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



Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Octyl Phthalate (DnOP)

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Preface

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

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

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

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

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

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

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Information about the NTP is available on the web at <*http://ntp-server.niehs.nih.gov*> or by contacting the NTP Office of Liaison and Scientific Review at the NIEHS:

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Introduction

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

These chemicals were selected because:

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

These seven phthalates are as follows:

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

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

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

The NTP has prepared an NTP-CERHR monograph for each phthalate. This monograph includes the NTP brief on DnOP, a list of the expert panel members (Appendix I), the expert panel's report on DnOP (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 DnOP 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 monograph presents the NTP's interpretation of the potential for exposure to DnOP to cause adverse reproductive or developmental effects in people. It is based upon information about DnOP 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 DnOP 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 two 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, postnatal 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.

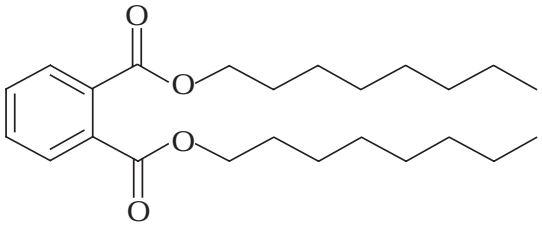
NTP Brief on Di-*n*-Octyl Phthalate (DnOP)

What is DnOP?

DnOP is an oily substance manufactured by reaction of phthalic anhydride and n-octanol in the presence of a catalyst. The structure of DnOP is shown in Fig. 1. Its chemical formula is $C_{24}H_{38}O_4$. It is one of a group of industrially important chemicals known as phthalates. Phthalates are used primarily as plasticizers to add flexibility to plastics. Available information indicates that there are no commercial uses of pure DnOP. However, it makes up approximately 20% of the commercially important phthalate mixture known as C6–10 phthalate. This mixture is used in the manufacture of a variety of commercial products including flooring and carpet tiles, tarps, pool liners, and garden hoses. DnOP is approved by the FDA as an indirect food additive and is used in seam cements, bottle cap liners, and conveyor belts. DnOP-containing compounds are not known to be used in medical devices or toys.

Based on an estimated annual production of 50 million pounds of C6–10 phthalate, 10 million pounds (20% of 50 million) of DnOP is produced each year.

Figure 1. *Chemical structure of DnOP*



Are People Exposed to DnOP?*

Yes. There are several ways that people may be exposed to DnOP at home or at work. Human exposure can occur during the manufacture of DnOP, during the manufacture of DnOP-containing products, during the use of such products, or through the presence of DnOP in the environment. Environmental exposures can occur through air, water, food, or contact with DnOP-containing products. Studies have shown that DOP (isomer not specified) is detectable in a variety of food products and household dust samples but the data are not sufficient to permit a confident estimate of general population exposure. Because of inadequate information on human exposure to DnOP, the expert panel took the conservative position of assuming that general population exposures in the United States would be 3–30 μg/kg bw/day (micrograms per kilogram body weight per day). This is the range of exposures estimated for the more widely used phthalate, DEHP. By comparison, a small drop of water weighs approximately 30,000 μg and a grain of table salt weighs approximately 60 μg.

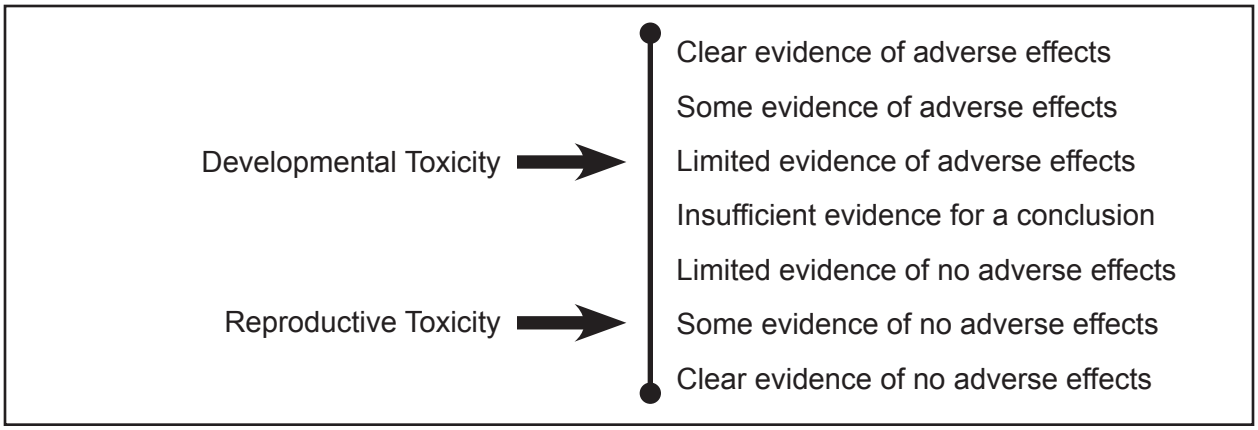
Can DnOP Affect Human Development or Reproduction?

Probably not. There is no direct evidence that exposure of people to DnOP adversely affects reproduction or development. Limited studies with mice and rats show that high exposures to DnOP may cause adverse developmental effects but no evidence of reproductive effects was observed (Fig. 2).

Scientific decisions concerning health risks are generally based on what is known as "weight-of-the-evidence." There are no exposure or effects data in humans. There is limited evidence of developmental effects in animals at high doses, and some evidence of no reproductive effects in animals. The NTP judges the scientific evidence to indicate DnOP is not likely to affect human reproductive systems. The data

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

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



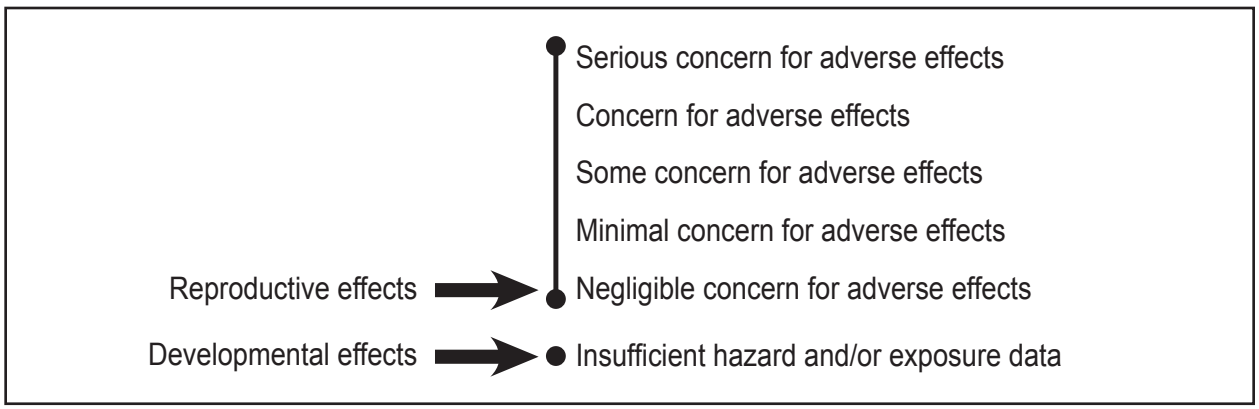
are insufficient to make a judgment on possible developmental effects. (Fig. 3).

Summary of Supporting Evidence

As presented in the expert panel report, two developmental toxicity studies—one in rats and one in mice—were available for evaluation. In the rat study, pregnant dams were treated with DnOP by intraperitoneal injection on gestation days 5, 10, and 15. Fetuses were evaluated on gestation day 20. The two dose levels used were very high, approximately 5,000 and 10,000 mg/kg bw/day. Increased malformations and reduced body weights were observed in fetuses at both dose levels. In the mouse study, pregnant dams were treated by gavage with approximately 10,000 mg/kg bw/day on gestation days 6–13 and allowed to deliver pups. DnOP treatment resulted in smaller litter sizes and reduced weight gain on postnatal days 1–3. However, birth weights and pup survival to day 3 were not affected.

The reproductive toxicity of DnOP was evaluated in a mouse continuous breeding study. Animals were exposed through dosed feed to approximately 1,800, 3,600, or 7,500 mg/kg bw/day. No adverse reproductive effects were observed in the treated parents or their offspring. Likewise, no reproductive effects were observed in two limited studies in male rats where oral exposure to DnOP for 4 days or 13 weeks did not affect testes weights or histology.

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



No new data on the developmental or reproductive effects of DnOP have become available since the expert panel report was completed.

Are Current Exposures to DnOP High Enough to Cause Concern?

Probably not. No studies have been conducted in humans and only a few animal studies are available. However, the animal studies evaluated indicate that there are no effects on reproduction and that developmental effects may occur at high exposure levels. Although no firm data are available on human exposures, it appears that the U.S. general population is not exposed to DnOP levels that are of immediate concern for causing adverse reproductive or developmental effects. The NTP offers the following conclusions.

The NTP concurs with the CERHR Phthalates Expert Panel's conclusion that there is negligible concern for effects on adult reproductive systems.

This conclusion is based on the assumption that humans are exposed to levels of DnOP less than 30 μg/kg bw/day.

Although DnOP is reported to be lethal to mouse fetuses at high doses, data are not available to determine exposure levels at which no adverse effects occur. Therefore, the NTP concludes that there is insufficient information on developmental toxicity to reach a conclusion regarding the potential for DnOP to adversely affect human development.

