrit

Table 7.3: DBP General Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Animal Number /Sex	Dose*	Body Weight Gain	Organ/Body Weight Ratio	Histopathology	Hematology	Chemistry	Other
F344/N Rats Marsman 1995 (2)	Rats were exposed to 0 or 10,000 ppm DBP during prenatal development until 8 weeks of age. At 8 weeks of age, the rats were then fed DBP in the diet for 13 weeks, killed and necropsied.	10	0**						
		10	0	↑[a]	↑Te[a]	NE	NE	↓Test[a]	↑PCoA at weaning.
		10	138(M)/ 147(F)		↑Ki(F)[b], Li(F)[a] ↑Te[a]	NE	NE		
		10	279(M)/ 294(F)		↑Ki(F)[b](M)[a] ↑Li (F)[a](M)[a,b] ↑Te[a]	NE	NE	↑Alb(F)[a]	↑PCoA(M)[a,b] No-effect level for liver and testes.
		10	571(M)/ 593(F)	↓F[b], M[ab]	↑Ki(F)[b](M)[a,b] ↑Li[a,b] ↑Te[a]	Hepatic and testicular lesions.	↓ Hct ↓Hb ↓ RBC(M)[b] ↑Pl(M)[b]	↑Alb[a,b] ↓Trigl(M)[a,b]	↑PCoA[a,b]
		10	1,262(M)/ 1,182(F)	↓[ab]	↑Ki[a,b] ↑Li[a,b] ↓Te[a,b]	Hepatic and testicular lesions. ↓Sperm counts and hypospermia of epididymis.	NE	↑Alb[a,b] ↓ Chol[a,b] ↓Trigl[a,b] ↑ AP[a,b]	↑Zn in serum(M)[a,b] ↑PCoA[a,b]
		10	2,495(M)/ 2,445 (F)	↓[a,b,c]	↑Ki[a,b] ↑Li[a,b] Te[a,b]	Hepatic lesions, peroxisomal proliferation, and testicular lesions. ↓Sperm counts and hypospermia of epididymis.	↓Hct ↓Hb ↓ RBC[a,b] ↑Pl(M)[a,b]	↓Tot Prot[a,b] ↑Alb(M)[a,b] ↓ Chol[a,b] ↓Trigl[a,b] ↑ AP[a,b] ↑Bile Ac (F)[b], (M)[a,b] ↓Test[a]	↑Zn in serum(M)[b] ↓Testicular Zn[a,b] ↑PCoA[a,b]

*Dose in mg/kg bw/day. ** No prenatal exposure [a]Significant compared to control with no perinatal DBP exposure
[b]Significant compared to control with 10,000 ppm DBP perinatal exposure [c] Significant reduction in food consumption, rats emaciated

NA=Not Analyzed
NE=No Effect
↑= Statistically Significant Increase
↓=Statistically Significant Decrease

M = Male
F = Female
Li= Liver
Ki = Kidney

Te = Testes
Tot Prot = Total Protein
Alb = Albumin
Chol = Cholesterol

Trigl = Triglycerides
AP = Alkaline Phosphatase
Bile Ac = Bile Acids
Hct = Hematocrit

Zn = Zinc
Pl = Plateets
Test = Testosterone
PCoA = Palmitoyl-CoA Oxidase

Hb = Hemoglob
RBC = Red Blood cells

Table 7.4: DBP General Toxicity, Mice

Species, Strain, and Source	Experimental Regimen	Animal Number/ Sex	Dose*	Body Weight	Organ/Body Weight Ratio	Histopathology	Hematology	Chemistry
$B6C3F_1$ Mice Marsman 1995 (2)	13 week sub-chronic study. 6 week-old mice were exposed to DBP in the diet at levels of 0,1,250, 2,500, 5,000, 10,000, or 20,000 ppm for 13 weeks and then killed and necropsied. Organ weights, histological exam of tissues. Hematology, sperm morphology and vaginal cytology.	10	0					
		10	163(M)/ 238(F)	NE	↑Ki(F)	NE	NE	NA
		10	353(M)/ 486(F)	NE	↑Ki(F)	NE	NE	NA
		10	812(M)/ 971(F)	↓	↑Li ↑Ki(F)	NE	NE	NA
		10	1,601(M)/ 2,137(F)	↓	↑Li ↑Ki(F)	Liver lesions (M).	NE	NA
		10	3,689(M)/ 4,278(F)	↓	↑Li ↑Ki(F)	Liver lesions. No testicular lesions or other adverse reproductive effects	↓ Hct (F)	NA

*Dose in mg/kg bw/day.

