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-isononyl Phthalate (DINP)

March 2003 *NIH Publication No. 03-4484*

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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 DINP, a list of the expert panel members (Appendix I), the expert panel's report on DINP (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 DINP to adversely affect human reproduction or development. Those interested in reading this report may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP brief included within this report presents the NTP's interpretation of the potential for exposure to DINP to cause adverse reproductive or developmental effects in people. It is based upon information about DINP 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 DINP exposures to result in adverse health effects on development and reproduction.

Developmental Toxicity versus Reproductive Toxicity

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

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

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

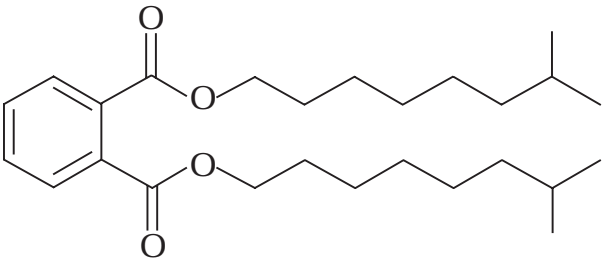
NTP Brief on Di-isononyl Phthalate (DINP)

What is DINP?

DINP is an oily, viscous liquid with the chemical formula $C_{26}H_{42}O_4$. It is a complex substance that contains a mixture of DINP isomers such as the one shown in Fig. 1. It is one of a group of industrially important chemicals known as phthalates. Phthalates are primarily used as plasticizers to add flexibility to plastics. DINP is used to manufacture a broad range of consumer products such as garden hoses, pool liners, flooring tiles, tarps, and toys. It is not used in medical devices and finds only limited use in food packaging.

Recent information indicates that approximately 178 million kilograms (392 million pounds) of DINP were used in the United States in 1998.

Figure 1. *Chemical structure of the DINP isomer, Di (7-methyloctyl) phthalate*



Are People Exposed to DINP?*

Yes. There are several ways that people may be exposed to DINP at home or at work. Human exposure to DINP can occur during the manufacture of DINP, during the manufacture of DINP-containing products, during the use of such products, or through the presence of DINP in the environment. Food does not appear to be a significant source of exposure. While inhalation, ingestion, or skin contact may expose people to DINP, consumer exposure is thought to occur primarily by ingestion and skin contact. Public concern has been raised for exposure of infants and children through the mouthing of soft toys made of DINP-containing plastics. The U.S. Consumer Product Safety Commission (CPSC) convened a Chronic Hazard Advisory Panel on Diisononyl Phthalate (CHAP-DINP) to determine if DINP in consumer products poses chronic health hazards. This panel considered hazards to children from mouthing DINP-containing toys. Their report was issued in June 2001, subsequent to release of the Phthalates Expert Panel Reports (CPSC, 2001).

Because of inadequate information on human exposure to DINP, the expert panel took the conservative position of assuming that general population exposures in the United States would be less than 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. This assumption was supported by exposure calculations (Kohn et al., 2000; David, 2000) using the urine data from Blount et al., 2000. Calculated daily exposure estimates indicate that 95% of the study population exposed to DINP was exposed to less than 1.7 µg/kg bw/day, and that the maximum exposure was 22 µg/kg bw/day. The CHAP-DINP (CPSC, 2001) estimated that, as a result of mouthing DINP-containing toys, children 0-18 months old could be exposed to up to 280 µg/kg bw/day. Children 19-36 months old could be exposed to up to 70 µg/kg bw/day. A 15 pound child exposed to 280 µg/kg bw/day would be exposed to approximately 2000 µg DINP/day. A 30 pound

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

child exposed to 70 µg/kg bw/day would be exposed to approximately 1000 µg DINP/day. By comparison, a small drop of water weighs approximately 30,000 µg and a grain of table salt weighs approximately 60 µg.

Can DINP Affect Human Development or Reproduction?

Possibly. Although there is no direct evidence that exposure of people to DINP adversely affects reproduction or development, studies in laboratory animals have shown that exposure to DINP can adversely affect development, but not reproduction, in rodents (Fig. 2).

Scientific decisions concerning health risks are generally based on what is known as the "weight-of-the-evidence." In this case, recognizing the lack of human data, some evidence of developmental effects, and limited evidence of no reproductive effects in animals, the NTP judges the scientific evidence sufficient to conclude that DINP might adversely affect development of the human fetus if the levels of exposure are sufficiently high (Fig. 3).

It is important to note that the levels of DINP exposure that lead to adverse effects on development in rodents are generally far higher than those experienced by people.

Summary of Supporting Evidence

As detailed in the expert panel report, studies of reproductive and developmental toxicity in rats have shown that exposure of the pregnant female to relatively high doses of DINP can affect development of the kidneys and skeletal system of the fetus and result in reduced birth weights. The reproductive toxicity studies reviewed by the expert panel reported no evidence of adverse effects on the reproductive system of rats.

Subsequent to release of the expert panel report, a rodent study was conducted to determine if DINP produced antiandrogenic-like effects in male rats following exposure to 750 mg/kg bw/day from gestation day 14 through postnatal day 3 (Gray et al., 2000). Treatment resulted in female-like areolas/nipples in some male pups, and limited evidence of effects on the structure of the male reproductive tract. No effects were observed for reproductive tract development endpoints that included testis weight, anogenital distance, age of preputial separation, hypospadias or undescended testes. This study provides some evidence that DINP, like other phthalates such as DEHP and DBP, adversely affects development of the male rat reproductive system. However, the use of a single, high dose level of DINP limits the utility of

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

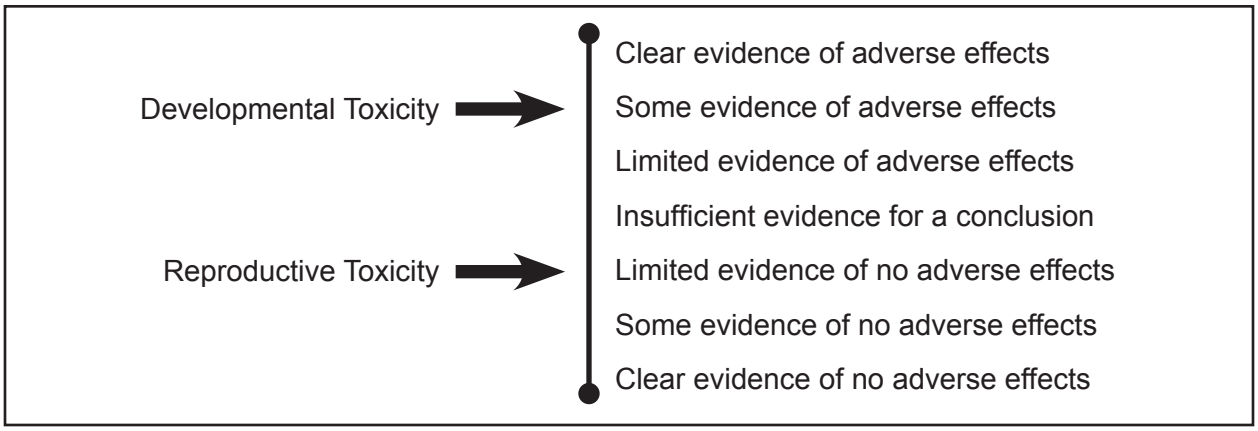
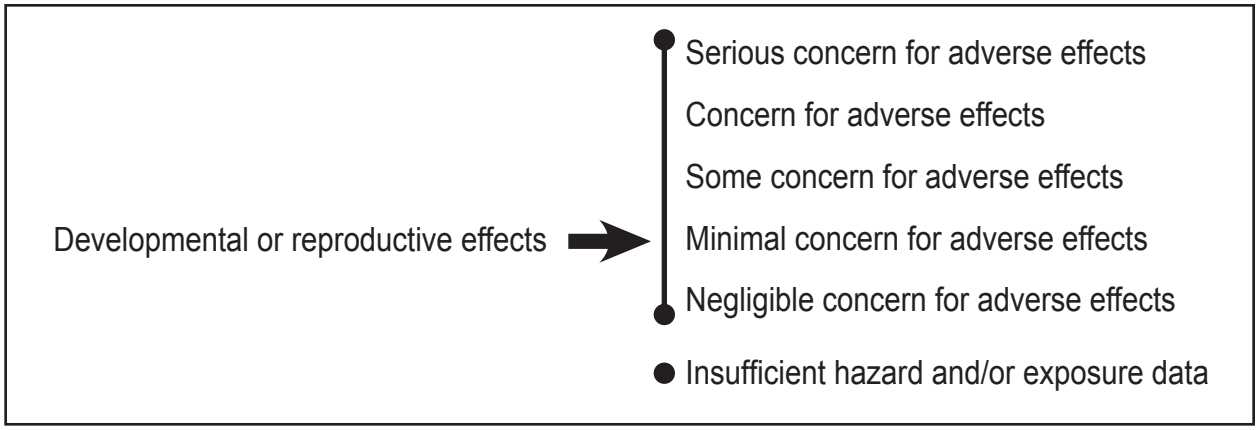


Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to DINP*



this study in evaluating human health risks.

Are Current Exposures to DINP High Enough to Cause Concern?

Probably not. More data are needed to better understand the levels to which people are exposed to DINP and how these exposures vary across the population. Although the general U.S. population presently appears to be exposed to DINP at levels that are not of immediate concern for causing adverse reproductive or developmental effects, data are not available to permit conclusions regarding the possibility of effects in various age groups, occupations, or socioeconomic strata. Thus, the NTP offers the following conclusions.

The NTP concurs with the conclusions of the CERHR Phthalates Expert Panel and has minimal concern for DINP causing adverse effects to human reproduction or fetal development.

This is based on recent estimated DINP exposures in the U.S. general population. The CHAP-DINP (CPSC, 2001) convened by the U.S. Consumer Product Safety Commission concluded, "...the risk to reproductive and developmental processes in humans due to DINP exposure is extremely low or nonexistent."

The NTP has minimal concern for developmental effects in children.

This is lower than the "low concern" expressed by the expert panel and is based on the CHAP-DINP estimates of potential DINP exposures in children from mouthing DINP-containing toys. The exposure levels at which developmental effects were reported in rats (143-285 mg/kg bw/day) is about 1000 times higher than the upper end of the range estimated for children's exposure (70-280 µg/kg bw/day).