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:

No new publications were available.

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

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

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

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National Toxicology Program
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Center For The Evaluation Of Risks To Human Reproduction

Appendix II

NTP-CERHR EXPERT PANEL REPORT

on

Di-*n*-Octyl Phthalate

October 2000 *NTP-CERHR-DNOP-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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1800 Diagonal Road, Suite 500
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A Report of the CERHR Phthalates Expert Panel:

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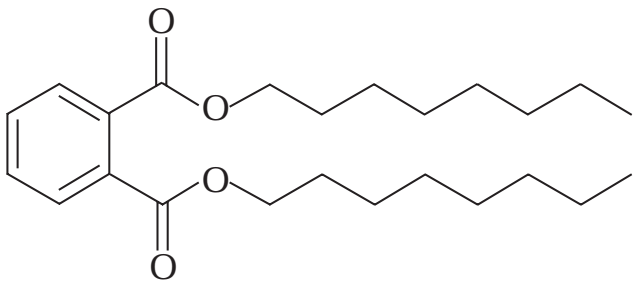
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1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: *Chemical Structure of a Di-n-Octyl Phthalate*



Di-n-octyl phthalate (DnOP) (CAS Registry Number 117-84-0) is produced by reacting phthalic anhydride and n-octanol in the presence of an acid catalyst (*1*).

Synonyms: 1,2-benzenedicarboxylic acid, dioctyl ester; phthalic acid, dioctyl ester; n-dioctyl phthalate; n-octyl phthalate; dioctyl o-benzenedicarboxylate; bis(n-octyl) phthalate.

DnOP is a significant component (20%) of C6–10 phthalate mixtures (*1*).

Table 1: Physicochemical Properties of DnOP

Property	***Value***
Chemical Formula	$C_{24}H_{38}O_4$
Molecular Weight	390.54
Vapor Pressure	1.0×10^{-7} mm Hg at 25 °C
Melting Point	-25 °C
Boiling Point	390 °C
Specific Gravity	0.978
Solubility in Water	Essentially Insoluble (0.5 µg/L)
Log K_{ow}	8.06

(2)

1.2 Exposure and Usage

There is sometimes confusion reporting data for DnOP because DEHP is often referred to in the literature as dioctyl phthalate (DOP). Unless otherwise stated, the information discussed in this exposure section refers specifically to DnOP to the best of CERHR's knowledge.

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of C6–10 phthalate substance. Commercial production of 50 million pounds of C6–10

phthalate in the United States in 1994 (*3*) equates to 10 million pounds of DnOP. C6–10 phthalate substance is used in PVC utilized in the manufacture of flooring and carpet tile, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys, vinyl gloves, garden hoses, weather stripping, flea collars, and shoes (*1*). DnOP-containing phthalate substances are also used in PVC intended for food applications such as seam cements, bottle cap liners, and conveyor belts.

Release of DnOP to the environment can occur during the production of C6–10 phthalates and during the incorporation of the phthalates into plastic resins. Because phthalates are not bound to plastics, DnOP can be released during the use or disposal of the product. 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 human food supply.

General Population Exposure

The general population is exposed to phthalates primarily through the oral and dermal routes. Based on data for other phthalates, the most likely source of DnOP exposure to humans is dietary intake. DnOP may be found in food as a result of environmental uptake during cultivation or as a result of migration from processing equipment or packaging materials. DnOP is approved by the FDA for use as an indirect food additive in sealants used for food packaging (*4*). In a survey of packaged fatty foods purchased from grocery stores in the UK, the total concentration of dioctyl phthalate (DOP, isomer not specified) *excluding* DEHP was 2.3 mg/kg in milk (*5*). A paper published in 1995 reported the detection of DnOP in two samples of vodka; concentrations of 57 ppb in a 100 proof sample and 131 ppb in an 80 proof vodka (*6*).

DOP (isomer not specified) excluding DEHP was detected in 8 of 12 infant formulas from the UK at concentrations ranging from 0.21–1.42 mg/kg (*7*). Using manufacturer recommendations for feeding rates and by assuming that formula was the only nutritional source for infants, exposures to DOP isomers other than DEHP were estimated at <0.1–43 μg/kg bw/day at birth and <0.1–24 μg/kg bw/day at 6 months of age by the UK Ministry of Agricutural Fisheries and Food (MAFF) (*7*). In a follow-up survey DOPs were not specifically targeted, but there was no evidence of their presence in 39 samples of infant formulas examined (*8*).

Pfordt and Bruns-Weller (*9*) reported the phthalate content of various household items in Germany. DnOP was detected in nutmeg at 0.02 mg/kg, but levels were below the limit of detection of 0.01 mg/kg in milk (breast and commercial), cream, nuts, and baby food. DnOP was detected in the dust from a vacuum cleaner bag at 40 mg/kg; 20 mg/kg DNOP was detected in a dust wipe sample from one of three homes tested. DNOP levels in textiles were measured at 0.01–0.08 mg/kg. A concentration of 0.01 mg/kg DNOP was detected in one of three samples of flooring textiles.

There appears to be little or no use of DnOP-containing compounds in toys. According to the American Chemistry Council (ACC, formerly CMA) (*1*), DnOP was only detected in some teethers that were tested for phthalate ester migration by the Danish Ministry of Environment and Energy. No other studies have reported the detection of DnOP in toys.

Exposure to DnOP through air is also possible but expected to be minimal. Reported concentrations of DnOP in ambient air range from 0.06 to 0.94 ng/m^3. The highest reported concentration resulted

in a calculated inhaled dose of 0.29 ng/kg bw/day for an adult (*10*). Reported concentrations in river water have ranged from 0.024 to 1 ppb. EPA estimates that DNOP levels in drinking water influents are less than 0.5 ppb (*10*). These levels are several orders of magnitude lower than levels found in food.

The available data do not allow the confident estimation of DnOP exposures to the general population. However, a comparison of production volumes for DnOP-containing compounds versus those that contain DEHP suggests that human exposure to DnOP is well below the exposure estimate for DEHP of 3–30 μg/kg bw/day (*11*). Exposures may be higher in children due to dietary preferences or mouthing of DnOP-containing articles.

Medical Exposure
There are no known current uses of DnOP-containing compounds in medical devices.

Occupational Exposure
Workers may be exposed to DnOP primarily through inhalation and dermal contact. Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tank cars (*1*). Higher exposures to phthalates can occur during the production of flexible PVC because the processes are open and run at higher temperatures. The ACC (*1*) reviewed six publications demonstrating that phthalate levels in air are generally less than 1 mg/m^3 and 2 mg/m^3 during the production of phthalates and flexible PVC, respectively. Exposure levels were estimated by the ACC (*1*) using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 μg/kg bw/workday and 286 μg/kg bw/workday for workers employed in phthalate and flexible PVC manufacturing operations, respectively.

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

Acute Studies

The LD_{50} values for mice and rats are given as 13 g/kg and 53.7 g/kg, respectively (*1*). Dermal LD_{50} values have been determined at 75 mL/kg for guinea pigs (*1*).

Repeat-dose Toxicity

Four studies in rodents were available for review (*12-15*).

Systemic effects following DnOP treatment for 3, 10, or 21 days were examined in 4-week-old Wistar rats. These effects were compared to effects produced by other groups of rats being fed diet containing 20,000 ppm of di-n-hexyl phthalate (DnHP), another straight chain phthalate, or di(2-ethylhexyl) phthalate (DEHP), a branched-chained phthalate (*14*). A group of 12 male rats was a fed a diet containing 20,000 ppm DnOP and a control group of 18 rats was fed the basal diet. Using actual food intake levels and rat body weights on the day of sacrifice, a DnOP dose of 1,821 mg/kg bw/day was calculated. Four treated and 6 control rats were killed and necropsied after 3, 10, or 21 days of treatment. Liver histopathology, enzyme activity, and peroxisome proliferation were examined. Levels of thyroid hormones in serum and thyroid histopathology were also examined (*12*).

DnOP treatment had no effect on testes weight or the gross appearance of testes, kidney, or pancreas (*14*). However, liver weight was significantly increased at 10 and 21 days of DnOP treatment with liver histology and chemistry changes seen at all 3 assessment times. After 3 days of exposure, centrilobular necrosis and glycogen loss were observed. At 10 days, centrilobular fatty accumulation was seen, this effect became more pronounced with increasing treatment duration. Electron microscopy (EM) showed effects on the smooth endoplasmic reticuli (proliferation and dilation) and microvilli shortening in the bile canaliculi at 3 days. At 10 days, EM also showed lipid droplets in the hepatocytes and a small increase in lysosomes and peroxisomes at 3 and 21 days respectively. Biochemical evidence for peroxisome proliferation was seen with significant increases in cyanide insensitive palmitoyl CoA oxidase at 10 and 21 days of treatment. Total catalase activity was unchanged; however, in the particulate sub-fraction it was significantly increased at 10 and 21 days of treatment. Other liver enzymes that were changed included significant decreases in 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphate at 21 days of treatment. There was a significant, DnOP-treatment related decrease in serum thyroxine (T4); serum triiodothyronine levels (T3) were not significantly affected and microscopic changes were suggestive of thyroid hyperactivity. These changes included increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage.