NA=Not Analyzed
NE=No Effect
↑= Statistically Significant Increase
↓=Statistically Significant Decrease

M=Male
F=Female
Li=Liver
Ki=Kidney

Zn=Zinc
Hct=Hematocrit

Table 7.5: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal Effects	Fetal Effects
ICR-JCL Mice Shiota et al. 1980; Shiota and Mishimura 1982 (3, 4)	Prenatal developmental toxicity study. Mice were fed diets with 0, 0.05, 0.1, 0.2, 0.4, or 1% DBP from gd 0–18. Body weights were measured on gd 0–18. Dams were sacrificed on gd 18. Corpora lutea were counted and pups were examined for skeletal and soft tissue malformations.	8	0		
		7	80	NE	LOAEL[b] Delayed ossification.
		8	180	NE	Delayed ossification.
		6	350	NE	Delayed ossification.
		9	660	NOAEL	↓ Fetal weight. Delayed ossification.
		15	2,100	↓ Bodyweight gain.	↑ Resorptions (98.4 vs 5%). ↓ Fetal weight. Delayed ossification. ↑ Neural tube defects (2/3 fetuses).[c]

*Dose in mg/kg bw/day.

[a]Number of pregnant females at sacrifice.

[b]Differs from author's selection of effect level.

[c]Effect not statistically significant.

↑=Statistically Significant Increase
↓=Statistically Significant Decrease
NE=No effects

Table 7.6: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal Effects	Fetal Effects
Wistar Rats Ema et al. 1993 (5)	Prenatal developmental toxicity study. Rats were gavaged with DBP from 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.	11(11)	0		
		11(11)	500	NOAEL	NOAEL
		12(12)	630	↓Weight gain.	Complete resorption in 2/12 litters. ↓Live fetuses/litter (43%). ↓ Fetal weight (9–10%).
		12(12)	750	↓Adjusted weight gain (38%).	Complete resorption in 10/12 litters. ↓ Live fetuses/litter (93%). ↓ Fetal weight (14–18%). ↑ External malformations (cleft palate) in 6/10 fetuses (2 litters) vs 0/118 fetuses in control.
		11(9)	1,000	↓Adjusted weight gain (71%).	Complete resorption in 9/9 litters.

*Dose in mg/kg bw/day.
[a]Number of pregnant rats (Number of litters evaluated)

↑=Statistically Significant Increase

↓=Statistically Significant Decrease

Table 7.7: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal Effect	Fetal Effects
Wistar Rats Ema et al. 1998 (6)	Prenatal developmental toxicity study. Rats were fed diets with 0, 0.5, 1.0, or 2.0% DBP on gd 11–21. Body weights and food intake were measured. Dams were sacrificed on gd 21. 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.	11	0		
		11	331	NOAEL	NOAEL.
		11	555	↓Corrected weight gain.[b] ↓Food intake.	↓Anogenital distance in males. ↑Undescended testes (15% vs 0 in 7/11 litters).
		11	661	↓Corrected weight gain.[b] ↓Food intake.	↓Fetal weight (22%). ↓Anogenital distance in males. ↑Undescended testes (53% vs 0 in 11/11 litters). ↑External (cleft palate; 4% vs 0 in 4/11 litters) and skeletal (fused sternebrae; 55% vs 0 in 11/11 litters) malformations

*Dose in mg/kg bw/day.
[a]Number of pregnant rats and litters evaluated [b]Body weight excluding gravid uterus