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:

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

CPSC. Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP). June, 2001. (cited June 2002). *<http://www.cpsc.gov/LIBRARY/FOIA/Foia01/os/dinp.pdf>*

David RM. Exposure to Phthalate Esters. *Environmental Health Perspectives* **108**: A440 (2000).

Gray LE, Ostby J, Furr J, Price M, Rao Veeramachaneni DN, Parks L. 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 (2000).

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

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

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 DINP is provided in Appendix II and the public comments received on that report are in Appendix III. Input from the public and interested groups throughout the panel's deliberations was invaluable in helping to assure completeness and accuracy of the reports. The Phthalates Expert Panel Reports are also available on the CERHR website <*http://cerhr.niehs.nih.gov*>.

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

Appendix I. NTP-CERHR Phthalates Expert Panel
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National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

Appendix II

NTP-CERHR EXPERT PANEL REPORT

on

Di-isononyl Phthalate

October 2000 *NTP-CERHR-DINP-00*

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PREFACE

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

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

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

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

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

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

CERHR
Sciences International, Inc.
1800 Diagonal Road, Suite 500
Alexandria, VA 22314-2808
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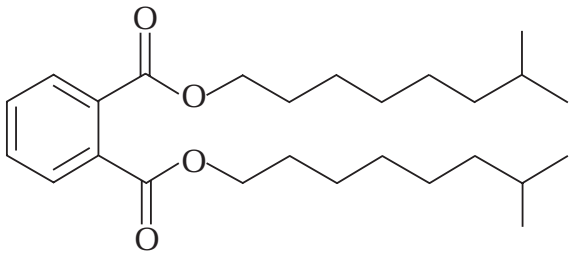
1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of a Di-isononyl Phthalate Isomer
Di (7-methyloctyl) phthalate



DINP is a complex substance assigned two different CAS Registry Numbers (RN). CAS RN 68515-48-0 (designated DINP-1 in this document) is manufactured from octene that is converted to alcohol moieties consisting mainly of 3,4-, 4,6-, 3,6-, 3,5, 4,5-, and 5,6-dimethyl-heptanol-1. CAS RN 28553-12-0 (DINP-2) is produced from n-butene that is converted primarily to methyloctanols and dimethylheptanols. The 28553-12-0 CAS RN also represents DINP-3 which is produced from n-butene and isobutene that are converted to alcohols, with 60% consisting of methylethyl hexanols. According to the American Chemistry Council (ACC; formerly CMA), DINP-3 is no longer produced (1). The ACC (2) has stated that although DINP is a complex substance, it is not variable due to the stability of the alcohol manufacturing process. The two types of DINP are considered commercially interchangeable.

DINP is an oily, viscous liquid at standard temperature and pressure.

Table 1: Physicochemical Properties of DINP

Property	***Value***
Chemical Formula	$C_{26}H_{42}O_4$
Molecular Weight	419
Melting Point	-48 °C
Boiling Point	370 °C
Specific Gravity	0.97
Solubility in Water	Insoluble (<0.001 mg/L)
Log K_{ow}	9

(3)

1.2 Exposure and Usage

Humans may be exposed to DINP by the oral, dermal, and inhalation routes of exposure. Occupational exposure occurs primarily through inhalation and dermal contact, while consumer exposure occurs primarily through oral and dermal routes. Exposure of children to DINP through children's products is a public concern.

Occupational exposure

DINP, like other phthalate esters, is manufactured within a closed system under negative pressure. However, some exposures may occur during the loading and unloading of railroad cars and trucks. Slightly higher exposures may occur during the production of PVC products because of elevated temperatures and more open processes. ACC (1) cites six studies that indicate exposures are below 1 mg/m^3 during production of phthalates and below 2 mg/m^3 during production of PVC. As discussed in Section 2.2, dermal exposure is not expected to result in significant absorption into the body.

Consumer exposure

The range of products that contain DINP is quite broad. The use, categories, and amounts used of DINP in 1998 are given in Table 2.

Table 2: Calculated 1998 US Consumption of DINP (thousands of metric tons)

End Use	***Subtotal***	***Total***
Film and Sheet		13
Stationary and Wood Veneer	6	
Pool Liners	1	
Other	6	
Flooring		48
Tiles	23	
Sheets	25	
Artificial Leather		3
Coated Fabrics		21
Tarps	16	
Conveyor Belts	1	
Other	4	
Dip Coating/Slush Molded		30
Gloves	15	
Toys	6	
Traffic Cones	<1	
Other	~9	
Tubings and Profiles		7
Profiles	5	
Garden Hoses	2	
Wire and Cables		32
Shoes/Shoe Soles		9
Under-Body Coating		7
Sealants (carpet backing)		8
GRAND TOTAL		178

(1)

DINP is a general purpose plasticizer with a broad range of applications used in flexible PVC. It is widely used in the toy, construction, and general consumer product markets. It has limited use in food packaging and is not used in medical applications.

Because of physicochemical similarities between DINP and DEHP, general exposure to DINP is probably very similar to exposure to DEHP, but few monitoring data were located. Based on data for other phthalates, one could speculate that environmentally-contaminated food represents a primary route for human exposure. However, the data are scant in support of this view.

DINP's solubility in water is extremely low; levels are often below the analytical detection limit. Vapor pressure is also extremely low, so measured concentrations in air are not available. Modeling based on physicochemical properties of DINP can be compared to similar models for DEHP.

Food

In 1996, dinonyl phthalate (isomer not specified) was identified but not quantified in 4 of 12 infant formulas from the UK (4). In a follow-up survey conducted by the Ministry of Agriculture, Fisheries, and Food (MAFF) (5), DINP was not specifically targeted, but there was no evidence of its presence in 39 samples of infant formula from the UK. In a UK survey of fatty foods (e.g., dairy products, meats, fish, eggs, and oils), DINP was not detected at an analytical limit of 0.01 mg/kg (6).

Toys

PVC plastics are often used in children's products. Different phthalates are constituents of PVC; DINP is currently the predominant plasticizer (7). Other phthalates, including DEHP, have been or are also used (8, 9). US toy manufacturers began voluntary removal of DEHP from pacifiers and nipples in 1986 (10). Few studies pertaining to plasticizers in children's products were found in the peer-reviewed literature. Additional information is available from industry groups and several government agencies. The Expert Panel did not perform a comprehensive review of available data, but believes the information it reviewed reflects the general state of knowledge.

As reported by the Consumer Product Safety Commission (CPSC) (7), Chen measured DINP in 31 of 35 toys and found a concentration range of 15.1–54.4 % dry weight. Health Canada (11) analyzed 41 children's products made in the US, China, and Thailand for the presence of DINP and DEHP. DINP was detectable in 27 of the 41 products in concentrations that ranged from 3.9 to 44% dry weight. Criteria for the selection of products were not discussed in any of these surveys. No information on market share, length of availability on the market, or estimates of the numbers of products in circulation was noted in any study. Only Health Canada listed product number, country of origin, manufacturer/distributor, and brand. All studies listed a product description. Marin (9) analyzed 15 samples of materials used in toys in Spain. The authors noted that the PVC contained a mixture of plasticizers including DINP, DEHP, and DIDP, but reported only the DEHP content.

The estimation of actual exposure of children to phthalates contained in children's products has been studied. *In vitro* studies using various agitation and impaction approaches yield a wide range of extraction of DINP from toys. CPSC used stainless steel pistons, 11 cm^2 of each product, and simulant saliva to obtain extraction rates for their 31 DINP-positive children's products. Migration

was log normally distributed with a mean rate of 8.2 – 9.83 µg/11 cm^2/hour and a range of 1– 48 µg/11 cm^2/hour. Both CPSC (7) and Health Canada (11) failed to find any correlation between release rate of DINP under experimental conditions and total DINP content.

The Dutch Consensus Group reported a small study by Meuling and Rijk (12) using 20 adult volunteers. A control specimen without DINP and three specimens with DINP were used; specimen 1 contained 38% DINP. Specimens 2 and 3 came from different parts of the same commercially-available teething ring, representing different shapes for mouthing, and contained 43% DINP each. All three were 10 cm^2 total area. All 20 volunteers were instructed to suck and bite on the control specimen for 10–15 minutes, all saliva was collected, volunteers rested 5 minutes and then they performed 4 separate sessions on the same test piece of specimen 1, resting 5 minutes between each session. This procedure was repeated with half (n=10) of the volunteers on specimen 2 and the other half (n=10) on specimen 3. DINP extraction from specimen 1 was 1.38 (0.3–8.3) µg/min, from specimen two 2.44 (0.9–8.9) µg/min, and from specimen three 1.63 (0.9–5.7) µg/min. The mean across all groups was 1.8 µg/10cm2/min (or 120 µg/11cm^2/hour). There was no correlation between extraction and pH or protein content of the saliva. Release rates over the various 15-minute intervals seemed consistent. The increase in extraction of Specimen 2 was thought to be due to the finger-like shape resulting in different mouthing behaviors from those employed on the disk-like shape of Specimens 1 and 3.

CPSC (7) reported a similar protocol using 10 adult volunteers and 5 toys, and found a mean migration rate of 241.3 µg/11cm^2/hour. This rate was 39.5 times higher than the average rate obtained by impaction with disks cut from the same 5 toys, but was similar to the ranges in the Dutch simulation study.

Exposure of children to DINP from PVC toys was estimated by Fiala et al. (13) in Austria. DINP levels were measured in the saliva of 10 adult volunteers who first sucked on and then sucked and chewed on 10–15 cm^2 pieces of teether (containing about 36% DINP) for 1 hour. In the experiment where the volunteers only sucked on the sample, the migration rates of DINP ranged from 297–1,452 µg/dm^2/hour with a mean migration rate of 832±397 µg/dm^2/hour. Using assumptions of an 8 kg body weight, 3-hour exposure time (12), and 10 cm2 mouthing area, mean and maximum exposure levels of 31.25 µg/kg bw/day and 54.4 µg/kg bw/day, respectively, were estimated. For the experiment where the adults chewed on the sample, migration rates of DINP in 9 adults ranged from 768−2152 µg/dm^2/hour. Using the same assumptions from the first experiment, a maximum exposure level of 84.5 µg/kg bw/day was estimated.