When compared to the other two co-tested phthalates, DnOP induced effects on hepatic lipid accu-

mulation and peroxisomal proliferation that were similar to the effects caused by DnHP, but dissimilar to those caused by DEHP. DEHP caused greater increases in liver weight and greater increases in mitotic activity. Less fat accumulation was seen with DEHP treatment which, when it occurred, was seen in the midzone and periportal zones rather than centrilobular regions. Biochemical evidence for peroxisome proliferation (cyanide insensitive palmitoyl CoA oxidation) occurred earlier with DEHP (after 3 days of treatment) and was approximately 7-fold higher than levels observed with DnOP. DnOP values were twice those observed in the control rats.

An effect level of 1,821 mg/kg bw/day was observed in this study after 3 days of treatment. Although DEHP induced peroxisome proliferation more strongly, rats exposed to DnOP did show evidence for proliferation after longer treatment. Additional liver effects suggestive of other types of liver damage were seen with DnOP.

Liver metabolism and the biochemical changes associated with peroxisome proliferation were also studied by Lake et al. (*13*) who treated rats with 1,000 mg/kg DnOP for 14 days. DnOP produced a marginal increase in liver weight compared with DEHP. There were no increases in peroxisomal enzyme activities at 1,000 or 2,000 mg/kg.

Systemic effects were studied in groups of young (~4–6 weeks old) Sprague-Dawley rats (*15*). Groups (10/sex) were fed DnOP at dietary concentrations of 0, 5, 50, 500, or 5,000 ppm (males: 0, 0.4, 3.5, 36.8, or 350 mg/kg bw/day; females: 0, 0.4, 4.1, 40.8, or 403 mg/kg bw/day) for 13 weeks (Table 7-1). Negative controls (10/sex) were fed basal diet and positive controls (10/sex) were fed 5,000 ppm DEHP (males: 345 mg/kg bw/day; females: 411 mg/kg bw/day). Rats were observed daily, and body weights and food intake were measured weekly. At the end of the exposure period rats were killed and necropsied. Parameters evaluated included histopathology (reproductive organs preserved in Zenker's solution), hematology, blood chemistry, liver enzyme activity, peroxisome proliferation, and DnOP levels in tissues.

DnOP exposure did not affect organ or body weight at any dose concentration. No hematological effects or testicular changes were observed. At the dose of 4.1 mg/kg bw/day, female rats experienced significant increases in plasma phosphate level. At 36.8 (M) or 40.8 (F) mg/kg bw/day, no effects were observed and this level was designated by the authors as a NOAEL. At the highest DnOP exposure tested, 350.1 (M) and 402.9 (F) mg/kg bw/day, liver and thyroid effects were observed. Authors reported dose-related hepatic effects including anisokaryosis, nuclear hyperchromicity, vesiculation, cytoplasmic vacuolation, nuclear endothelial prominence, and accentuation of zonation. Increases in hepatic ethoxyresorufin-o-deethylase activity were also seen in this high-dose group. Thyroid effects, observed at the highest dose of DnOP tested, included decreases in follicle size and colloid density. Serum T3 or T4 analyses for thyroid function were not performed. Plasma calcium levels were significantly increased in male rats at the high dose only.

The DEHP-positive control group of rats, exposed to 345 (M) and 411(F) mg/kg bw/day, had effects in the liver and thyroid with respect to severity of lesions and biochemical changes that were similar to those observed in the high-dose DnOP group. However, DEHP also induced peroxisomal proliferation, seminiferous tubule atrophy, Sertoli cell vacuolation, and decreased sperm levels. Also, numerous additional biochemical changes were observed with the DEHP-exposed rats, such

as increases in plasma levels of albumin and inorganic phosphate, total protein (in female rats), and hepatic aminopyrine-N-demethylase and analine hydroxylase. DEHP also produced significant hematological effects such as increased platelet counts in rats of both sexes, increased WBC, and decreased mean corpuscular volume and decreased hemoglobin in females.

Poon et al. (*15*) also evaluated levels of DnOP and DEHP in liver and fat. The authors stated that the findings suggest that both DnOP and DEHP are rapidly metabolized and excreted and that their distribution in body tissues is determined by the lipophilicity of the compounds. The reliability of the actual levels reported in the paper has been questioned (*16*) by noting the failure of mass spectrometry to confirm the chemicals detected in tissues and the absence of analytical blanks when performing the analyses.

2.2 Toxicokinetics

Phthalate Moiety Toxicokinetics

Absorption
Few data are available describing the toxicokinetics of DnOP. Albro and Moore (*17*) dosed male CD rats by gavage with 0.2 mL DnOP and collected urine for analysis of metabolites. They recovered 31% of the dose in the urine by 48 hours. The monoester and some free phthalic acid were detected, but no parent DnOP was observed. Blood levels of the monoester, mono-octylphthalate, were measured in rats following administration of 2,000 mg/kg of DnOP by gavage (*18*). The biological half-life in the blood was 3.3 hours with an area under the curve (AUC) of 1,066 μg·h/mL. Peak blood levels were observed at 3 hours following administration.

Biotransformation
Humans: In a study comparing the relative rates of monohydrolysis of DnOP by rat, baboon, and human gut preparations, Lake et al. (*19*) demonstrated that these species possess similar intrinsic esterase activity. Rates observed in human intestinal preparations were similar enough to the other species to conclude that human intestinal metabolism of DnOP would be expected to result in absorption of the monoester similar to what occurs in rats.

Rodents: Six dialkyl phthalates, including DnOP, were found to be metabolized to their monoesters and alcohol by enzymes present in gut tissues. It is generally accepted that orally-ingested phthalate diesters are primarily hydrolyzed by esterases in the wall of the small intestine, not by intestinal flora, and absorbed almost entirely as the corresponding monoester (*20*).

Distribution
Following an oral dose of 2,000 mg/kg DnOP to rats, mono-octylphthalate was found in blood and testes in 3–6 hours (*21*).

Excretion
Following a gavage dose of 559 mg/kg for 2 days to rats, metabolites accounting for 31% of the administered dose were found in urine. The major metabolites found in urine of rats were derived from the monoester (*17*).

Side Chain-associated Toxicokinetics

n-Octanol is a metabolite of DnOP. Octanol is oxidized to the fatty acid and metabolized by the fatty acid oxidation pathway.

2.3 Genetic Toxicity

Mixtures containing DnOP have not shown conclusive evidence of mutagenicity. Barber et al. (*22*) tested C6–10-phthalate, which contains approximately 20% DnOP, in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency that occurred in the presence and absence of S9 metabolic activation.

The ACC (*1*) reviewed two studies of Di(n-octyl, n-decyl) phthalate, which contains DnOP as a component. Di(n-octyl, n-decyl) phthalate was reported to be negative in the Ames test and the Chinese hamster ovary cell/HPRT locus assay.

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

Two studies were found, one in rats by intraperitoneal (IP) injection and one in mice by gavage. A third study examined effects of metabolite exposure in rats.

Singh et al. (*23*) administered DnOP at 0, 5, or 10 mL/kg (equivalent to 0, 4,890, and 9,780 mg/kg based on the specific gravity of DnOP of 0.978 g/mL) by IP injection to pregnant Sprague Dawley rats (Table 7–2). The rats, 5 per group, were dosed on gd 5, 10, and 15. The control group was untreated or dosed with distilled water, normal saline, or cottonseed oil. Dams and fetuses were evaluated on gd 20. Information on maternal toxicity was not reported. Fetal body weight was reduced at both doses, and incidences of gross malformations were increased in a dose-related manner (0–2% in controls, 16% at 4,890 mg/kg, and 27% at 9,780 mg/kg). The abnormalities were predominantly missing tail, anophthalmia, twisted hind legs, and hematomas.

Hardin et al. (*24*) evaluated DnOP in the Chernoff-Kavlock assay in CD-1 mice. The mice, 40/group, were dosed by gavage, with 9,780 mg/kg bw/day (undiluted chemical, 10 mL/kg/day) or corn oil on gd 6–13. Dams were allowed to deliver their litters; dams and pups were terminated on pnd 3. No dams died, 39/40 had live litters, and maternal weight change was similar to controls. Litter size on pnd 0 was significantly reduced (10.2) versus the control value (11.5). Birth weight was normal as was pup survival to pnd 3. However, weight gain on pnd 1–3 was significantly reduced (0.6 g) versus the control value (1.0 g).

There was no effect on ability to produce litters, litter size, sex ratio, or pup weight or viability in F_1 and F_2 litters in a continuous breeding study in CD-1 mice. Mice were exposed to 0, 1.25, 2.5, or 5% DnOP (0, 1,800, 3,600, or 7,500 mg/kg bw/day) (*25, 26*). Complete details of this study are included in Section 4.

Hellwig and Jackh (*27*) investigated the prenatal toxicity of n-octanol, a primary metabolite of DnOP, when administered by gavage to pregnant Wistar rats on days 6–15 of gestation. There were 6 groups studied, 8–10 females/group: a distilled water control, an emulsifier control, and DnOP doses of 1, 5, 7.5, and 10 mmol/kg (130, 650, 945, and 1,300 mg/kg bw/day DnOP, respectively). Dose-related symptoms of clinical intoxication of the nervous system were observed with maternal death seen in the three highest dose levels. A slight decrease in food consumption and body weight gain was also recorded at these doses. However, no effects on fetal weight, viability, or developmental toxicity were observed. The incidence of malformations was similar to that of controls.