↑=Statistically Significant Increase
↓=Statistically Significant Decrease

Table 7.8: DBP Developmental Toxicity, Rats

Species, Strain, Source	Experimental Regimen	Animal Number	Dose*	Maternal Effects	F_1 Offspring Effects
F344/N Rat Marsman et al. 1995 (2)	Pre- and post-natal exposure study. DBP administered in feed to dams throughout gestation and lactation. Dams were weighed on gd 0 and 18, and weekly during lactation. Uteri of nulliparous rats in high-dose group were stained with ammonium sulfide. Gestation index[a], litter size, and pup survival were examined. Pups were weighed at birth and pnd 0, 4, and weekly thereafter.	28[b]	0		
		15	92 (1,250 ppm)[d]	NE	NE
		15	184 (2,500 ppm)	NOAEL	↓ Weight days 21–28.
		13	368 (5,000 ppm)	↓ Gestation index(68 vs. 93%)[a] ↓ Gestation length.	↓ Weight days 1–28.
		14	551 (7,500 ppm)	NE	↓ Weight days 0-28.
		16	736 (10,000 ppm)	↓ Weight gain during lactation.	↓ Weight days 0-28. ↓ Percent live pups/litter (89 vs. 96%).
		14	1,472 (20,000 ppm)	↓ Gestation index (21 vs. 93%)[a] ↓ Gestational weight gain.	↓ Pup weight day 0. ↓ Litter size (72%) and % live pups/litter (29 vs 99) Complete pup mortality by pnd 1.
	After weaning on day 28, pups were administered DBP in feed for 4 weeks at the same levels administered to their mothers (1,250, 2,500, 5,000, 7,500, 10,000 ppm). Body weights were measured weekly. Necropsies were conducted and organ weights determined for all groups. Histopathology was evaluated in control and high dose rats. Testis evaluated in dose groups receiving 2,500 ppm and higher.	10[c]	0		
		10	133(F)/143(M)[e]		↑ Kidney (M) & liver to body weight ratio (M). ↑ Weight gain in females.
		10	275(F)/284(M)		↑ Kidney (M) to body weight ratio (M). ↑ Liver to body weight ratio.
		10	500(F)/579(M)		Hypospermia in 4/10 males. ↑ Kidney & liver to body weight ratio .
		10	836(F)/879(M)		Hypospermia in 10/10 males. ↓ Weight gain in males. ↑ Kidney & liver to body weight ratios.
		10	1,104(F)-1165(M)		Hypospermia in 10/10 males. ↓Testis to body weight ratio (11%). ↓ Weight gain in males. ↑ Kidney & liver to body weight ratios .

*Dose in mg/kg bw/day.
[a]Delivery of ≥ 1 live pup per sperm positive female
[b]Number of rats delivering litters
[c]Number of pups/sex
[d]Doses estimated by CERHR, see Section 3 for explanation.
[e]Author calculated doses for females and males, respectively
NE=No effect
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

Table 7.9: DBP Developmental Toxicity, Mice

Species, Strain, Source	Experimental Regimen	Animal Number	Dose*	Maternal effects	Offspring effects
$B6C3F_1$ Mouse Marsman et al. 1995 (2)	Pre- and post-natal exposure study. DBP administered in feed to dams throughout gestation and lactation. Dams were weighed on gd 0 and 17, and weekly during lactation. Uteri of nulliparous mice in high-dose group were stained with ammonium sulfide. Litter size, and pup survival were examined. Pups were weighed at birth and pd 0, 4, and weekly thereafter.	11[a]	0		
		10	227 (1,250 ppm)[c]	NE	NE
		12	454 (2,500 ppm)	↑ Gestation length (2%).	↓ Litter size.
		9	908 (5,000 ppm)	↑ Gestation length (3%).	NE
		11	1,359 (7,500 ppm)	↓ Gestational weight gain (18%). ↑ Gestation length (5%).	↓ Litter size (28%). ↓ Live pups/litter (48%).
		5	1,816 (10,000 ppm)	↓ Gestational weight gain (34%). ↑ Gestation length (6%).	↓ Litter size (48%). ↓ Live pups/litter (89%). ↓ Pup birth weight (14%).
		0	3,632 (20,000 ppm)	No live deliveries.	
	After weaning, pups were administered DBP in feed for 4 weeks at the same levels administered to their mothers (0, 1,250, 2,500, 5,000, 7,500, 10,000 ppm). Body weights were measured weekly. Necropsies were conducted and organ weights determined for all groups. Histopathology was evaluated in controls and the 1,060–1,286 mg/kg bw/day group.	10[b]	0		
		10	170(F)/199(M)[d]		↑ Liver to body weight ratio (M). ↑ Kidney to body weight ratio (F).
		10	399(F)/437(M)		↓Male body weights (7%). ↑ Liver to body weight ratio in males. ↑ Kidney to body weight ratio in females.
		10	714(F)/750(M)		↓Male body weights (11%). ↑ Liver to body weight ratio in males. ↑ Kidney to body weight ratio in females.
		10	1,060(F) /1,286(M)		↓Male body weights (12%). ↓ Female body weight (11%). ↑ Liver to body weight ratio in males. ↑ Kidney to body weight ratio in females.
		1	3,804(M)		