CPSC (7), the Dutch Consensus Group (12), and Health Canada (11) have attempted to calculate daily intake based upon the leaching rates described above. The Dutch Group used Monte Carlo simulation and estimates of mouthing time and the leaching rates from the *in vivo* study of 20 adults. Mouthing time was derived from parent observations and logging of mouthing time of 42 children aged 3−35 months (Table 3). Mouthing time was calculated for the time children were awake, but not eating, during ten 15 minute observation periods over 2 days. Logs were kept of objects mouthed; the objects were divided into those intended for mouthing and those not intended for mouthing. The Dutch calculations used total mouthing time excluding time spent mouthing pac-

ifiers. Because the greatest exposure levels were determined for children within the ages of 3−12 months, the results for that age group are summarized in Table 4.

Table 3: Total Mouthing Time

Age (months)	Sample Size	Mean in Minutes (SD)	Min (Minutes)	Max (Minutes)
3–6	5	36.9 ±19.1	14.5	67.0
6–12	14	44.0 ± 44.7	2.4	171.5
12–18	12	16.4 ± 18.2	0	53.2
18–36	11	9.3 ± 9.8	0	30.9

Table 4: Toy Exposure Estimates for Children Aged 3–12 Months.

	Estimated Intake Level (µg/kg bw/day)			
Agency	**Mean**	**95th Percentile**	**99th Percentile**	**Maximum**
RIVM*	6.53–14.4	20.7–39.7	39.8–77.3	70.7–204
CPSC	5.7	94.3	-	-
Health Canada**	44	73.9***	173.5***	320

** Exposure range for 3−6 month-old and 6−12 month-old children; range includes results from 3 specimens tested.*

*** Calculated with mouthing times for teethers and other objects intended for mouthing.*

**** Results using Monte Carlo simulations in children aged 3–6 months.*

The approach taken by Health Canada used published data and 10,000 Monte Carlo simulations and total mouthing time from the Dutch observation study including mouthing of pacifiers, teethers and other objects intended for mouthing. CPSC used the same mouthing-time data, but limited its calculations to the mouthing time of objects not intended for mouthing. They performed a log transformation of the time because of the extreme skewness in the sample and calculated a geometric mean mouthing time of 12.03 minutes (95% CI 6.2–23.3). Exposure estimates were made using a log linear model, the mean leaching rate from mechanical extraction from 31 consumer products and a 39.5 factor (to adjust for differences between *in vitro* and *in vivo* extraction rates). The differences in the analyses resulted in quite different exposure estimates, which explains the different conclusions and recommendations of the agencies.

The differences also highlight the uncertainties inherent in these calculations. Because extraction of DINP does not correlate with DINP content, because extraction is highly variable across both laboratory procedures and human subjects, and because the number and distribution of children's products containing DINP is unknown, the amounts of DINP presented to a child cannot be well characterized. Furthermore, the estimates of mouthing behavior in the youngest and potentially highest risk group, 3–12 months, are based upon only 19 children. No discussion of developmental age, physical condition, ethnicity, or other socio-demographic indicators is included in the small parental observation study. These numbers are preliminary estimates at best. Standardization of laboratory techniques with correlation with *in vivo* simulations, better data on product distribution and use, and independent studies of mouthing behavior in babies and young children are needed. None-

the-less, existing models show this is a potentially significant exposure for young children. A study using larger numbers of children has been submitted by Juberg et al. (14), but could not be cited at the time of this review. According to the ACC (2), the CPSC and EU Joint Research Laboratory are working on standardizing laboratory techniques with *in vivo* simulations.

Dermal exposure to DINP from toys may also occur, but has not been studied specifically in children.

Exposure Estimate

Based on the physicochemical characteristics of DINP and limited monitoring data, the Expert Panel believes it reasonable to assume that exposure to DINP in the general adult population is lower than exposure to DEHP, which is estimated at 3–30 µg/kg bw/day (15). Children may incur significantly greater nondietary exposures from mouthing toys and other articles containing DINP.

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

BIBRA (16) (Table 7-1) conducted a 21-day dietary study in 6-week-old F344 rats where groups of 5 males and 5 females were fed concentrations of 0, 0.6, 1.2, or 2.5% DINP (M: 639, 1,192, or 2,195 mg/kg bw/day; F: 607, 1,193, or 2,289 mg/kg bw/day). The test material most likely consisted of a mixture of DINP represented by CAS numbers 68515-48-0 and 28553-12-0 (DINP-1 and DINP-2). A positive control group of 5 rats per sex was exposed to 1.2% DEHP (M: 1,084 mg/kg bw/day; F: 1,063 mg/kg bw/day). Body weight and food intake were measured twice weekly. On day 21, rats were killed and necropsied. Liver, kidney, and testes were preserved in formalin and examined histologically. Peroxisomal proliferation was assessed by measuring activities of peroxisomal proliferation enzymes and by examining liver tissue by electron microscopy.

A significant decrease in weight gain was observed in the mid- and high-dose groups. Food intake was significantly reduced in males. Organ to body weight ratios that were significantly increased in all treatment groups included liver (M: 136, 173, and 232%, F: 131, 175, and 237% of control values) and kidney (M: 115, 122, and 124%, F: 107, 108, and 114% of control values). Histopathological changes were not observed in kidneys; changes in liver were limited to reduced cytoplasmic basophilia in the mid- and high-dose group and increased cytoplasmic eosinophilia in the high-dose group. Palmitoyl-CoA (PCoA) oxidase activity was significantly increased in the mid- and high-dose groups (M: 452 and 1,035%; F: 376 and 1,104% increases, respectively, compared to controls) and an increase in peroxisome numbers was observed by electron microscopy in livers from the high-dose group. The activity of 11-hydroxylase and 12-hydroxylase was significantly increased in males of all dose groups and in females of the high-dose group. Significant changes observed in all treatment groups included increased total liver proteins and reductions in serum levels of cholesterol. Serum triglyceride levels were significantly reduced in all treated males, but increased in mid- and high-dose females. The testes to body weight ratio was significantly increased in the high-dose males (135% of control value), but absolute testes weights were not significantly affected. Testicular lesions were not observed with the exception of severe unilateral atrophy in one male of the mid-dose group. Treatment with 1,063−1,084 mg DEHP/kg bw/day resulted in similar effects including decreased weight gain, increased liver and kidney to body weight ratio, increased liver enzyme activities, and reduced serum levels of cholesterol and triglycerides. Moderate testicular atrophy was noted in one male. Peroxisomal proliferation is of particular interest and an increase in peroxisome numbers was observed after treatment with DEHP. PCoA activity was significantly increased to 683 and 540% of control values for males and females, respectively. The increase in peroxisomal enzyme activity in rats treated with 1,063−1,084 mg/kg bw/day DEHP was greater than that obtained by treatment with DINP at 1,192−1193 mg/kg bw/day (452 and 376% of control values in males and females, respectively).

This study provides evidence that the liver is a target organ of DINP. A pattern similar to effects noted with DEHP is seen: increased liver weight and induction of hepatic peroxisome proliferation. The testes do not appear to be a target organ at these dose levels. The study provided a LOAEL of 0.6% (607[F] and 639[M] mg/kg bw/day) and no NOAEL was identified.

In a 2-year dietary study, (17) (Table 7-2) systemic effects resulting from DINP-1 exposure in adult (6 week old) Fischer 344 rats were evaluated. Groups of 110 rats per sex were fed diets containing 0, 0.03, 0.3, and 0.6% DINP-1 (males: 0, 15, 152, and 307 mg/kg bw/day; females: 0, 18, 184, and 375 mg/kg bw/day). Body weight and food intake were measured weekly. Ten rats/sex/group were killed and necropsied at 6, 12, and 18 months; the remaining rats were killed and necropsied at the end of the 2-year study. Evaluation of hematology, urine, and blood chemistry effects was performed at 6, 12, 18, and 24 months. Histopathological evaluations were conducted on the liver and the kidney from all dose groups and in the remaining organs of the control and high-dose groups. Evidence of peroxisome proliferation was determined by microscopic examination of livers of 2 rats/sex/group at 24 months.

Significant reductions in body weight gain were observed in males from 18–24 months in the 152 mg/kg bw/day group and from 12–24 months in the 307 mg/kg bw/day group. Food intake levels were not reported. Survival was significantly decreased in females of the 184 and 375 mg/kg bw/day groups. Liver and kidney to body weight ratios were significantly increased throughout the study in both sexes in the mid- and high-dose groups (152–375 mg/kg bw/day). Spleen to body weight ratios were significantly increased in males and females of the high-dose group (307–375 mg/kg bw/day) at 24 months. A small but significant increase in adrenal to body weight ratio was reported for females in the 375 mg/kg bw/day group at 6–12 months, and in both sexes in the high-dose group (307–375 mg/kg bw/day) at 24 months. Adrenal weights were not listed in tables. Dose-related changes in liver included hepatocyte enlargement in high-dose males and females throughout treatment. At 24 months, dose-related liver effects included regenerative nodules and focal necrosis in males and females of the two highest dose groups, and spongiosis hepatitis in males of the high-dose group. In males of the mid- and high-dose groups, consistent increases in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities were observed. However, for SGOT, statistical significance was obtained only at 6 and 12 months in the mid-dose group and at 6–18 months in the high-dose group. In males, increases in SGPT activity were statistically significant at 24 months in the mid-dose group and at 6 and 18 months in the high-dose group. An increased incidence of mononuclear cell leukemia (MNCL) was observed in both sexes of the mid- and high-dose groups. Peroxisome proliferation did not occur and there was no evidence of treatment-related lesions in testes or female reproductive organs. The only significant dose-related changes in hematology were a reduction in red blood cell count and hemoglobin and hematocrit values in males of the 307 mg/kg bw/day group at 24 months. Urinalysis results were not listed in tables, but authors reported increased urine volumes in high-dose males at all time points and transient increases in potassium and glucose. A NOAEL of 17 mg/kg bw/day was selected by the authors.