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data located for Expert Panel review.

4.2 Experimental Animal Toxicity

One rodent reproductive toxicity study was found for DnOP reported by Heindel et al. (*26*) (also (*25*)) (Table 7-3). In this continuous breeding study, CD-1 (Swiss) mice, 20 pairs/dose level, 40 in controls) were fed DnOP in the diet at 0, 1.25, 2.5, or 5% (w/w). Body weights and food consumption were monitored, and these concentrations gave calculated daily DnOP consumption estimates of 1,800, 3,600, and 7,500 mg/kg bw/day. Following a week of pre-mating exposure, mice were housed as breeding pairs for 14 weeks. Litters born during the 14-week period were evaluated and removed so that the adult pair could continue breeding. Reproductive function was measured by determining the fertility index; litters/pair; live pups per litter; and pup sex, body weight, and gross external malformations.

There were no effects on ability to produce litters, litter size, sex ratio, or pup weight or viability over five successive litters. For this protocol, when no effect on fertility was seen, the last litter from both the high dose and control group was reared, and used to evaluate fertility and toxicity of the F_1 generation. In addition to the reproductive parameters evaluated in the F_0 mice, sperm morphology, estrous cycles, and selected organ weights were evaluated in the F_1 mice. The F_0 mice were discarded without necropsy after weaning the last litter. The F_1 animals were mated within dose groups at sexual maturity. DnOP had no effect on indices of fertility, litter size, or pup weight or viability. The control and high-dose F_1 adults were killed and necropsied after delivery of a single litter. DnOP at 7,500 mg/kg bw/day in the diet had no effect on male body weight, but increased absolute and relative liver weights and decreased relative seminal vesicles weight. Sperm indices were unchanged. In females, body weight was unchanged, while relative liver and kidney weights were increased; estrous cycle was unchanged by 7,500 mg/kg bw/day DnOP consumption.

As discussed in Section 2.2, Poon et al. (*15*) (Table 7-1) reported a subchronic-type study of DnOP. Pubertal SD rats were exposed to DnOP in diet at doses as high as 5,000 ppm (350 mg/kg bw/day) for 13 weeks when the rats were killed and necropsied. Testes were weighed and fixed in Zenker's solution; no sperm measures were taken. Terminal weights of whole body and testis were unaffected by DnOP consumption. Testis histology was normal. No reproductive effects were seen, so no LOAEL was determined. The reproductive NOAEL in this study is 5,000 ppm, ~350 mg/kg bw/day, based on lack of changes in testis weight and histology as observed by light microscopy. Confidence in the quality of the study is moderate-to-high. Confidence that this study found the true NOAEL is moderate-to-low, because guidelines for subchronic studies do not require the examination of functional reproductive effects.

Foster et al. (*28*) gavaged 12 male Sprague-Dawley rats (70–90 g) with DnOP at 2,800 mg/kg bw/day for 4 days. Control animals received the corn oil vehicle. No testicular lesions were observed in the treated animals.

Mode of Action

Following exposure to a variety of phthalate monoesters over a range of doses, germ cell detach-

ment was examined in *in vitro* co-cultures of Sertoli-germ cells isolated from pubertal rats. Results indicate that the n-octyl monoester is ~100-fold less potent than the 2-ethylhexyl monoester in producing this effect (*29*). These co-culture *in vitro* studies suggest that DnOP produces a similar effect to other phthalates in this model system, albeit at concentrations two orders of magnitude higher. There are no *in vivo* data to suggest effects on either germ cells or Sertoli cells due to DnOP exposure.

Several studies have examined the ability of selected phthalate esters to compete with labeled estradiol (E2) for binding to the estrogen receptor (ER). Sources of ER protein included rat uterine cytosol (*30*), rainbow trout hepatic cytosol (*31*), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (*32, 33*) and rainbow trout ERs expressed in yeast (*34*). Triated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. The estrogenic activity of DnOP has been examined using a battery of short-term *in vitro* and *in vivo* assays. DnOP did not compete with tritiated estradiol for binding to the rat uterine cytosolic estrogen receptor (*30*).

Selected phthalate esters have been examined in a number of in vitro gene expression assays systems. The assays have used stably transfected cells (*30*), transiently transfected cells (*30, 31*), yeast based assays (*30, 34-36*) and vitellogenin induction in rainbow trout hepatocyte cultures (*34*). DnOP did not induce any activity in *in vitro* gene expression assays systems and did not induce reporter gene activity in transiently transfected MCF-7 cells (*30*).

DnOP, in contrast to the positive control estradiol, did not significantly induce a vaginal cornification response at any of the concentrations tested (20, 200, and 2,000 mg/kg) over the course of a 5-day experiment using immature and adult ovariectomized Sprague Dawley rats (*30*). The effects of subcutaneous injection of 10^{-4} mol of DBP, BBP, and DnOP on uterine vascular permeability following a 4-hour incubation were examined in mature ovariectomized Swiss albino mice (*37*). Although no significant effect on uterine vascular permeability was reported, it is unclear whether the authors tested DnOP or DEHP. The publication states that "dioctyl phthalate" was purchased from Aldrich chemical company.

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

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of the commercial mixture C6–10-phthalate. This commercial mixture has a variety of home and consumer product uses including as a plasticizer for PVC used in flooring and carpet tile, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys, vinyl gloves, garden hoses, weather stripping, flea collars, and shoes. Uses of PVC containing DnOP with possible food applications include seam cements, bottle cap liners, and conveyor belts.

Dietary: In a survey of infant formulas from the UK, the level of dioctyl phthalates (DOP) other than DEHP ranged from 0.21–1.42 mg/kg (*7*). In a subsequent survey in 1998, DOP isomers were not targeted, but there was no evidence that they were present in any of 39 samples of infant formula tested (*8*). There was a published report in 1995 that documented the detection of DnOP in two samples of vodka at concentrations of 57 and 131 ppb (*6*). In a German survey (*9*), DNOP was detected in nutmeg at 0.02 mg/kg but DNOP levels were below the detection limit of 0.01 mg/kg in milk (breast and commercial), cream, nuts, and baby food. Dioctyl phthalate is approved for use as an indirect food additive in sealants used for food packaging (*4*).

Exposure Estimates: Based on levels of DOP isomers (excluding DEHP) detected in baby formula, infant exposures to DOP isomers other than DEHP were estimated at <0.1–43 μg/kg bw/day at birth and <0.1–24 μg/kg bw/day at 6 months of age by MAFF (*7*). However, there was no evidence that DOP isomers were present in infant formulas in a survey conducted 2 years later by MAFF (*8*).

Based on production volumes of DnOP-containing compounds versus those containing DEHP, exposure to DnOP in the general population is likely lower than exposure to DEHP, which was estimated at 3–30 μg/kg bw/day (*11*). Exposures may be higher in children due to dietary preferences and mouthing of DnOP-containing articles. Variability in food exposure estimates is possible due to the inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations. In occupational settings, exposure is thought to be highest in workers of flexible PVC manufacturing facilities. Based on general levels of phthalates reported, the ACC (*1*) estimated an occupational exposure level of 286 μg/kg bw/workday in this activity.

5.1.1.1 Utility of Data to the CERHR Evaluation

There is very limited information on exposure and exposure pathways to DnOP in humans. DnOP is not known to be produced directly for commercial use, but is a component (20%) of commercial 6–10 phthalate substances. 6–10 Phthalates are used in a variety of consumer products.

5.1.2 General Biological and Toxicological Data

Data presented in this section are derived from experimental animal and laboratory studies. Human data were not found.

General toxicity
A 3-week dietary study (*12, 14*) and a 90-day dietary study (*15*) in rats have been conducted. Liver effects were noted when rats were fed 1,821 mg/kg bw/day for 3, 10, or 21 days, or 350 mg/kg bw/day for 90 days. Thyroid effects also were noted in rats fed 350 mg/kg/d for 90 days and 1,821 mg/kg bw/day for 21 days. No effects were observed in the testes in either study. The sub-chronic dietary NOAEL in rats is 36 (M)–40 (F) mg/kg bw/day.

Toxicokinetics
DnOP is metabolized and rapidly absorbed in the gut as the monoester and primarily excreted in the urine of rats (*17, 18, 20*). The major metabolites found in urine of rats were derived from the monoester (*17*).

Genetic toxicity
DnOP has not been tested for genetic toxicity. Mixtures containing DnOP have not shown conclusive evidence of mutagenicity. Barber et al. (*22*) tested C6–10-phthalate in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. 6–10-Phthalate mixture was considered equivocal in the mouse lymphoma mutation assay due to a non-dose related increase in mutations in the presence and absence of metabolic activation, but tested negative in the Balb/3T3 cell transformation assay. According to two studies reviewed by ACC (*1*), di(n-octyl, n-decyl) phthalate, which contains DnOP as a component, has been reported to be negative in the Ames test and the Chinese hamster ovary/HPRT locus assay.