*Dose in mg/kg bw/day.
[a]Number of mice delivering litters [b]Number of pups/sex [c]Doses estimated by CERHR, see section 3 for explanation.
[d]Author calculated doses for females and males respectively ↑=Statistically Significant Increase
NE=No Effect (M)=Male (F)=Female

Table 7.10: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal effects	Offspring effects
CD Rat Mylchreest et al. 1998 (7)	Pre- and post-natal developmental toxicity study. Rats were gavaged with DBP from gd 3 until the end of lactation. Body weights were measured daily and food intake was measured weekly. Dams were killed and necropsied following weaning of pups. Implantation sites were examined. Pups were sexed, weighed, and evaluated for sexual maturation. Pups were sacrificed on pnd 100–105. All males and up to 3 females/litter were necropsied. Histological exams were conducted on malformed rats and ≤ 2 normal rats/litter. Sperm analysis was conducted at necropsy.	9	0		
		8	250	NE	↑Hypospadia (1/32 pups), underdeveloped or absent epididymis (3/32 pups; 2 litters) and seminal vesicles (0 pups), and undescended testes (1/32 pups).
		7	500	↓Uterine weight.	↓Anogenital distance in males (pnd 1). ↑Hypospadia (7/34 pups; 4 litters), underdeveloped or absent epididymis (17/34 pups; 6 litters) and seminal vesicles (2/34 pups; 2 litters), and undescended testes (2/34 pups; 2 litters). ↓Testis (24%) and seminal vesicle weight (16%).
		4	750	↓Uterine weight (non-significant).	↓Live pups/litter (27%). ↓Pup survival during lactation (85 vs 96%). ↓Anogenital distance in males (pnd 1). ↑Hypospadia (6/14 pups; 2 litters), underdeveloped or absent epididymis (10/14 pups; 3 litters) and seminal vesicles (7/14 pups; 3 litters), and undescended testes (4/14 pups; 2 litters). ↓Testis (33%), seminal vesicle (32%), epididymis (34%), and prostate weight (27%) . ↓Kidney weight. No effects on female sexual development or estrous cycles.

*Dose in mg/kg bw/day.

[a]The number of ~~dams that littered~~litters evaluated. ↑ Statistically Significant Increase ↓ Statistically Significant Decrease

Table 7.11: DBP Developmental Toxicity, Rats

Species, Strain, Source	Experimental Regimen	Number*	Dose*	Maternal effects	Offspring effects
CD Rat Mylchreest et al. 1999 (8)	Pre- and post-natal developmental toxicity study. Rats were gavaged with DBP from gd 12–21. Body weights were measured daily during dosing and weekly at other times. Food intake was measured weekly. Dams were killed and necropsied following weaning of pups. Implantation sites were examined. Pups were sexed, weighed, and evaluated for sexual maturation. Male pups were sacrificed on pnd 100–105 and a histological examination of sex organs was conducted. Females were sacrificed on pnd 25–30 and their reproductive tracts were evaluated for gross abnormalities.	10	0		
		9	100	NE	↑Age of preputial separation (5%).
		10	250	NE	↓Anogenital distance in males (9%). ↑Retained thoracic nipples (35/62 pups; 5 litters). ↑Absent or underdeveloped epididymis (6/62 pups; 4 litters).
		9	500	Large weight loss (16%) and complete litter death in one dam.	↓Anogenital distance in males (24%). ↑ Retained thoracic nipples (47/54 pups; 8 litters). ↑Age of preputial separation (9%). ↑Hypospadias (21/52 pups; 4 litters), absent prostate (3/52 pups; 1 litter), absent or underdeveloped epididymis (26/52 pups; 8 litters) and vas deferens (14/52 pups; 4 litters). ↑Testicular and epididymal lesions. ↑Interstitial adenoma (2/45 in 1 litter versus 0/51 pups in control). ↑Intra-abdominal testes (5/52 pups; 3 litters). ↓Absolute testes (16%), epididymis (26%), and seminal vesical (21%) weight. ↓Absolute kidney weight.
	Results were compared to those induced by the antiandrogenic drug, flutamide.	5	100 flutamide	↓Body weight gain.	↓Anogenital distance in males. ↑ Retained thoracic nipples. ↑Hypospadias, underdeveloped or absent seminal vesicles, complete lack of prostate and epididymis, and vas deferens development. ↑Testicular lesions. ↑Suprainguinal testes. ↓Absolute testes, epididymis, and seminal vesical weight.