A second 2-year dietary feeding study in F344 rats was reported by Moore et al. (18) (Table 7-3). Groups of 70–85 F344 rats/sex/group (6 weeks old) were fed concentrations of 0, 500, 1,500,

6,000, and 12,000 ppm DINP-1 (males: 0, 29.2, 88.3, 359, or 733 mg/kg bw/day; females: 0, 36.4, 109, 442, or 885 mg/kg bw/day). Body weight and food intake were measured weekly through weeks 16–17 and monthly thereafter. Standard hematological, clinical chemistry, and urinalysis parameters were measured every 26 weeks. Peroxisome proliferation was measured in 5 rats/sex in control and high-dose groups at weeks 1, 2, and 13, and in 3–5 rats/sex in the control and 2 highest dose groups at week 104. Five rats/sex/group were sacrificed and necropsied at weeks 1, 2, and 13. Fifteen rats/sex/group were killed and necropsied at week 79. The remaining rats were sacrificed and necropsied at week 104. Another group of 55 rats/sex was exposed through diet to 12,000 ppm (males 637 mg/kg bw/day; females 774 mg/kg bw/day) DINP for 78 weeks and sacrificed at week 104 in order to determine if recovery occurs after exposure to DINP has ended. Histopathological evaluations were conducted on major organs from rats in all dose groups.

Clinical signs of toxicity were observed in rats exposed to 359 mg/kg bw/day and higher, and included hunched posture, decreased activity, bodies that appeared pale and thin, and fewer feces. Rats exposed to 733–885 mg/kg bw/day experienced a statistically significant reduction in weight gain accompanied by a decrease in food intake. Survival was significantly reduced in the high-dose males with only 54% surviving to the end of the study. The body weight effect was shown to be partially reversible because male weight gain in the recovery group was not reduced at week 104; reduced weight gain in females was less pronounced. Survival was not significantly affected in the recovery group. The authors reported that the dose-related depression of body weight gain in the two highest doses was associated with clinical chemistry findings or histomorphologic effects in liver and kidney. A significant increase in the incidence of anemia, as observed by decreases in erythrocyte, hemoglobin, and hematocrit levels, was observed throughout the study in rats exposed to 359 mg/kg bw/day and higher, but was not observed in the recovery group. A significant increase in kidney to body weight ratio was observed in rats exposed to 359 mg/kg bw/day and higher from week 79 to 104 (M: 8.1 and 25% and F: 14.4 and 22% increases in 2 highest dose groups, respectively, at week 104). Liver to body weight ratios were significantly increased in both sexes exposed to 359 mg/kg bw/day and higher throughout the study (M: 35 and 61% and F: 26 and 71% increases at 2 highest doses, respectively, at week 104). Histological effects observed in kidneys of rats exposed to 359 mg/kg bw/day and higher at weeks 79 and 104 included an increased incidence and severity of renal papilla mineralization in males (59/85 and 57/85 at 2 highest doses). An increase in tubule cell pigmentation was also reported by the authors, but the incidence of the lesion appeared equal among control and dose groups (55−59 sex/dose). Urinalysis findings at week 104, which included significant increases in urine output and corresponding decreases in potassium, calcium, creatinine, and chloride levels in high-dose males, suggested compromised ability to concentrate in the renal tubule epithelium. Serum urea levels were significantly increased during the second half of the study in rats exposed to 359 mg/kg bw/day and higher. Increases in urine volume and kidney lesions were observed in the recovery group exposed to 733 mg/kg bw/day and greater with severity approximately equal to that of the 359−442 mg/kg bw/day treatment group. Livers of rats exposed to 359 mg/kg bw/day and higher appeared enlarged and granular at weeks 79 and 104. Histopathological effects in the livers of the high-dose group included diffuse hepatocyte enlargement (37/85 males and 52/85 females), cytoplasmic eosinophilia (43/85 males and 45/85 females), and Kupffer cell/bile canaliculi pigmentation (12/85 males and 17/85 females). These effects were first detected at weeks 2, 13, and 79, respectively. The authors also reported alterations in serum alanine aminotransferase and aspartate aminotransferase activity, but the changes did not appear to

be consistent or dose related. Non-neoplastic liver changes were found to be reversible in the recovery group. Peroxisomal enzyme activity was significantly increased at week 104 in females exposed to 442 mg/kg bw/day and in both sexes of the high-dose group throughout the study. The recovery group was not tested for peroxisomal enzyme activity. Histopathological changes in testes or female reproductive organs were not observed.

Neoplastic effects included a significant increase in liver adenomas (10/80 vs 4/80) and carcinomas (11/80 vs 1/80) in male rats of the high-dose group at week 104. At week 104, renal tubule cell carcinoma was observed in 2 males of the high-dose group and 4 males of the recovery group. Mononuclear cell leukemia was found in 45–49% of rats in the 2 highest dose groups. Liver neoplasms were not observed in the recovery group, but the incidence of renal tubule cell carcinoma in males and mononuclear cell leukemia remained elevated compared to controls. The authors selected a NOAEL of 1,500 ppm (88.3−109 mg/kg bw/day) for this study.

In a 2-year dietary study in 6-week-old B6C3F1/CrlBR mice (19) (Table 7-4), groups of 70 mice/sex/group ate diets that contained 0, 500, 1,500, 4,000, and 8,000 ppm DINP-1 (males: 0, 90.3, 276, 742, 1,560 mg/kg bw/day; females: 0, 112, 336, 910, 1,888 mg/kg bw/day). Body weights and food intake were measured weekly through week 16–17 and monthly thereafter. Standard, hematological, clinical chemistry, and urinalysis parameters were measured every 26 weeks. Peroxisome proliferation was measured in five mice/sex in the control and high-dose group at the midpoint and end of the study. Fifteen mice/sex/group were sacrificed and necropsied at week 79. The remaining mice were sacrificed and necropsied at the end of the 2-year study. Histopathological evaluations were conducted on major organs from mice in all dose groups. Another group of mice was exposed to 8,000 ppm DINP in the diet for 78 weeks and sacrificed at week 105−106 in order to determine if recovery would occur after exposure to DINP ended.

Toxicological and non-neoplastic effects were observed in mice that received the 2 highest doses, 742 mg/kg bw/day and greater. A statistically significant reduction in weight gain occurred throughout the study; this reduction was not accompanied by a decrease in food intake. The effect was shown to be partially reversible because female weight gain in the recovery group was not reduced at week 104; reduced weight gain in males was less pronounced. Clinical signs of toxicity were observed and included abdominal swelling in males exposed to 742 mg/kg bw/day and greater, and hunched posture, decreased activity, and fewer feces in the high-dose males. Survival was significantly reduced in the high-dose males (1,560 mg/kg bw/day), with only 63% of males surviving until the end of the study. Survival was not significantly affected in the recovery group. A significant reduction in kidney to body weight ratio was observed in males of the 2 highest dose groups (13 and 25% reduction, respectively), whereas a significant increase in liver to body weight ratio occurred (7 and 24% increase, respectively). Females exposed to the highest dose (1,888 mg/kg bw/day) had a 37% increase in liver to body weight ratio from week 79 to 104. Histological examination revealed an increased incidence and severity of renal nephropathy in female mice of the high-dose group. Urinalysis findings, which included significant increases in urine output and corresponding decreases in sodium, potassium, and chloride levels in high-dose mice from week 52–104, suggested compromised ability to concentrate in the renal tubule epithelium. The effects on renal structure and function proved to be partially reversible as they were less pronounced in mice of the recovery group by the end of the study. Histopathological liver changes were observed

in mice of the highest dose group and included diffuse hepatocyte enlargement (56/70 males and 65/70 females) and cytoplasmic eosinophilia (67/70 males and 68/70 females) and pigment (64/70 males and 53/70 females). Other hepatic effects included increased serum alanine aminotransferase and aspartate aminotransferase levels in the high dose males at various time points throughout the study. Non-neoplastic liver changes were found to be reversible in the recovery group. An increase in peroxisomal enzyme activity in mice exposed to 1,560−1,888 mg/kg bw/day indicated that hepatocyte enlargement was due to peroxisomal proliferation. Histopathological changes in testes or female reproductive organs were not observed.

Neoplastic effects included increased incidences of hepatic adenomas and carcinomas combined in females exposed to 336 mg/kg bw/day (10/60 versus 3/70), and adenomas (15/60 and 13/60 versus 10/70) and carcinomas (17/60 and 20/60 versus 10/70) in males exposed to 742 and 1,560 mg/kg bw/day, respectively, and in females exposed to 1,888 mg/kg bw/day (18 adenomas and 18 carcinomas/70 versus 2 adenomas and 1 carinoma/70). The occurrence of hepatic neoplasms was lower in the recovery group compared to the high dose mice exposed for the duration of the study with an incidence of 37−39% versus 50−56%. Based on hepatic neoplasms, the authors selected a NOAEL of 500 ppm (112 mg/kg bw/day) for females and 1,500 ppm (276 mg/kg bw/day) for males.

Hall et al. (20) (Table 7-5) exposed sixteen 25-month-old marmosets (2/sex/group) by gavage for 13 weeks with DINP (CAS number not provided) in 1% methylcellulose and 0.5% Tween at concentrations of 0, 100, 500, or 2,500 mg/kg bw/day. Clofibrate was administered as a positive control at 500 mg/kg bw/day. Analysis was conducted for hematology (weeks 0, 6, and 13), blood chemistry (weeks 0, 4, and 13), estradiol and testosterone levels (week 12), and urine composition (weeks 0, 5, and 12). The main organs (including but not limited to liver, testes, and epididymides) were weighed and examined histologically (testes and epididymides were preserved in Bouin's). Peroxisomal proliferation was determined by measuring cyanide-insensitive PCoA oxidase activity.

Clinical signs observed in the marmosets included ungroomed coats and localized reddening of the skin around the anus and legs which was likely caused by excretion of test substance in feces. One male exposed to 2,500 mg/kg bw/day experienced a 13% weight loss and had reduced activity and a hunched posture. Weight loss or decreased weight gain was observed in 2 males and 1 female exposed to 2,500 mg/kg bw/day. Peroxisome proliferation was not evident as indicated by a lack of dose-related increases in PCoA oxidase activity. There were no DINP-treatment related changes in estradiol or testosterone levels, hematology, blood chemistry, organ weights, urine composition, or microscopic findings. The authors identified a NOAEL of 500 mg/kg bw/day.

Administration of the positive control, clofibrate, did result in an approximate 100% increase in PCoA oxidase activity. Other effects in positive control animals included an increase in 11-hydroxylase activity in males, reduced weight gain, anemia, and a slight increase in relative and absolute kidney weight.