5.1.2.1 Utility of Data the CERHR Evaluation
The data is adequate for the examination of systemic effects and identification of the liver and thyroid as target organs. The dataset consisted of one useful study that examined systemic effects in groups of rats exposed for 90 days to multiple doses of DnOP by the oral route, a route relevant to human exposure. Levels of DnOP in the diet were verified. The evaluation included a histological examination of various organs, including reproductive organs that were fixed in Zenker's solution. One concern is that male rats were at the pubertal stage at the start of the study and were therefore past the age of maximum sensitivity to phthalate-induced testicular damage. However, mice were exposed during prenatal development (the most sensitive period for testicular toxicity) in a continuous breeding study described in the reproductive toxicity section.

There is adequate general toxicokinetic data for DnOP, consisting of absorption, distribution, metabolism, and excretion in rodents. While studies of toxicokinetics in humans have not been located, the DnOP toxicokinetic data in rodents is consistent with the large body of data on phthalates that includes data on rodents and primates. It is reasonable to assume that the DnOP rodent data are relevant to humans.

5.1.3 Developmental Toxicity
There are no data on the developmental toxicity of DnOP in humans. Two studies, where massive doses of DnOP were administered (4,890 and 9,780 mg/kg bw/day) to rats or mice by gavage or IP injection, suggest a potential for adverse prenatal effect or effect during the perinatal period expressed as death, growth retardation, and/or malformations (*23, 24*). However, litter size and pup weight and mortality were unaffected in a continuous breeding study where mice were exposed to dietary concentrations up to 7,500 mg/kg bw/day (*25, 26*). A primary metabolite, n-octanol, gave no

sign of developmental toxicity at doses up to 1,300 mg/kg bw/day in rats (*27*). This dose caused severe intoxication and some deaths in the dams and prompts speculation whether the DnOP rat study that administered 4,890 mg/kg bw/day (*23*) led to severe maternal intoxication as well. The authors were silent on maternal effects. The limited study designs do not provide a basis for comparing consistency of response in the two species, nor do they allow meaningful assessment of dose-response relationships and determination of either LOAELs or NOAELs with any degree of confidence. The available studies do suggest a developmental toxicity response with gavage or IP administration with very high doses.

Table 2: Summaries of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol and Study	***NOAEL (mg/kg bw/day)***	***LOAEL (mg/kg bw/day) and Effects***		***Developmental Effects Observed at Higher Dose Levels***
		Maternal	***Developmental***	
Prenatal developmental toxicity study with IP exposure in Sprague-Dawley rats. 5 dams/group received 0, 4,890, or 9,780 mg/kg bw on gd 5, 10, and 15. Fetuses were evaluated on gd 20. (*23*)	Maternal: Not reported. Developmental: None.	Not reported.	4,890 ↑ Fetuses with external malformations. ↓ Fetal weight.	↑ Fetuses with external malformations. ↓ Fetal weight.
Prenatal gavage toxicity screening assay in CD-1 mice. 40 dams/group received 0 or 9,780 mg/kg bw/day on gd 6–13. Dams and pups evaluated on pnd 3 for litter size, survival, and body weight changes only. (*24*)	Maternal: Not reported. Developmental: None.	Not reported.	9,780 ↓ Litter size on pnd 0. ↓ Pup weight gain on pnd 1–3.	No higher doses.
Continuous breeding study in CD-1 mice. 20 dams/group received DnOP in feed at 0, 1,800, 3,600, or 7,500 mg/kg bw/day throughout mating and gestation. (25, *26*)	Maternal: * Developmental: 7,500	*	No effects on live pups/litter or pup weight.	

*Only developmental effects reported in this table. See Table 3 in Section in 5.1.4 for a description of effects in parental rats.
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

5.1.3.1 Utility of Data to the CERHR Evaluation

The data set is inadequate for an evaluation of developmental toxicity. In one study, small numbers of rats (n=5/group) were exposed by intraperitoneal injection, a route that is not relevant to human exposure, and there was no information on maternal toxicity. In a screening study of mice, only a single dose was administered and there was no internal examination of offspring or dams.

5.1.4 Reproductive Toxicity

There were no data located on the reproductive toxicity of DnOP in humans. The continuous breeding design with DnOP in mice was negative at what can only be considered massive dietary doses up to 7,500 mg/kg bw/day (*26*). This was not a true multigeneration study because effective evaluation of the second generation was not performed. This lack of effect is loosely corroborated by the dietary study in rats (*15*) which found no histologic effects on reproductive organs after sub-chronic exposure to concentrations as large as 350 or 403 mg/kg bw/day for males and females, respectively. In addition, testicular lesions were not observed in male Sprague-Dawley rats gavaged with DnOP at 2,800 mg/kg bw/day for 4 days (*28*). Since there are no adverse reproductive effects in any study, no LOAEL can be estimated. The reproductive toxicity NOAEL in mice is 7,500 mg/kg bw/day and in rats is 350 mg/kg bw/day.

The data are sufficient to conclude that DnOP causes no detectable reproductive toxicity in adult mice at doses up to ~7,500 mg/kg bw/day. The data also find no reproductive toxicity at doses up to 403 mg/kg bw/day in a subchronic dietary study in adult rats or 2,800 mg/kg bw/day in a 4-day gavage study in young rats, but there are no data on functional measures of reproduction. The data are insufficient to conclude that DnOP does not cause reproductive toxicity in developing rats or mice. It can be reasonably speculated, based upon both *in vivo* and *in vitro* studies, that DnOP is certainly less potent in producing male reproductive effects than the shorter-chain phthalate congeners.

Following exposure to a variety of phthalate monoesters over a range of doses, germ cell detachment was examined in *in vitro* co-cultures of Sertoli-germ cells isolated from pubertal rats. Results indicate that the n-octyl monoester is ~100-fold less potent than the 2-ethylhexyl monoester in producing this effect (*29*). These co-culture *in vitro* studies suggest that DnOP produces a similar effect to other phthalates in this model system, albeit at concentrations two orders of magnitude greater. There are no *in vivo* data to suggest effects on either germ cells or Sertoli cells due to DnOP exposure.

DnOP did not exhibit estrogenic activity in a variety of *in vitro* assays (*30*). It did not induce a significant *in vivo* response in ovariectomized rats (*30*). The results suggest that adverse effects as a result of exposure to DnOP would not be due to estrogenic activities of this phthalate.

Table 3: Summaries of NOAELs, LOAELs, and Major Effects in Reproductive Toxicity Studies

Protocol & Study	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day) and Effects*		*Effects Observed at Higher Dose Levels*
		Reproductive	*Systemic*	*Reproductive*
Continuous breeding study with mating of high dose and control F_1 offspring. 20 pairs of CD-1 mice were fed diets with DnOP (0, 1,800, 3,600, 7,500 mg/kg bw/day) for 7 days prior to mating and during a continuous 98-day mating period.* (25, *26*)	Reproductive: 7,500 Systemic: Not identified due to limited examination of lower dose groups.	None No effect on fertility, mating, sperm or estrous cycles in F_0 or high dose F_1 rats.	7,500 ↑Liver and kidney weight in F_1 rats	No higher doses in study

* Developmental effects are reported in Table 2 in Section 5.1.3.
↑=Statistically Significant Increase

5.1.4.1 Utility of Data to the CERHR Evaluation

Data are sufficient to indicate that oral DnOP exposures are not associated with detectable effects on reproduction at doses of up to 7,500 mg/kg bw/day in mice. Adequate numbers of mice (20 pairs/group) were exposed to multiple doses of DnOP for a sufficient duration. Feed was analyzed for DnOP levels. Reproductive function and sperm quality were assessed in the F_1 mice exposed during prenatal development; thus, mice exposed during the most sensitive age were evaluated. A concern with this study is that several postnatal maturation effects (found to be the most sensitive indicators of toxicity for other phthalates) were not evaluated. Other concerns included no reporting of histopathological effects, examination of only the F_1 mice from the high-dose group, and a lack of necropsies at the lower dose levels.

5.2 Integrated Evaluation

There are no human data from which to judge the health effects of DnOP. Based on experimental literature, including toxicity studies in rats and mice with DnOP and other structurally-related phthalates, there is a reasonable basis for assuming relevance of these data for judging potential hazard to humans.

There are no data indicating that DnOP is currently used in medical devices. Exposure to DnOP results from its presence as a 20% constituent of a commercial mixture of C6–10 phthalates. Humans would gain contact from household and consumer products. Absorption through skin from such contacts are expected to be low. Absorption into the body would result from dietary sources. Presence in food might reflect migration from food packaging and a legacy of fate and transport of phthalates into the environment. Like other phthalates, DnOP is readily absorbed from the intestinal tract as a monoester, and is rapidly metabolized and excreted.

The experimental animal data are insufficient to permit a firm judgment about DnOP's potential to

pose a developmental toxicity hazard to humans. Studies that suggest potential developmental effects were of inadequate design for confident interpretation and effects were observed only at very high doses. A study of n-octanol, a primary metabolite of DnOP, reported severe maternal intoxication without any effect on growth, viability, or development. It was noted that adequate data are available on DnOP to indicate adverse effects on liver at doses lower than doses that suggest developmental toxicity. There are data to indicate that DnOP does not demonstrate estrogenic properties.

There are experimental data on the reproductive toxicity of DnOP. The data indicate no effects in adult mice fed high doses (7,500 mg/kg bw/day). The data in rats, while negative at dietary doses up to 350 mg/kg bw/day in adults and gavage doses up to 2,800 mg/kg bw/day in young rats, did not assess a sufficient array of reproductive measures to be considered a complete evaluation. The data, while indicating a lack of effect, are insufficient to conclude with complete confidence that exposure by the oral route poses no hazard to adult reproduction. While there was a continuous breeding study that assessed the effects of exposure to DnOP during development on subsequent reproductive function later, the protocol did not completely assess two generations.