*Dose in mg/kg bw/day.
*Numbers of litters evaluated NE=No Effect ↑=Statistically Significant Increase ↓=Statistically Significant Decrease

Table 7.12: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal effects	Offspring effects
Sprague-Dawley Rat Mylchreest et al. 2000 (9)	Pre- and post-natal developmental toxicity study. Rats were gavaged with DBP in corn oil from gd 12–21. Dams delivered litters and pups were examined and weighed at birth. After the pups were weaned, dams were killed and organ weights and implantation sites were evaluated. Pups were weighed weekly and evaluated for sexual maturation until killed at puberty. Male and female pup organs were weighed and testes and epididymides were examined histologically.	19	0	No effects observed at any dose level.	
		20	0.5		NE
		19	5		NE
		20	50		NOAEL
		20	100		↑Seminiferous tubule degeneration (3% of rats in 2/10 litters). ↑Retained areolas or nipples in males (31% of rats in 16/20 litters).
		11	500		↑Seminiferous tubule degeneration (56% of rats in 3/5 litters). ↑Retained areolas or nipples in males (90% of rats in 11/11 litters). ↓Anogenital distance in males. ↑Hypospadias (9% of rats in 4/11 litters). ↑Agenesis of epididymis (36% of rats in 9/11 litters), vas deferens (28% of rats in 9/11 litters), and prostate (1/58 rats). ↓Testis, epididymis, prostate, and levator ani muscle weight. ↑Interstitial cell hyperplasia (35% of rats in 3/5 litters) and adenoma (1/23 rats). ↑Intra-abdominal testes (4 rats/3 litters). No effect on vaginal opening or on female reproductive organ weight or histology.

*Dose in mg/kg bw/day.
[a]Number of litters evaluated. ↑=Statistically Significant Increase ↓=Statistically Significant Decrease NE=No Effect

Table 7.13: DBP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[a]	Dose*	Maternal effects	Offspring effects
Sprague Dawley Rat	Pre- and post-natal developmental toxicity study.	9	0		
	DBP administered in oil by gavage from gd 14 to lactation day 3. Male pups were examined for sexual maturation. At 5 months of age, male offspring were killed and necropsied. Organ weights were measured and a histological examination was conducted on reproductive organs.	8	500	Not reported	↓Anogenital distance (2.79 vs 3.70mm). ↑Percentage of areolas (55 vs 0%) and numbers of areolas/nipples at birth (n=2.7 vs 0) and adulthood (2.2 vs 0). ↑% Hypospadias (6.2 vs 0%) and testicular and epididymal atrophy or agenesis (46 vs 0%). ↓Seminal vesicle, prostate, epididymis, testes, levator ani, and penis weight.
LE Hooded Rats	LE Hooded Rats were gavaged with DBP from gd 16–19.	6	0		
Gray et al. 1999 (10)	All other details are as described above for longer exposure in Sprague-Dawley rats.	4	500	Not reported	↓Anogenital distance (2.83 vs 3.21 mm). ↑Percentage of areolas (87 vs 0%) and numbers of areolas/nipples at birth and adulthood (1.9 vs 0). ↓Seminal vesicle, prostate, and levator ani muscle weight.

*Dose in mg/kg bw/day.
[a]Number of pregnant rats. ↑ Statistically Significant Increase ↓ Statistically Significant Decrease