Pugh et al. (21) gavaged 2-year-old (prepubertal) cynomolgus monkeys (4/group) with 0 or 500 mg/kg bw/day DINP-1 in methylcellulose for 14 days (Web Table 9). According to Short et al. (22),

500 mg/kg bw/day is the maximum dose that can be absorbed by the monkeys. On day 15, the animals were sacrificed and the tissues were removed, weighed, and fixed in formalin for histopathological evaluation. Hematology, serum chemistry, and urine analysis were conducted. Peroxisomal proliferation was examined by measuring peroxisomal beta oxidation activity and replicative DNA synthesis. Gap junctional intercellular communication was determined in liver. There were no clinical signs or changes in body weight gain. A significant increase in blood neutrophil numbers and decrease in lymphocyte count were the only effects reported. There were no testicular or hepatic lesions and no effects on any of the systemic parameters examined.

Mode of Action

The renal neoplasia in male rats appears to be due to alpha-2-microglobulin nephropathy which is a mechanism not considered relevant to humans (23). However, an increased rate of nephropathy was seen in female mice exposed to 1,888 mg/kg bw/day which would not be consistent with the alpha-2-microglobulin mechanism. The Moore (18) study demonstrated liver tumors in rats only in the highest-dose males. Peroxisome proliferation in rats was observed at the highest dose in males and females, and the second highest dose in females but not males. No liver tumors were observed in either sex at the second highest dose level. In addition, no liver tumors were noted in the recovery groups. These results are consistent with a peroxisome proliferation mode of action for hepatic tumor induction. Unfortunately, peroxisome proliferation was assayed in mice only at the highest dose, and liver tumors were also observed at lower doses.

2.2 Toxicokinetics

Phthalate Moiety

Absorption

Rodents: Dermal

Dermal absorption of ^{14}C-DINP was studied in male Fischer 344 rats (24) in both conditioned (pre-treatment with non-labeled DINP) and non-conditioned skin. Following exposure, the dosed area was occluded. Under all conditions, the amount absorbed after 7 days ranged from 2 to 4% of the dose. Approximately 93−99% of the administered radioactivity was recovered at the site of application. Radioactivity in feces and gut of the exposed rats suggested some excretion via the biliary route. In *in vitro* studies comparing absorption of DEHP through human and rat skin (25), absorption through human skin was slower than through rat skin. Therefore, the dermal absorption rate of DINP is also expected to be slower through human versus rat skin. Studies conducted by Deisinger et al. (26) have demonstrated that dermal absorption of DEHP from a plasticized film is slower than dermal absorption of neat DEHP. It is reasonable to assume that these results apply to DINP.

Rodents: Oral

Oral absorption of ^{14}C-DINP (dose=2,500 mg/kg) was studied (27) in conditioned (pre-treatment with non-labeled DINP) and non-conditioned male albino rats. The rats were administered 0.5 mL of radiolabeled DINP by gavage and the dose was estimated at approximately 2,500 mg/kg bw by the Expert Panel based on the density of DINP and reported rat body weights. Within 72 hours, 85% of the administered dose was excreted in the feces, most within the first 24 hours. The rest of

the dose was excreted in urine (average of 12%) or remained in the tissues (trace amounts). Thus, the oral absorption was approximately 12%. In studies at Midwest Research Institute (28), male and female Fischer 344 rats were dosed orally either in a single or in 5 daily doses of 50, 150, or 500 mg/kg. At least 49% of the single low dose was absorbed. Absorption was decreased at the high single dose and at all doses following repeated exposures.

Biotransformation

Most of the ^{14}C collected in the urine of rats following a single oral dose of ^{14}C-DINP was in the form of phthalic acid or side-chain oxidation products of the monoester (MINP) (28). The relative amount of phthalic acid in the urine decreased at the high dose. The monoester itself, as well as the diester, was present in only trace amounts. In feces, 8 and 41% of the radioactivity was associated with the diester following administration of a low (50 mg/kg) or a high (500 mg/kg) oral dose of ^{14}C-DINP. This indicates saturation of metabolism at the high dose. The remainder of the fecal radioactivity was associated with the monoester or its side-chain oxidation products. Major metabolites in the liver were the monoester and its side-chain oxidation products. The same metabolites and phthalic acid were in testes. Fat contained the monoester and its oxidation products. Repeated exposures revealed similar metabolites in the tissues. In summary, in the rat, DINP was de-esterfied to the monoester, which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. Formation of oxidation products appeared to increase following the high dose or repeated dosing, while the hydrolysis to phthalic acid decreased (28).

Distribution

In albino rats receiving 0.5 mL of ^{14}C-DINP (approximately 2,500 mg/kg bw as estimated by the Expert Panel) after 5 days of dosing with the same amount of unlabeled DINP (27), no tissue studied had over 0.001% per gram of the administered dose after 3 days. The liver contained the most radioactivity on a total tissue basis. In male and female Fischer 344 rats receiving single or repeated oral doses of ^{14}C-DINP (28), radioactivity also cleared from the tissues rapidly, but analysis of tissues soon (within 1 hour) after the exposure indicated that the highest levels were in liver (4.7% of administered dose), kidneys (0.31%), and blood (1.62 %). Fat and testes contained small amounts of metabolites. No bioaccumulation occurred over 72 hours postdosing.

Excretion

The major routes of excretion for orally administered DINP in rats were urine and feces, with about equal amounts excreted by either route at low doses, but more excreted in feces at high doses (28). Repeated dosing caused no accumulation of DINP or its metabolites in blood or tissue, but resulted in increased formation and elimination of the monoester side-chain oxidation products (28).

Side Chain-associated Toxicokinetics

A major metabolite of DINP, the monoester, MINP, is further oxidized in the side chain.

2.3 Genetic Toxicity

DINP was tested in the Ames assay, Chinese hamster ovary (CHO) cells for chromosomal aberrations, the mouse lymphoma forward mutation assay (L5178Y TK -/- cell line), the primary rat hepa-

tocyte unscheduled DNA synthesis assay, and in an *in vitro* transformation assay using clone 1–13 of Balb/c-3T3 A31 mouse cells. Where appropriate, exogenous metabolic activation systems were used. Many of the assays were conducted according to GLP standards (29). Based on the results of these studies, DINP is not considered mutagenic in bacterial mutation assays and mammalian gene assays and is not clastogenic in one cytogenetic assay *in vitro* with CHO cells and in one *in vivo* assay with bone marrow cells of Fischer rats. This suggests that DINP is not genotoxic *in vivo* or *in vitro* (29)

Cell transformation studies give various results. The experimental conditions in the assays were not quite identical and the results are not inconsistent. Such positive results are in accord with those of well known peroxisome proliferators (29). DINP tested negative in the L5178Y mouse lymphoma mutation assay and the Balb/3T3 cell transformation assay (30). The data from the mutation and cell transformation assay were reviewed by OECD.

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 Data

Two rat studies evaluating prenatal developmental toxicity of DINP by gavage were reviewed, as were the developmental toxicity aspects of a two-generation study in rats. Prenatal developmental toxicity of isononyl alcohol, a primary metabolite, was also evaluated.

Using Sprague Dawley rats, Waterman et al. (31) (Table 7-7) evaluated DINP-1 (CAS No. 68515-48-0) and diisodecyl phthalate (DIDP) in 24 rats/group gavage treated with 0, 100, 500, and 1,000 mg/kg bw/day on gd 6−15. For both compounds, maternal toxicity was observed at 1,000 mg/kg bw/day expressed as reduced weight gain and food consumption. Fetal results were presented in terms of affected litters and fetuses. Skeletal variations were observed at the 500 and 1,000 mg/kg bw/day treatment levels. A dose-related increase in litters with lumbar ribs (25.0, 20.2, 54.2, and 78.3%) was observed, which was statistically significant at the high dose. A dose-related increase in the percent of fetuses with rudimentary lumbar ribs was observed (3.7, 5.4, 18.6, and 34.5%) with statistical significance attained in the mid- and high-dose groups. The percent of fetuses with supernumerary cervical ribs was statistically increased only in the high dose group (1.6, 1.6, 1.0, and 5.7%), but the 2.5-fold increase in litter incidence in the high-dose group was not statistically significant (12.5, 12.0, 8.3, and 30.4%). There was a dose-related increase in the percentage of litters with dilated renal pelves (0, 12.0, 16.7, and 26.1%) that attained statistical significance at the highest dose. The percentage of fetuses with dilated renal pelves was significantly increased at all treatment levels (0, 3.7, 4.0, and 4.5% at 0, 100, 500, and 1,000 mg/kg bw/day, respectively). The interpretation of results by Waterman et al. (31) included the maternal and developmental LOAEL of 1,000 mg/kg bw/day with a NOAEL of 500 mg/kg bw/day, with a conclusion that DINP "is not teratogenic or a selective developmental toxicant." The Expert Panel agreed with the authors selection of a maternal NOAEL; however, the Panel concluded that fetal data indicated that developmental toxicity was present at 500 mg/kg bw/day. The Expert Panel communicated to the study sponsor that there were improved and more current approaches to the analysis of fetal incidence data. The sponsor reanalyzed the fetal incidence data of interest using the GEE approach (32). This is a pup-level analysis within a model that uses the generalized estimating equation approach to account for the litter effect, i.e., the correlation between outcomes measured on pups within the same litter. The dose groups were tested pairwise versus controls; this gave similar results to a trend test based on a dose-response model fit with all dose levels up to that of interest included. These reanalysis data (33), presented below, are consistent with the Expert Panel's interpretation of the vertebral data.

Table 5: Mean Percent of Pups in Litter with Effect of Interest (significance level)

	Dose Group (mg/kg bw/day DINP)			
	0	***100***	***500***	***1,000***
Skeletal Variations	16.4	15.0 (0.91)	28.3* (0.05)	43.4** (0.001)
Visceral Variations	0.5	3.3 (0.08)	3.7 (0.09)	5.8* (0.04)
Rudimentary Lumbar Ribs	3.5	4.7 (0.18)	18.1** (0.001)	34.2** (0.001)
Supernumerary Cervical Ribs	1.6	1.5 (0.81)	1.0 (0.64)	5.5* (0.05)

**p≤0.05, **p≤0.01*

The GEE methodology could not be used to test the dilated renal pelves data because of the zero incidence in the control. Two solutions were tried. First, the zero incidence in the control was altered by changing one pup to "affected." Second, an alternative statistical analysis that considers litter effects was used (34). The results of these statistical tests show reasonable agreement and are shown below.