5.3 Expert Panel Conclusions

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of the commercial mixture C6–10 phthalate. This commercial mixture has a variety of home and consumer product uses. DnOP is approved for use as an indirect food additive in sealants used for food packaging (*4*). There is very limited information on exposure and exposure pathways to DnOP in humans. To allow for an integrated evaluation of exposure and toxicity information, the Expert Panel made a conservative estimate (i.e., an overestimation) of general population exposure to DnOP that would be at or lower than DEHP exposures of 3–30 μg/kg bw/day (see DEHP review). A similar approach for studying occupational exposures in chemical manufacturing was done using measured DEHP levels to estimate that chemical worker exposure would be less than 286 μg/kg bw/workday.

Two studies where massive doses of DnOP were administered intraperitoneally to rats or by gavage to mice suggest a potential for adverse prenatal effects or effects during the perinatal period expressed as death, growth retardation, and/or malformations (*23, 24*). However, litter size and pup weight and mortality were unaffected in a continuous breeding study where mice were orally exposed to concentrations up to 7,500 mg/kg bw/day (*25, 26*). A primary metabolite, n-octanol, gave no sign of developmental toxicity at doses up to 1,300 mg/kg bw/day in rats (*27*). Higher doses resulted in maternal lethality. The dataset is inadequate for an evaluation of developmental toxicity for the following reasons. The limited study designs do not provide a basis for comparing consistency of response in the two species, nor do they allow meaningful assessment of dose-response relationships and confident determination of either LOAELs or NOAELs. The available studies suggest a developmental toxicity response with gavage or IP administration at very high doses. The developmental toxicity response suggests minimal concern from exposure during pregnancy or the perinatal period.

There is a single dietary exposure multigeneration study on DnOP in mice which was negative at exposures of 7,500 mg/kg bw/day (*26*). This lack of effect is loosely corroborated by the dietary study in rats (*15*) which found no histologic effects on reproductive organs after sub-chronic expo-

sure to concentrations as large as 350 or 403 mg/kg bw/day for males and females, respectively. In addition, no testicular lesions were observed in young male rats that were gavage dosed with 2,800 mg/kg bw/day for 4 days. Since there are no adverse reproductive effects in either study, no LOAEL can be estimated. The reproductive toxicity NOAEL in mice is 7,500 mg/kg bw/day and in rats is 350 mg/kg bw/day.

Data are sufficient to indicate that oral DnOP exposures are not associated with detectable effects on reproduction at doses of up to 7,500 mg/kg bw/day in mice. Therefore, the Panel has only negligible concern for effects on the adult reproductive system. Taken together, the Expert Panel has determined that although all of the databases are limited or inadequate, the existing data do not suggest that DnOP is a potent developmental or reproductive toxicant in rodents.

5.4 Critical Data Needs

The critical data needs are to determine if, and at what levels, humans are exposed to DnOP. DnOP is a significant constituent (20%) of a C6–10 phthalate product that has major commercial production and use. The public would be best served if data needs for the evaluation of risks to human reproduction focus on the commercial mixture that contains DnOP, rather than pure di-n-octyl phthalate. Information on the use of DnOP as a food additive would be useful in determining if there are critical data needs for pure DnOP.

6.0 REFERENCES

1. CMA. Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. FR Doc. 99-9484. Washington, DC: Chemical Manufacturers Association, 1999.

2. Staples CA, Peterson DR, Parkerton TF, Adams WJ. The environmental fate of phthalate esters: A literature review. Chemosphere 35:667-749 (1997).

3. ACC. Meeting with Aristech Chemical Company, 1999.

4. FDA. Code of Federal Regulations. 21 (1999).

5. MAFF. Phthalates in food. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996; 9p.

6. HSDB. Hazardous Substances Data Bank. Bethesda:National Institutes of Health, 1999.

7. MAFF. Phthalates in infant formulae. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996; 7p.

8. MAFF. Phthalates in infant formulae. Food surveillance information sheet 168: Joint Food Safety and Standards Group, 1998.

9. Pfordt J, Bruns-Weller E. Die phthalsaureester als eine gruppe von umwelt-chemikalien mit endokrienem potential: Niedersachsisches Ministerium fur Ernahrung, Landwirtschaft und Forsten, 1999.

10. ATSDR AfTSaDR-. Di-n-octylphthalate. Toxicological Profile. Prepared by: Sciences International, Inc. Under Subcontract to: Research Triangle Institute.: U.S. Department of Health and Human Services, Public Health Service., 1997.

11. Doull J, Cattley R, Elcombe C, Lake B, Swenberg J, Wilkinson C, Williams G. Expert panel report on DEHP.: U.S. Environmental Protection Agency, 1998.

12. Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P, Bridges JW. Effects of phthalic acid esters on the liver and thyroid. Environ Health Perspect 70:195-210 (1986).

13. Lake BG, Gray TJB, Gangolli SD. Hepatic effects of phthalate esters and related compounds--in vivo and in vitro correlations. Environ Health Perspect 67:283-90 (1986).

14. Mann AH, Price SC, Mitchell FE, Grasso P, Hinton RH, Bridges JW. Comparison of the short-term effects of di(2-ethylhexyl) phthalate, and di (n-octyl) phthalate in rats. Toxicol Appl Pharmacol 77:116-132 (1985).

15. Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Subchronic oral toxicity of di-n-octyl phthalate and di (2-ethylhexyl) phthalate in the rat. Food Chem Toxicol 35:225-

239 (1997).

16. American Chemistry Council Phthalate Esters Panel. Comments on Draft CERHR Evaluation of Di-n-Octyl Phthalate (DnOP):ACC, 2000.

17. Albro PW, Moore B. Identification of the metabolites of simple phthalate diesters in rat urine. Journal of Chromatography 94:209-218 (1974).

18. Oishi S. Effects of phthalic acid esters on testicular mitochondrial functions in the rat. Arch Toxicol 64:143-147 (1990).

19. Lake BG, Phillips JC, Linnell JC, Gangolli SD. The invitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. Toxicol Appl Pharmcaol 39:239-248 (1977).

20. Rowland IR, Cottrell RC, Phillips JC. Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. Food Cosmet Toxicol 15:17-21 (1977).

21. Oishi S, Hiraga K. Testicular atrophy induced by phthalic acid monoesters: Effects of zinc and testosterone concentrations. Toxicology 15:1997 (1990).

22. Barber E, Cifone M, Rundell J, Przygoda R, Astill B, Moran E, Mulholland A, Robinson E, Schneider B. Results of the L5178Y mouse lymphoma assay and the Balb/3t3 cell invitro transformation assay for eight phthalate esters. J Appl Toxicol 20:69-80 (2000).

23. Singh AR, Lawrence WH, Autian J. Teratogenicity of phthalate esters in rats. J Pharm Sci 61:51-55 (1972).

24. Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogen Carcinogen Mutagen 7:29-48 (1987).

25. Gulati DK, Chambers R, Shaver S, Sabehrwal PS, Lamb JC. Di-n-octyl phthalate reproductive and fertility assessment in CD-1 mice when administered in feed. Research Triangle Park: National Toxicology Program, 1985.

26. Heindel JJ, Gulati DK, Mounce RC, Russell SR, Lamb JCI. Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. Fundam Appl Toxicol 13:508-518 (1989).

27. Hellwig J, Jackh R. Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats. Food Chem Toxicol 35:489-500 (1997).

28. Foster PMD, Thomas LV, Cook MW, Gangolli SD. Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. Toxicol Appl Pharmacol 54:392-398 (1980).

29. Gray TJ, Beamand JA. Effects of some phthalate esters and other testicular toxins on pri-

mary cultures of testicular cells. Food Chem Toxicol 22:123-131 (1984).

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

31. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582-587 (1995).

32. Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W. Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environ Health Perspect 106:551-7 (1998).

33. Nakai M, Tabira Y, Asa D, Yakabe Y, Shimyozu T, Noguchi M, Takatsuki M, Shimohigashi Y. Binding characteristics of dialkyl phthalates for the estrogen receptor. Biochemical and Biophysical Research Communications 254:311-314 (1999).

34. Petit F, Le Goff P, Cravedi J-P, Valotaire Y, Pakdel F. Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and troup hepatocyte cultures. Journal of Molecular Endocrinology 19:321-335 (1997).

35. Coldham NG, Dave M, Sivapathasundaram S, McDonnell DP, Connor C, Sauer MJ. Evaluation of a recombinant yeast cell estrogen screening assay. Environ Health Perspect 105:734-742 (1997).

36. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect 1997 105:802-811 (1997).

37. Mulligan SR, Balasubramanian AV, Kalita JC. Relative potency of xenobiotic estrogens in an acute in vivo mammalian assay. Environ Health Perspect 106:23-26 (1998).