Table 7.14: DBP Reproductive Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Number[b]	Dose[c] (mg/kg bw/day)	Effects
CD Rats	Fertility assessment through a continuous breeding study.	40	0	
Wine et al. 1997 (11)[a]	DBP administered in feed at 1,000, 5,000 or 10,000 ppm. Breeding pairs housed together for 112 days; female body weight was measured on days of littering and both sexes at necropsy; clinical signs, and food intake were recorded; litters were counted, sexed, weighed, and removed following birth. In a crossover breeding study, high-dose F_0 males and females were mated with control animals for 1 week. At the end of the study Necropsy and a histopathological examination were conducted.	20	52(M)/80(F)	↓ Live pups/litter.
		19	256(M)/385(F)	↓ Live pups/litter. ↓ Pup weight.
		20	509(M)/794(F)	↓ Live pups/litter. ↓ Pup weight. ↑ Liver and kidney to body weight ratio. ↓ Pup weight from treated females in crossover.
	Final F_1 litters from continuous breeding study were weaned and mated within dose groups for 1 week. Rats continued to receive the same DBP concentrations as their parents.	20	0	
		20	52(M)/80(F)	↓ F_2 Pup weight.
		20	256(M)/385(F)	↑ Kidney to body weight ratio (M). ↓ F_2 Pup weight. ↑ Degeneration of seminiferous tubules.
		20	509(M)/794(F)	30% mating, 5% pregnancy, 17% fertility indices ↓ Sperm count (49%). ↑ Degeneration of seminiferous tubules, interstitial cell hyperplasia, underdeveloped epididymis, and malformed penises and prepuces. ↓ Prostate and seminal vesicle to body weight ratio. ↓ Testis weight. ↓ Body weight in males and females. ↑ Liver and kidney to body weight ratio in males. ↓ F_2 Pup weight

[a]This study is also addressed in Marsman et al. 1995
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

[b]Number of male and female pairs
(F)=Female
(M)=Male

[c]Author-calculated male and female doses, respectively.

Table 7.15: DBP Reproductive Toxicity, Rats

Species, Strain, and Source	Experimental Regimen	Animal Number	Dose*	Effects
LE Hooded Rats Gray et al. 1999 (10)	Multigeneration reproductive study. Male and female rats (F_0) were gavaged with DBP from puberty through adulthood, mating, and lactation. Sexual maturation and estrous cycles were evaluated. Treated rats were mated with untreated controls. Following weaning of F_1 pups, F_0 rats were killed. At necropsy, serum hormone levels, organ weights, testicular histology, and implantation sites were examined.	24[a]	0	
		10	250	↑Age of F_0 preputial separation (42.6 vs 39.5 days). ↑Malformed F_1 pups (14.5 vs 0.7%) and litters with malformed pups (50 vs 5.5%).
		4	500	↑Age of F_0 preputial separation (43.4 vs 39.5 days). ↑Malformed F_1 pups (33 vs 0.7%) and litters with malformed pups (100 vs 5.5%). ↓Fertility in F_0 males and females. ↑Testicular atrophy in F_0 males. ↓Sperm production in F_0 males. ↑Midterm abortions in F_0 females.
		8–12[b] (males only)	1,000	↑Age of F_0 preputial separation (44.4 vs 39.5 days). ↑Testicular atrophy in F_0 males. ↓Sperm production in F_0 males.
	The F_1 rats were not exposed to DBP following weaning. Some F_1 pups from treated dams were mated within dose groups for 11 cycles, and the remainder were necropsied. F_2 pups were counted and discarded.	18[c]	0[d]	
		18	250	↓Fecundity in F_1. ↓Number of F_2 pups born. ↓Caudal sperm levels in F_1 (non-significant; 19%).
		4	500	↓Fecundity in F_1. ↓Number of F_2 pups born. ↓Caudal sperm levels in F_1 (34%).

*Dose in mg/kg bw/day.

[a]Number of litters evaluated [b]Number of males, only males treated with highest dose [c]Number of breeding pairs

[d]Maternal (F_0) exposure levels ↑=Statistically Significant Increase ↓=Statistically Significant Decrease

Table 7.16: DBP Reproductive Toxicity, Mice

Species, Strain, and Source	Experimental Regimen	Number[b]	Dose[c] (mg/kg bw/day)	Effects
CD-1 Mice Reel et al. 1984; Lamb 1987 (12, 13)[a]	Fertility assessment through a continuous breeding study. DBP administered in feed. Breeding pairs housed together for 98 days; body weight was measured on 7 days, clinical signs, and food intake were recorded; litters were counted, sexed, weighed, and removed following birth. In a crossover breeding study, high dose males and females were mated with control mice. Breeding pairs were housed together for 7 days or until a copulatory plug was observed. Necropsy and a histopathological examination were conducted.	39	0	
		20	52.5	NE
		18	525	NOAEL
		20	1,750	↓ Number of fertile pairs. ↓ Number of litters delivered/pair. ↓ Litter size. ↓ Live pups. ↑ Percentage of male pups. ↓ Pup weight. ↓ Uterus to body weight ratio in F_0 females. ↓ Body weight in F_0 males. ↑ Liver to body weight ratio in F_0 males and females. No effects on estrous cycles, sperm morphology, or sex organs in F_0 mice.