Table 6: Mean Percent of Pups in Litter with Dilated Renal Pelves (significance level using two methods)

	Dose Group (mg/kg bw/day DINP)			
	0	***100***	***500***	***1,000***
Renal Pelves	0.0	3.3	3.7	5.3
Sig. using added control event Sig. using nested analysis		(0.06) (0.18)	(0.10) (0.14)	(0.05)* (0.04)*

These results diminish the Expert Panel's initial concern that developmental toxicity effects based on dilated renal pelves may extend to lower doses. The Panel now concludes that the 100 mg/kg bw/day dose is a NOAEL.

Using the model-fitting approach, the sponsor also calculated benchmark doses (BMDs) at the 5 and 10% excess risk level, based on a multiplicative (or 'extra') excess risk function, for the rudimentary lumbar rib variant. At the 5% excess risk level, the BMD05 (and 95% lower confidence interval, estimated by a bootstrap approach) was 193 mg/kg bw/day (162 mg/kg bw/day). Benchmark doses were not calculated for other variants.

Hellwig et al. (35) (Table 7-6) evaluated the comparative developmental toxicity of a number of phthalates including three separate DINP materials. The material with CAS RN 68515-48-0 was identified as DINP-1, and two materials with CAS RN 28553-12-0, but from different production lines, were identified as DINP-2 and DINP-3. See Section 1.1 for description of chemical differences. Each DINP was administered by gavage in olive oil at 0, 40, 200, and 1,000 mg/kg bw/day to 8−10 sperm-positive Wistar females/group on gd 6–15. The dams were killed on gd 20 and implantation

sites were examined. Fetuses were weighed and examined for external malformations; half of the fetuses were examined for skeletal malformations and the other half for visceral malformations.

For DINP-1, maternal toxicity at the high dose consisted of reduced food consumption and increased relative liver (~6%; not statistically significant) and kidney (~13%) weights. There were no treatment-related effects on the number of live fetuses/dam or fetal weight. Developmental toxicity was evident at the highest dose by a statistically significant increase in percent fetuses/litter with variations (35.3, 41.5, 29.5, and 58.4% in the 0, 40, 200, and 1,000 mg/kg bw/day groups, respectively). These variations consisted of rudimentary cervical and/or accessory 14th rib(s). A modest increase in dilated renal pelves in the high dose group was also noted by the Expert Panel (8.9% of fetuses in 78% of control litters versus 16.8% of fetuses in 90% of treated litters). There were no maternal or developmental effects at 40 or 200 mg/kg bw/day.

For DINP-2, there was no statistically significant, dose-related evidence of maternal toxicity. However, a non-significant increase in relative liver (~5%) and kidney (~7%) weight did occur. The authors stated that developmental toxicity effects were limited to an increased fetal incidence of accessory 14th lumbar ribs at the high dose. The Expert Panel also noted a modest increase in dilated renal pelves in the high-dose group (8.9% of fetuses in 78% of control litters versus 10.6% of fetuses in 80% of treated litters).

For DINP-3, maternal toxicity was present at the high dose, expressed as reduced mean body weight gain and reduced food consumption during some portions of the treatment period. Relative liver weights (~11%) were also increased at the high dose and a non-significant increase in relative kidney weights (~9%) was observed. Developmental toxicity was evidenced by a statistically significant increase in percent fetuses/litter with variations at the highest dose (35.3, 29.6, 39.5, and 60.7% in the 0, 40, 200, and 1,000 mg/kg bw/day groups, respectively). Specific types of developmental toxicity observed in the high-dose group at increased incidences included skeletal retardation (unossified or incompletely ossified sternebrae) and skeletal variations (rudimentary cervical and/or accessory 14th rib[s]). The authors assumed that low incidences of soft tissue variations (hydroureter), visceral malformations affecting the urogenital tract (agenesis of kidneys and ureters), and skeletal malformations affecting the long bones (shortened and bent humerus and femur) observed at the high dose were treatment-related. The Expert Panel also observed that dilated renal pelves were slightly increased in the high dose group (8.9% of fetuses in 78% of control litters versus 16.7% of fetuses in 100% of treated litters). A maternal and developmental NOAEL of 200 and LOAEL of 1,000 mg/kg bw/day, respectively, were identified by the Expert Panel and were in concurrence with effect levels identified by authors.

In a two-generation reproductive toxicity study, postnatal weight gain was examined in pups of F0 and F1 dams exposed to DINP in feed at concentrations of 0, 0.2, 0.4, and 0.8% during mating (0, 182–197, 356–397, and 696–802 mg/kg bw/day) , gestation (0, 143–146, 287–288, 555–560 mg/kg bw/day), and lactation (0, 254–285, 539–553, and 1,026–1,129 mg/kg bw/day) (2, 36). Complete details of the experiment are included under Section 4. Weight gain for the F1 pups was reduced by DINP; males of the high-dose group were affected on postnatal day (pnd) 0, pups of the mid- and high-dose groups were affected on pnd 7 and 14 and all dose groups were affected by pnd 21. Weight gain of the F2 young during lactation was reduced in primarily the mid- and high-dose

groups; females of the low-dose group were affected on pnd 7, females of the mid- and high-dose groups were affected on pnd 4, 7, 14, and 21, and males of the mid- and high-dose groups were affected on pnd 7, 14, and 21. Postnatal sexual maturation was not examined. The Expert Panel identified a developmental NOAEL of 0.2%.

Hellwig and Jackh (37) evaluated the prenatal development toxicity of two types of isononyl alcohol in Wistar rats. The type 1 alcohol consisted of isomers with a medium degree of branching and 16% isodecanol and the type 2 alcohol consisted of isomers with a low degree of branching. On gd 6–15, 10 rats/group were gavaged with the alcohols in water with 0.005% Cremophor EL at concentrations of 0, 1, 5, and 10 mmol/kg bw/day which the authors stated equated to 0, 144, 720, and 1,440 mg/kg bw/day. A supplementary study was later conducted in 10 rats/group exposed to 0 or type 1 or type 2 alcohol at 7.5 mmol/kg bw/day (~1,080 mg/kg bw/day). In the main and supplementary studies, two groups of 10 control rats each were administered water or vehicle. Fetuses and dams were evaluated on pnd 20.

For the type 1 isononyl alcohol, complete maternal lethality occurred in the 1,440 mg/kg bw/day group and 1 of 10 dams died at the 1,080 mg/kg bw/day dose. Clinical signs/symptoms were reported to have been observed in a dose-related manner in dams that received 720 mg/kg bw/day and the two higher doses. A significant reduction in maternal body weight gain and increased fetal resorptions were observed in the 1,080 mg/kg bw/day group. Numerical reductions in fetal body weight occurred in the 720 mg/kg bw/day group, but were not statistically significant. Malformations that primarily affected the heart were significantly increased in fetuses and litters of the 1,080 mg/kg bw/day group. Skeletal variations (cervical ribs) or retardations (reduced ossification of sternebrae) were increased in the 1,080 mg/kg bw/day group (statistically significant) and 720 mg/kg bw/day group.

Maternal mortality was also observed in the 1,440 mg/kg bw/day group treated with type 2 isononyl alcohol with death occurring in 3/10 dams. Maternal signs and symptoms were observed in the three highest doses. Non-significant reductions in body weight gain and marginal increases in resorption rates were observed in dams exposed to 720 mg/kg bw/day and higher. Fetal body weights were significantly reduced in the 1,440 mg/kg bw/day group. The authors reported that malformations were not significantly increased, but also reported that there were significant increases in fetuses with skeletal variations and retardations (reduced ossification) from the high-dose group (1,440 mg/kg bw/day). It is not clear which type of variation was increased. The authors stated that the number of fetuses with malformations (primarily affecting the thoracic vertebrae) was elevated in the 1,080 mg/kg bw/day group.

Data available in abstract form (38) and a study in press (39) report that oral exposure of SD rats to DINP at 750 mg/kg bw/day on gd 14 through pnd 3 resulted in reproductive malformations in male offspring (7.7%). The data were not available to the Panel for evaluation, therefore we merely note the existence of the abstract.

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

The reproductive toxicity of DINP-1 (CAS 68515-48-0) was reported by Waterman et al. (36) (Table 7-8). This report describes the results of both a one-generation and a two-generation study. In the two-generation study, SD rats (30/group) were given DINP in the diet at 0.2, 0.4, or 0.8% (w/w) for 10 weeks prior to mating, and through gestation and lactation. The study sponsor estimated doses of 0, 165, 331, and 665 mg/kg bw/day for males during premating, 0, 182, 356, and 696 mg/kg bw/day for females during premating, 0, 146, 287, and 555 mg/kg bw/day for females during gestation, and 0, 254, 539, and 1,026 mg/kg bw/day for females during lactation (2). Body weights and food consumption were measured weekly. After 10 weeks of pre-mating exposure, males and females were paired 1:1 within dose groups, and the females were monitored for vaginal sperm for up to 3 weeks. F_0 males were treated until after the delivery of their last litter and then killed and necropsied; females were killed after weaning their litters. Litters were culled to yield four male and four female pups per litter on pnd 4. At weaning, one male and one female from each litter were selected to grow to adulthood for mating; the remaining animals were examined externally, then killed and discarded without necropsy. F_1 animals were fed the same diet as their parents throughout the rest of the study. As adults, the F_1 rats were mated within dose groups for 3 weeks after confirmation of vaginal sperm. Estimated doses for the F_1 rats were 0, 189, 379, and 779 mg/kg bw/day for males during premating, 0, 197, 397, and 802 mg/kg bw/day for females during premating, 0, 143, 288, and 560 mg/kg bw/day for females during gestation, and 0, 285, 553, and 1,129 mg/kg bw/day for females during lactation (2). Dams were allowed to litter and raise young until pnd 1, at which time they and their litters were killed, the adults were necropsied, and their organs weighed and preserved. Sperm measures were not made; testes were fixed in Bouin's.

Weight gain for the F_0 rats was unaffected by DINP consumption until pnd 14 and 21, when the high-dose dams weighed less than the controls. In the F_0 rats, absolute liver weight was increased in the females of the mid dose group, and in both males and females of the high-dose group. Absolute kidney weight was increased in the two highest dose groups for males and in all exposed female groups. Absolute reproductive organ weights (testes, epididymis, prostate, and seminal vesicles) were unchanged by DINP. At the high dose, absolute left ovary weight was reduced versus control, although the weight of the right ovary was unchanged; the reduced weight of the left ovary appears anomalous. Fertility indices for the F_0 mating were unchanged by DINP; this includes litter size, measures of mating, number of dead offspring, and sex ratio. Weight gain was reduced in both F_1 and F_2 pups and these results are discussed in detail in Section 3. Body weights during the mating of the F_1 generation were variably reduced at the high dose by ~8−10%. When the F_1 animals were mated within dose groups as adults, DINP caused no change in ability to mate or bear young, litter size, pup weight or viability, or sex ratio. At the F_1 adult necropsy, absolute liver weight was increased in the high-dose females and absolute kidney weight was increased in the high-dose males. Absolute reproductive organ weights were unchanged. Livers appeared more eosinophilic in all treated F_0 and F_1 rats; kidneys of the mid- and high-dose males had minimal-to-mild pelvic dilation. Testes were microscopically equivalent to controls.