7.0 TABLES

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Table 7-1: DNOP General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose**	*Body Weight*	*Organ Weight*	*Histopathology*	*Hematology*	*Chemistry*	*Other*
Sprague-Dawley Rat Poon et al. 1997 (1)	Young male and female rats were fed diets containing DNOP for 13 weeks, then killed and necropsied. A positive control group was exposed to DEHP.	10	0						
		10	0.40	NE	NE	NE	NE	NE	
		10	3.5(M) 4.1(F)	NE	NE	NE	NE	↑PO_4 (F)	
		10	36.8(M) 40.8(F)	NE	NE	NE	NE	NE	NOAEL
		10	350.1(M) 402.9(F)	NE	NE	Mild lesions in liver. Thyroid follicle reduction and decreased colloid density. No peroxisome proliferation or testicular lesions.	NE	↑EROD ↑ Ca (M)	
		10	345(M) 411(F) [DEHP]	NE	↑Li, Ki (M) ↓Te	Testicular atrophy, liver and thyroid lesions, and peroxisomal proliferation.	↑WBC (F), PC ↓Hb (F), MCV (F)	↑Alb, PO_4, Ca (M), protein (F), APD, AH	

*Doses measured in mg/kg bw/day.
NE=No Effects
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

F=Female
M=Male

Ki=Kidney
Li=Liver
Te=Testes

Alb=Albumin
APD=Aminopyrine-N-Demethylase Activity
AH=Aniline Hydroxylase
Ca=Calcium
EROD=Ethoxyresorufin-O-deethylase
Hb=Hemoglobin
MCV=Mean Corpuscular Volume
PO_4=Phosphate
PC=Platelet Count
WBC=White Blood Cell

Table 7-2: DNOP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number*	*Dose**	*Maternal effects*	*Fetal Effects*
Sprague-Dawley Rat Singh et al. 1972 (2)	Prenatal developmental toxicity study DNOP administered by intraperitoneal injection on gd 5, 10, and 15. Dams killed on gd 20, corpora lutea counted and implantation sites examined Fetuses weighed, examined for viability and gross external malformations. 30–50% of fetuses examined for skeletal malformations.	5	0		
		5	4,890	Not mentioned in paper	↓Fetal weight. ↑External malformations (16% fetuses with gross abnormalities)
		5	9,780		↓Fetal weight. ↑External malformations (27% fetuses with gross abnormalities)

*Doses measured in mg/kg bw/day.
↓=Statistically Significant Decrease
↑=Statistically Significant Increase

Table 7-3: DnOP Reproductive Toxicity, Mice

Species, Strain, and Source	*Experimental Regimen*	*Number*[a]	*Dose*[b]	*Effects*
CD-1 Mice Heindel et al. 1989 Gulati et al. 1985 (3, 4)	Dose range finding study.		0–10,000	Rough hair coat in high-dose group
	Fertility assessment through continuous breeding for 14 weeks. DNOP administered in feed. Body weight measured at 6 time points, clinical signs, and food and water intake recorded. Litters counted, sexed, weighed, observed for abnormalities, and removed following birth. Final litter raised; some control and high-dose F_1 weanlings mated for fertility assessment; F_1 organ weights measured at necropsy.	36	0	
		20	1,800	NE
		18	3,600	NE
		20	7,500	No adverse effects on sperm morphology, estrous cycles, or other reproductive parameters in F_1 rats. No effect on fertility index, mating index, numbers of litters produced, live pups/litter, sex ratio, or pup weight. ↓Percent abnormal sperm in F_1 rats ↓Seminal vesicle to body weight ratio in F_1 rats ↑Liver and kidney (females) to body weight ratio in F_1 rats

[a]Number of male and female pairs; half the number of controls used for F_1 study
[b]Author-calculated doses (in mg/kg bw/day) based on male mice.
NE=No Effect
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

References

1. Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Subchronic oral toxicity of di-n-octyl phthalate and di (2-ethylhexyl) phthalate in the rat. Food Chem Toxicol 35:225-239 (1997).

2. Singh AR, Lawrence WH, Autian J. Teratogenicity of phthalate esters in rats. J Pharm Sci 61:51-55 (1972).

3. Heindel JJ, Gulati DK, Mounce RC, Russell SR, Lamb JCI. Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. Fundam Appl Toxicol 13:508-518 (1989).

4. Gulati DK, Chambers R, Shaver S, Sabehrwal PS, Lamb JC. Di-n-octyl phthalate reproductive and fertility assessment in CD-1 mice when administered in feed. Research Triangle Park: National Toxicology Program, 1985.

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



Center For The Evaluation Of Risks To Human Reproduction

PUBLIC COMMENTS ON THE PHTHALATES EXPERT PANEL REPORTS

Appendix III

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AdvaMed
Advanced Medical Technology Association

December 11, 2000

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

Dear Dr. Shelby:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sincerely,

[redacted]

James S. Benson
Executive Vice President
Technology & Regulatory Affairs

[redacted]

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

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

Attachment 1

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

Type of Study: GLP

Table 1. Study Design

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

*Dose had to be decreased to 600 mg/kg

Total Number of Animals: 112 pups

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

Observations: Daily

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

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

Sperm Count: At 90 day

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

Necropsy: Gross observations

Clinical Pathology: None

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

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

DEC - 7 2000

December 1, 2000

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council *Good Chemistry Makes It Possible*

Ms. Kate Rawson
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5550 Friendship Blvd., Suite One
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Dear Sir/Madam:

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

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

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

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

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

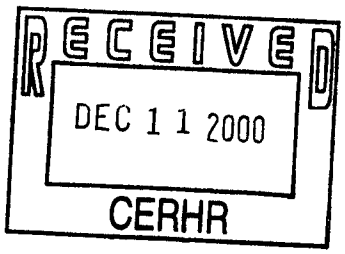
In summary, independent scientists, international government bodies and phthalate producers have conducted extensive studies about the safety, health and environmental effects of phthalates. This substantial body of scientific data does not present credible evidence that people are harmed by phthalates.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

Sincerely yours,

[redacted]

Courtney M. Price
Vice President, CHEMSTAR

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



December 11, 2000

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Re: Evaluations of Seven Phthalate Esters

Dear Dr. Shelby:

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

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

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

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

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

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

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

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

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

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3 Charge to Expert Panel (emphasis added).

4 *Id.*

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

Sincerely yours,

Courtney M. Price
Vice-President, CHEMSTAR

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

ATTACHMENT 1

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

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

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

General Comments

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

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Appendix III

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

ATTACHMENT 2

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ATTACHMENT 3

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Analysis of Fraction of Affected Pregnant Females
DnHP and DEHP

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

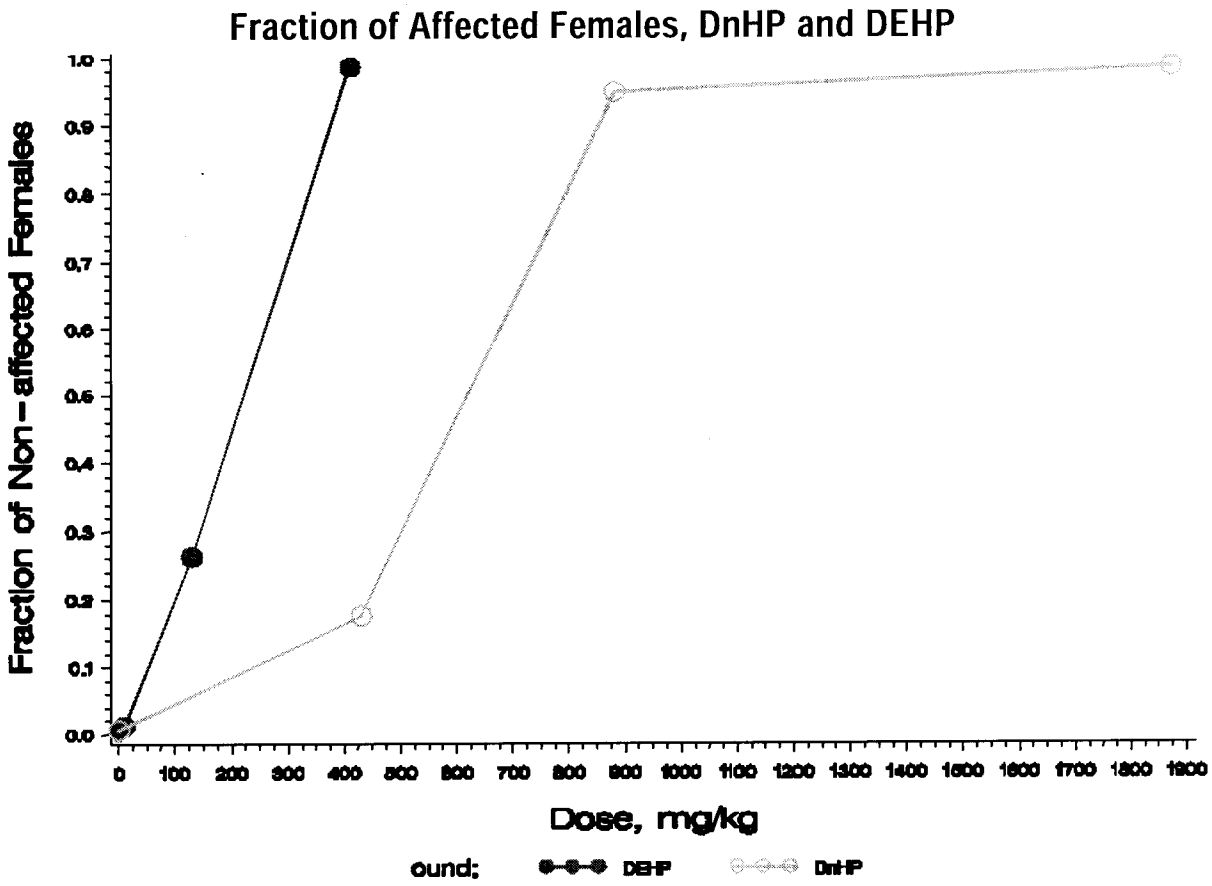
Compound	Dose (mg/kg)	Number Affected	Sample Size	Fraction Affected
DnHP	0	0	39	0.0
	430	3	17	0.18
	880	18	19	0.95
	1870	16	16	1.0
DEHP	0	0	40	0.0
	10	0	20	0.0
	130	5	19	0.26
	410	18	18	1.0