[a]This study is also addressed in Marsman et al. 1995
[b]Number of male and female pairs
[c]Author-calculated doses

NE=No Effect
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

Table 7.17: MBuP Developmental Toxicity, Rats

Species, Strain, and Source	Experimental Regimen		Dose*	Maternal effects	Offspring effects
Wistar-King A rats.	Pre and postnatal developmental toxicity study with prenatal exposure.	19 / 15	0		
Imajima et al. 1997 (14)	Rats were gavage dosed 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.
↑ Statistically significant increase

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

Center For The Evaluation Of Risks To Human Reproduction Ê

PUBLIC COMMENTS Ê ON THE PHTHALATES Ê EXPERT PANEL REPORTS Ê

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,

James S. Benson
Executive Vice President
Technology & Regulatory Affairs

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

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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
Chevy Chase, MD 20815-7278

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.

Appendix III

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,

Courtney M. Price/HCS

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

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December 11, 2000

Michael D. Shelby, Ph.D
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Research Triangle Park, NC 27709-2233

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.

Appendix III

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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.

[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

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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

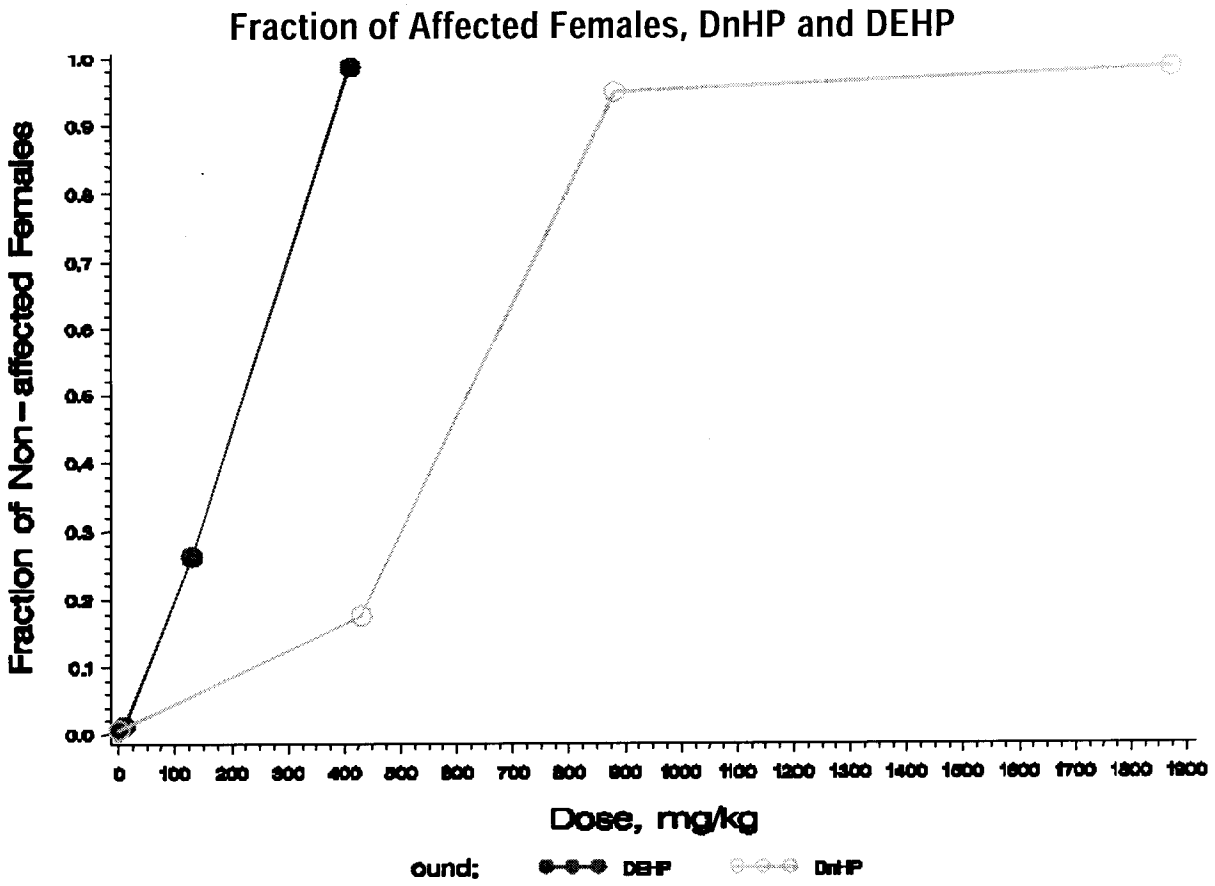
A probit regression analysis with compound and dose indicated a statistically significant difference in compounds ($p<0.001$). The model diagnostics indicated the statistical assumptions for the analysis were met.