The NOAEL for reproduction appears to be at least 0.8%, 665–779 mg/kg bw/day for males and 696–802 mg/kg bw/day in females. There is no LOAEL for reproduction, as there were no reproductive toxicities observed. The weight gain inhibition at 0.2% seen by pnd 21 in F_1 pups suggests a developmental LOAEL of 143–285 mg/kg bw/day during gestation through lactation. Using benchmark dose methodology (BMD) the authors reported that 250 mg/kg bw/day represented the 95% lower confidence limit for a 5% reduction in body weight.

In the one-generation study, groups of 30 male or female animals consumed DINP-1 in the feed at 0, 0.5,1.0, or 1.5% w/w for 10 weeks prior to mating (36). Study sponsor-estimated doses for males during the premating period were reported at 301–591, 622–1,157, and 966–1,676 mg/kg bw/day in the low- to high-dose groups respectively (2). Doses for the low to high dose females were 363–624, 734–1,169, and 1,114–1,694 mg/kg bw/day during premating, 377–404, 741–796, and 1,087–1,186 mg/kg bw/day during gestation, and 490–923, 1,034–1,731, and 1,274–2,246 mg/kg bw/day during lactation. The females were exposed throughout mating, gestation, and lactation until pnd 21. The males were killed immediately after the mating period. At necropsy, the liver, kidneys, and reproductive organs were removed and weighed.

In this one-generation study, body weight gain was reduced at 1 and 1.5% DINP. There were no effects on indices of mating or fertility (litter size), and a reproductive NOAEL of 1,000 mg/kg bw/day was identified. At necropsy, absolute liver and kidney weights were increased in both sexes at all dose levels. Testes absolute weights were increased at the high dose; ovary weights were reduced by ~30% at the highest dose. Offspring viability was reduced in the high-dose group. Offspring body weight gain at pnd 21 was reduced at all dose levels, as in the two-generation study.

Mode of Action

Several studies have examined the ability of selected phthalate esters to compete with labeled estradiol (E2) for binding to the estrogen receptor (ER). Sources of ER protein included rat uterine cytosol (40), rainbow trout hepatic cytosol (41), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (42, 43) and rainbow trout ERs expressed in yeast (44). Tritiated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. Selected phthalate esters have been examined in a number of *in vitro* gene expression assays systems. The assays have used stably transfected cells (40), transiently transfected cells (40, 41), yeast based assays (40, 44-46) and vitellogenin induction in rainbow trout hepatocyte cultures (44). DINP exhibited no activity in an *in vitro* assay that measured binding of phthalates to estrogen receptors (40) and in an assay of estrogen-induced gene expression (46). The assays did not include the addition of esterases or lipases to metabolize DINP to MINP. *In vivo* assays demonstrated that DINP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats treated with up to 2,000 mg/kg bw for 4 days. (40). There were no studies located on anti-androgenic activity, but an abstract and study in press have reported that gestational DINP exposure demasculinizes male pups (38, 39). Thyroid and estrogen serum levels were unaffected in adult marmosets at doses as high as 2,500 mg/kg bw/day for 13 weeks (20).

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

DINP, a complex substance of branched, predominantly C-9 isomers, is a general-purpose plasticizer for flexible PVC with a broad range of applications. It is widely used in the toy, construction, and general consumer product markets. It has limited use in food packaging. It is not used for medical applications.

The very limited monitoring data for DINP in air, drinking water, and surface and ground waters have usually yielded negative results (i.e., concentrations below detection limits). In the few studies of food and infant formula, the levels of DINP have not been quantitated or have been at or below the detection limit (0.01 mg/kg) (4-6). Occupational exposures to phthalates are reported to be below 1 mg/m^3 during the production of phthalates and 2 mg/m^3 during the manufacture of plasticized PVC (1).

Toys represent a unique childhood exposure to DINP since it is a major plasticizer used in children's toys (7, 8, 11). DINP content has been measured at 15.1–54.4% dry weight in 31 toys (7), and 3.9–44% dry weight in 27/42 toys (11). Using pneumatic piston impaction in saliva simulant, DINP migration ranged from 1.0–48.4 µg/11cm^2/hour, but there was no correlation between DINP content and migration rate (7). *In vivo* extraction has been studied using adult volunteers as surrogates for children (7, 12). In a comparison of extraction rates in 10 adults mouthing toys versus laboratory simulation, ratios varied from 22.9 to 72.6 (mean 39.5) for 5 toys (7). RIVM (12) tested two different pieces of one toy and a controlled disk using 20 adults and also found higher extraction *in vivo*. Using a 2-day parent observation study of 42 children, ages 3–36 months, mean mouthing times have been generated per age category with ranges from 0 minutes/day in older children to 171.5 minutes/day in the 6–12 month age group (see Section 1). These mouthing times have been used to model DINP exposure by several groups using a variety of assumptions as indicated in Table 7. Dermal exposure may also occur, but has not been studied specifically in children.

Table 7: Toy Exposure Estimates for Children Aged 3−12 Months

	Estimated Intake Level (µg/kg bw/day)			
Agency	***Mean***	***95th Percentile***	***99th Percentile***	***Maximum***
RIVM*	6.53–14.4	20.7–39.7	39.8–77.3	70.7–204
CPSC	5.7	94.3	–	–
Health Canada**	44	73.9***	173.5***	320

** Exposure range for 3−6 month-old and 6−12 month-old children; range includes results from 3 specimens tested.*

*** Calculated with mouthing times for teethers and other objects intended for mouthing.*

**** Results using Monte Carlo simulations in children aged 3–6 months.*

5.1.1.1 Utility of Data to the CERHR Evaluation

The Expert Panel believes it is reasonable to assume, based on the physicochemical characteristics of DINP and existing, though limited monitoring data, that *general population* exposure to DINP (excluding children) is expected to be lower than DEHP, which is estimated at 3–30 µg/kg bw/day (15). Children may be in the upper portion of the population range because of their physiologic differences compared to adults. The Panel also believes that some small children are likely to have exposures exceeding the general population estimates due to non-dietary ingestion from mouthing toys and other objects that contain DINP. Current models of non-dietary oral exposure predict that older infants and toddlers may incur exposures up to one order of magnitude higher than the upper limit of exposure expected in the general population.

5.1.2 General Biological and Toxicological Data

General Toxicity:

There were no human data identified. Animal data consisted of two subhuman primate studies and four rodent studies. In a 13-week gavage study, adult marmosets treated with up to 2,500 mg DINP/kg bw/day (CAS number not specified) experienced weight loss or decreased weight gain, but there was no biochemical evidence of peroxisome proliferation or microscopic changes in organs examined, including testes and epididymides (20). Prepubertal (2-year-old) cynomolgus monkeys that were gavaged with 500 mg/kg bw/day for 2 weeks experienced changes in white blood cell numbers, but there were no testicular lesions or hepatic effects, including peroxisome proliferation (21). A 21-day repeat-dose dietary study in adult rats focused on peroxisome-proliferating effects in liver, and a LOAEL of 607 (F) and 639 (M) mg/kg bw/day was identified; a NOAEL was not established (16). Effects included increased liver weight at all dose levels in males and in females, dose-related enzymatic evidence of peroxisome proliferation, and alterations in hepatic cytoplasmic basophilia and eosinophilia at the high dose. With the exception of severe unilateral atrophy in one male of the mid-dose group, testicular effects were not observed in males dosed with up to 2,195 mg/kg bw/day. Moderate testicular atrophy was observed in one DEHP-positive control that received 1,084 mg/kg bw/day.

There were three chronic (2-year) dietary studies reviewed that were of similar design and included toxicopathologic evaluation at several times during the study. Two studies were conducted using 6-week-old F 344 rats (17, 18), while the third used 6-week-old B6C3F_1 mice (19). Lesions in testes or female reproductive organs were not observed in any of the 3 studies, with the highest doses tested being 885 mg/kg bw/day in rats and 1,888 mg/kg bw/day in mice. Non-neoplastic liver lesions and/or changes in liver enzyme activity occurred at doses of 152 mg/kg bw/day and greater in rats, and 1,560 (M) to 1,888 (F) mg/kg bw/day in mice. Biochemical evidence of peroxisome proliferation was noted throughout the study in both sexes of rats in the Moore (18) study that were dosed with 733 (M) and 885 (F) mg/kg bw/day. Female rats receiving 442 mg/kg also had biochemical evidence of peroxisome proliferation when evaluated at the end of the study. Peroxisome proliferation was noted in high-dose mice (1,560 [M]; 1,888 [F] mg/kg bw/day), but the mid- and low-dose groups were not examined. The Lington et al. (17) rat study evaluated peroxisome proliferation by electron microscopy and saw none in two rats per sex per dose group at the end of the study. Non-neoplastic kidney lesions and changes in urinary excretion were seen in rats exposed to 307 mg/kg bw/day and higher, and in mice dosed with 1,560 (M) and 1,888 (F) mg/kg bw/day.

Indications of anemia, such as reductions in red blood cell numbers and hemoglobin levels, were seen in rats exposed to 307 mg/kg bw/day and higher. Hepatic neoplasia was observed only in male rats exposed to 733 mg/kg bw/day and in mice exposed to 336 (F) and 742 (M) mg/kg bw/day and higher. Renal neoplasia was only observed in male rats of the highest dose group (733 mg/kg bw/day). The apparent qualitative difference in liver and renal effects (i.e., tumors vs hepatotoxicity) in the rat studies may reflect differences in the range of doses tested.

There were no toxicity studies with inhalation exposure.