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

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

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

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



ATTACHMENT 4

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ATTACHMENT 5

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

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

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

General Comment

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

Comments on Potential Occupational Exposures

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

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

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

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

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

Additional Technical Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ATTACHMENT 6

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Comments Based on Recently Published Data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Typographical Errors

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

Appendix III

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

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

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

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

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

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

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

* Significant at $p < 0.05$

** Significant at $p < 0.01$

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

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

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

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

* Significant at $p < 0.05$

** Significant at $p < 0.01$

Appendix III

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

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

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

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

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

A. Biological Significance of Dilated Renal Pelves

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

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

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

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

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

** significant at $p<0.01$

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

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

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

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

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

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

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

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

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

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

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

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

C. Biological Significance of Total Visceral and Skeletal Variants

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

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

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

Appendix III

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

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

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

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

Table 7. Historical Control Data for Developmental Toxicity Studies

at Exxon and BASF

Exxon Data

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

BASF Data

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

A. Litter Based Data

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

Appendix III

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

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

B. Fetal Based Data

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

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

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

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

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

III. Conclusion

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

References:

N. Chernoff, R. Kavlock, P. Beyer and D. Miller (1987). The potential relationship of maternal toxicity, general stress and fetal outcome. *Teratogenesis, Carcinogenesis and Mutagenesis* 7:241-253.

N. Chernoff, J. Rogers, C. Turner and B. Francis (1991). Significance of supernumerary ribs in rodent developmental toxicity studies: Postnatal persistence in rats and mice. *Fundamental and Applied Toxicology* 17:448-453.

EPA (1991). Environmental Protection Agency: Guidelines for Developmental Toxicity Risk Assessment; Notice. *Federal Register* 56:63798-63826.

J. Hellwig, H. Freudenberger and R. Jackh (1997). Differential prenatal toxicity of branched phthalate esters in rats. *Food and Chemical Toxicology* 35: 501-512.

R. Kavlock, N. Chernoff and E. Rogers (1985). The effect of acute maternal toxicity on fetal development in the mouse. *Teratogenesis, Carcinogenesis and Mutagenesis* 5:3-13.

K. Khera (1981). Common fetal aberrations and their teratologic significance: a review. *Fundamental and Applied Toxicology* 1:13-18.

K. Khera (1984). Maternal toxicity: A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. *Teratology* 31:129-153.

J. Moore, G. Daston, E. Faustman, M. Golub, W. Hart, C. Hughes, C. Kimmel, J. Lamb, B. Schwetz and A. Scialli (1995). An evaluative process for assessing human reproductive and developmental toxicity of agents. *Reproductive Toxicology* 9:61-95.

B. Schwetz, G. Sparschu and P. Gehring (1971). The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. *Food and Cosmetic Toxicology* 9:801-817.

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.

G. Wickramaratne (1988). The post-natal fate of supernumerary ribs in rat teratogenicity studies. *Journal of Applied Toxicology* 8:91-94.

ATTACHMENT 7

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COURTNEY M. PRICE
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April 11, 2001

American Chemistry Council *Good Chemistry Makes It Possible*

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

Dear Drs. Shelby and Moore:

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

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

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

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

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

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

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

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

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

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

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

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

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

Sincerely yours,

[redacted]

Courtney M. Price
Vice-President, CHEMSTAR

Literature Cited

Anderson, W. et al (2000). A biomarker approach to quantify human dietary exposure to phthalates. Risk Communication and Food Safety. First Joint CSL/JIFSAN Symposium on Food Safety and Nutrition. 20-22 June 2000. Central Science Laboratory, Sand Hutton, U.K.

Anderson, W. et al. (2001). A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Additives and Contaminants. In press.

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.

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

(CDC) (2001). National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control. Available at http://www.cdc.gov/nceh/dls/report.

David, R. (2000). Exposure to phthalate esters. Environmental Health Perspectives 108:A440.

Kohn, M., et al. (2000). Human exposure estimates for phthalates. Environmental Health Perspectives 108:A 440-442.

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. Toxicological Sciences 58:350-365.

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

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.

[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,

[redacted]

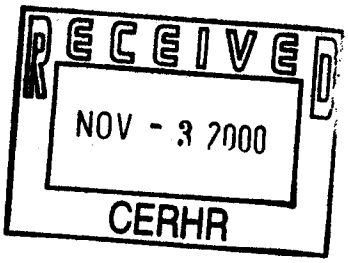
Doug Sallenbach
Director – Sales and Marketing
Discovery Medical, Inc.

Appendix III

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ENVIRONMENTAL WORKING GROUP™



October 30, 2000

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

Dear Dr. Shelby:

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

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

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

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

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

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

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

Thank you very much for your attention to this matter.

Sincerely,

[redacted]

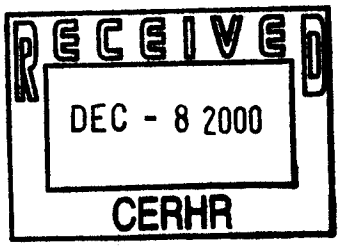
Richard Wiles
Vice President for Research

[redacted]

Jane Houlihan
Senior Analyst

Appendix III

ENVIRONMENTAL WORKING GROUP™



December 7, 2000

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

Dear Dr. Shelby:

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

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

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

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

Sincerely,

[redacted]

Richard Wiles
Vice President for Research

[redacted]

Jane Houlihan
Senior Analyst

Attachment

References

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

Appendix III

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DEHP STUDIES - MOLAR COMPARISON FOR DOWNSTREAM METABOLITES

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

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

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

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

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

DEHP STUDIES - MOLAR COMPARISON FOR DOWNSTREAM METABOLITES

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

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

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

Appendix III

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

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

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

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

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

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

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

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

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

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

a

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

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

References

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

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

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HEALTH CARE WITHOUT HARM

THE CAMPAIGN FOR ENVIRONMENTALLY RESPONSIBLE HEALTH CARE

December 8, 2000

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

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

Exposure:

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

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

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

Animal models:

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

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

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

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

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

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

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

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

Biotransformation:

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

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[1] Huber WH, Grasl-Kraupp B, Schulte-Hermann R. Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. Crit Rev in Toxicol 26(4):365-481, 1996.

[2] Wams TJ. Diethylhexylphthalate as an environmental contaminant-a review. Sci Total Environ 66:1-16, 1987.

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

[4] Muller J, Skakkeback N. The prenatal and postnatal development of the testis. Balliere's Clin Endocrin Metabol 6(2):251-271, 1992.

[5] Schlatt S, Weinbauer GF, Arslan M, Nieschlag E. Appearance of alpha-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. J Androl 14(5):340-350, 1993.
[6] Keys D, Wallace DG, Kepler T, Conolly R. Quantitative evaluation of alternative mechanisms of blood and testes disposition of di(2-ethylhexyl) phthalate and mono(2-ethyl hexyl) phthalate in rats. Toxicol Sci 49:172-185, 1999.
[7] Gray TJB, Gangolli SD. Aspects of the testicular toxicity of phthalate esters. Environ Health Perspect 65:229-235, 1986.
[8] Dixon RL, Lee IP. Pharmacokinetic and adaptation factors involved in their testicular toxicity. Fed Proc 39(1):66-72, 1980.
[9] Lee M, Gustafson M, Ukiyama E, et al. Developmental changes in Mullerian inhibiting substance in the cynomolgus monkey, Macaca fascicularis. J Clin Endocrin Metabol 78:615-621, 1994.
[10] Abbott D, Hearn J. Physical, hormonal, and behavioral aspects of sexual development in the marmoset monkey, Callithrix jacchus. J Reprod Fertil 53(1):155-166, 1978.
[11] Cortes D, Muller J, Skakkebaek N. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. Intl J Androl 10(589-596, 1987.

DEC 1 9 2000

Sept. 15, 2000

To;

National Institute of Environmental Health Sciences

P.O. Box 12233

Research Triangle Park, N.C. 27709

FROM: [redacted]

RECE
OCT 19 2000
LAB OF T

RE: 60 day public comment period on phthalates.

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

[redacted]

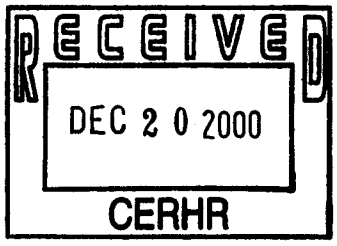
Appendix III

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Sincerely,

[redacted]

Kemikalieinspektionen



20th December 2000

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

Dear Dr. Shelby,

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

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

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

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

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

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

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

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

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

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

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

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

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