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

Appendix III

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.

Appendix III

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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
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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.

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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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S. Waterman, J. Ambroso, L. Keller, G. Trimmer, A. Nikiforov and S. Harris (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. *Reproductive Toxicology* 13:131-136.

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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
VICE PRESIDENT
CHEMSTAR

April 11, 2001

American Chemistry Council *Good Chemistry Makes It Possible*

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RECEIVED
APR 13 2001
CERHR

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Principal Investigator, CERHR
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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

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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

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

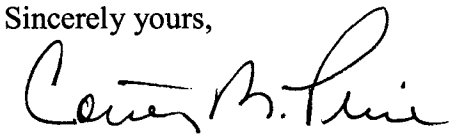
bulbocavernosus muscles in the 1500 mg/kg/day group. Weights of testes, ventral prostate, epididymis and bulbourethral glands were unaffected.

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,



Courtney M. Price
Vice-President, CHEMSTAR

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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.

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.

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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.

DISCOVERY MEDICAL, INC.

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,

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

Appendix III

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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,

Richard Wiles
Vice President for Research

Jane Houlihan
Senior Analyst

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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,

Richard Wiles
Vice President for Research

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>>

Appendix III

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.

Appendix III

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

DEHP STUDIES - MOLAR COMPARISON FOR DOWNSTREAM METABOLITES

	DEHP mg/kg	DEHP mmol/kg	MEHP mmol/kg	MEHP mg/kg	2-EH mmol/kg	2-EH mg/kg	2-EHA mmol/kg	2-EHA mg/kg
Tyl, et al.,								
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 mg/kg	MEHP mmol/kg		2-EH mg/kg	2-EH mmol/kg	2-EHA mmol/kg	2-EHA mg/kg
Price, et al.,			Tyl, et al.,				
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 mg/kg	DEHP mmol/kg	MEHP mmol/kg	MEHP mg/kg	2-EH mmol/kg	2-EH mg/kg	2-EHA mmol/kg	2-EHA mg/kg
Wistar									
Hellwig, et al.,									
	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 mg/kg	MEHP mmol/kg		2-EH mg/kg	2-EH mmol/kg	2-EHA mmol/kg	2-EHA mg/kg
Wistar				Wistar				
Ruddick, et al.,				Hellwig, et al				
	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		

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

Ih Chu*, Udai Gill, André Craan and Kunnath Subramanian, Healthy Environments and Product Safety Branch, Health Canada, Ottawa, ON, K1A 0L2, Canada

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

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

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.

Appendix III

HCWH AT THE CENTER FOR HEALTH, ENVIRONMENT AND JUSTICE

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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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Sept. 15, 2000

To:

National Institute of Environmental Health Sciences

P.O. Box 12233
Research Triangle Park, N.C. 27709

FROM:

Mrs. Beverly Smith
21 Rolling Hill Dr.
Fairport, N.Y. 14450

ECE
OCT 19 2000
LAB OF

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. My nephew was born with pyloric stenosis and an inguinal hernia which made it necessary for him to have intravenous feeding and blood transfusions during surgery. Now an adult desiring marriage and children he has only one incompletely developed testicle.

Appendix III

His sterility fits the pattern described in this article.

For him DEHP was not just a risk, it was a life long disaster. Please prevent this from happening to others.

Sincerely,

Beverly Smith

Kemikalieinspektionen

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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

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