Mode of Action:

The renal neoplasia in male rats appears to be due to alpha-2-microglobulin nephropathy which is a mechanism not considered relevant to humans (23). However, an increased rate of nephropathy, was seen in female mice exposed to 1,888 mg/kg bw/day which would not be consistent with the alpha-2-microglobulin mechanism. The Moore (18) study demonstrated liver tumors in rats only in the highest-dose males. Peroxisome proliferation in rats was observed at the highest dose in males and females, and the second highest dose in females but not males. No liver tumors were observed in either sex at the second-highest dose level. In addition, no liver tumors were noted in the recovery groups. These results are consistent with a peroxisome proliferation mode of action for hepatic tumor induction. Unfortunately, peroxisome proliferation was assayed in mice only at the highest dose, and liver tumors were observed at lower doses.

Toxicokinetics.

There are no human data. DINP was orally administered to adult male albino rats at doses of 50, 150, or 500 mg/kg bw/day. It is metabolized by pancreatic lipases in the lumen of the gut and rapidly absorbed (49%) as the monoester and rapidly excreted via urine and feces with no accumulation in tissues (28). Dermal absorption of DINP is slow (<4% in 7 days) in rats (24). Dermal absorption of DINP through human skin is expected to be lower than rat skin based on results of an *in vitro* study conducted with DEHP (47). There is evidence for excretion via the biliary route based on radioactivity in feces and GI tract of rats dosed dermally with ^{14}C-DINP. There are no inhalation studies available.

Genetic Toxicity.

DINP tested negative in experiments of mutagenicity and clastogenicity including the Ames, Chinese hamster ovary cell, and rat bone marrow chromosomal aberration, mouse lymphoma mutation, unscheduled DNA synthesis, and Balb/c-3T3 mouse cell transformation assays (29, 30).

Table 8: Summaries of NOAELs and LOAELs and Major Effects in General Toxicity Studies

Protocol & Study DINP Tested and Doses (mg/kg bw/day)	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day) & Effects*	*Major Effects at Higher Doses*
13-week repeat-dose gavage study in adult marmosets 16–25 months of age, 1–2 per sex/group Doses: 0, 100, 500, 2,500 DINP type not specified (20)	500	2,500 ↓Weight gain or weight loss No peroxisomal proliferation No microscopic findings in organs	No higher doses in study
2-week repeat-dose gavage study in male prepubescent cynomolgus monkeys 2 years of age, 4/group Doses: 0, 500 DINP-1 (21)	Not determined	500 Changes in neutrophil and lymphocyte counts No testicular lesions No liver effects including peroxisomal proliferation	No higher doses in study
21-day repeat-dose dietary study in young adult Fischer 344 rats 6 weeks of age at start of study, 5 rats per sex/group Doses: (M) 0, 639, 1,192, 2,195; (F) 0, 607, 1,193, 2,289 Mixture of different DINP types (16)	None	M: 639, F: 607 ↑ Liver weight ↑ Peroxisomal proliferation (M) ↑ Kidney weight	↑ Liver weight, and peroxisomal proliferation ↑ Kidney weight ↑Testes weight No testicular lesions
2-year repeat-dose dietary study in Fischer 344 rats 6-week-old at beginning of study, 110 per sex/group Doses: (M) 0, 15, 152, 307; (F) 0, 18, 184, 375 DINP-1 (17)	M: 15 F: 18	M: 152, F: 184 Hepatic effects ↑Liver weight Mononuclear cell leukemia ↑Kidney weight	Hepatotoxicity ↑Liver weight No testicular lesions Mononuclear cell leukemia Anemia ↑Kidney weight and excretion changes No peroxisomal proliferation
2-year repeat-dose dietary study in Fischer 344 rats 6 weeks of age at start of study, 70–85 per sex/group Doses: (M) 0, 29, 88, 359, 733; (F) 0, 36, 109, 442, 885 DINP-1 (18)	M: 88 F: 109	M: 359, F: 442 Nephrotoxicity Excretion changes Anemia ↑ Liver weight, peroxisomal proliferation (F) Mononuclear cell leukemia ↑Kidney weight	Hepatic & renal neoplasia at high dose (M) Anemia Nephrotoxicity ↑ Liver weight and peroxisomal proliferation Mononuclear cell leukemia ↑Kidney weight No testicular lesions
2-year repeat-dose dietary study in $B6C3F_1$ mice 6 weeks of age at beginning of study, 70/sex/group Dose: (M) 0, 90, 276, 742 or 1,560; (F) 0, 112, 336, 910, 1,888 DINP-1 (19)	M: 276 F: 112	M: 742, F: 336 Liver neoplasia ↑Liver weight (M) ↓Kidney weight (M)	Liver neoplasia, hepatocyte staining variations, peroxisomal proliferation, and nephrotoxicity (F) at highest doses ↑Liver weight ↓Kidney weight (M) No testicular lesions

5.1.2.1 Utility of Data to the CERHR Evaluation

There are adequate subchronic and chronic data available in rats and mice and adequate subchronic data in primates to assess general toxicity by the oral route, including liver and kidney effects (16-19). No effects have been noted in the male or female reproductive system, although these studies were not designed to fully assess this system.

Toxicokinetic data consist of oral and dermal studies in rodents. The data permit the Panel's conclusion that dermal absorption is slow; oral absorption is rapid for the monoester formed by lipases in the gut. Dose-related kinetics of absorption across species is not known. DINP and its metabolites are rapidly excreted via urine and feces with no accumulation in tissues.

5.1.3 Developmental Toxicity

There were no human studies located for Expert Panel review.

Two published prenatal developmental toxicity studies in rats were available for DINP (31, 35). The protocols for the two studies were similar and included dosing of dams by gavage on gd 6–15 with sacrifice and evaluation of fetuses on gd 20–21. Developmental toxicity was also noted in both a one-generation and a two-generation toxicity study. The effects on pup body weight are discussed below and summarized in Table 9; the reproductive effects are described in Section 5.1.4.

Hellwig et al. (35) performed their studies in Wistar rats (10/group) at doses of 0, 40, 200, and 1,000 mg/kg bw/day. Although sample size (n=10) was small, the aggregate of their work can logically be considered to be three separate studies of DINP. There was a degree of consistency across all studies. Effects were only observed at the highest dose. Relative kidney and liver weights were slightly increased in dams of the highest dose group (5–13%), but statistical significance was erratic. Fetal viability and body weight were unaffected in all three studies. Skeletal variations (rudimentary cervical ribs, accessory 14th ribs) were numerically increased with each DINP with the number of affected fetuses per litter significantly higher than controls in two instances. There was a tendency to see dilated renal pelves at the highest dose; in one study agenesis of kidneys and ureters was assumed by the authors to be DINP-related. Skeletal (shortened and bent humerus and femur) malformations were also observed in the high-dose group of this study. It is clear that organ effects are associated with kidney and the skeletal system. For maternal and developmental effects, a NOAEL of 200 and a LOAEL of 1,000 mg/kg bw/day were identified by the Expert Panel for each DINP and are in concordance with effect levels identified by Hellwig et al. (35).

The prenatal toxicity study of Waterman et al. (31) was more informative than the Hellwig study from the standpoint of number of rats per test group and completeness of data reported. Waterman et al. (31) tested DINP-1 in Sprague-Dawley rats (25/group) at doses of 0, 100, 500, or 1,000 mg/kg bw/day. Maternal toxicity at the highest dose consisted of decreased food intake and weight gain. The authors presented and analyzed effects on offspring as percent affected fetuses and percent affected litters. Waterman et al. (31) interpreted their results as indicating a LOAEL for maternal and developmental toxicity at 1,000 mg/kg bw/day and a NOAEL of 500 mg/kg bw/day. The Panel concurred with the maternal NOAEL, but concluded there was developmental toxicity at the 500 mg/kg bw/day dose. As discussed in Section 3.2, the Panel advised the study sponsor that there were more recent and improved methods for the statistical analysis of fetal incidence data. The

sponsor performed appropriate reanalyses that the Panel reviewed and found to be consistent with the Panel interpretation of skeletal variations. The Panel concludes there is a NOAEL in the study at 100 mg/kg bw/day. The BMD estimated a 5% excess risk level was 193 mg/kg bw/day (95% LCL=162 mg/kg bw/day) for rudimentary lumbar ribs, as provided by the study sponsor (33).

The Panel noted that developmental toxicity was observed in the prenatal rat studies by Waterman and Hellwig. In the study by Waterman, the urinary system was a target of effect as noted by a modest increase in dilated renal pelves at the 1,000 mg/kg dose. While only a mild increase in dilated renal pelves was observed in the three Hellwig et al. studies, in one instance more severe renal effects (hydroureter, agenesis) were seen. In studies by Waterman et al. (31) and Hellwig et al. (35), the skeletal system was the target for effect as observed by an increased incidence of cervical ribs and accessory 14th (lumbar) ribs. These studies also evaluated the closely related phthalate DIDP where the same target organs were identified. An increase in cervical ribs and lumbar ribs was observed at the common dose of 1,000 mg/kg bw/day in the two studies. While effect on lumbar ribs was more pronounced, the effect on cervical ribs is of greater toxicological concern. Cervical ribs are seen infrequently in controls, and their presence may indicate a disruption of gene expression. There is evidence that cervical ribs may interfere with normal nerve function and blood flow.

Differences in NOAELs between the Waterman et al. (31) and Hellwig et al. (35) studies, 100 and 200 mg/kg bw/day respectively, may be due to rat strain, and certainly to dose selection.

The two-generation reproductive study by Waterman et al. (36) suggests an adverse effect on weight gain in pups during the perinatal and pre-weaning period of life. Developmental landmarks of reproductive tract development, identified as a sensitive target with other phthalates, were not examined. F_1 mean pup body weight was significantly reduced on pnd 0 in males at 0.8% DINP (555 and 1,026 mg/kg bw/day during gestation and lactation, respectively, as calculated by study sponsors). On pnd 7 and 14, mean male and female pup body weights were significantly reduced at 0.4% (287 and 539 mg/kg bw/day during gestation and lactation, respectively) and 0.8%, and by pnd 21, mean male and female body weights were reduced at all dose levels. In the F_2 generation, mean female pup body weights were significantly reduced at 0.4 and 0.8% on pnd 4, 7, 14, and 21 and at 0.2% (143 and 285 mg/kg bw/day during gestation and lactation, respectively) at pnd 7. Mean male pup body weights were significantly reduced at 0.4 and 0.8% at pnd 7, 14, and 21. The LOAEL for developmental effects was therefore identified as 0.2% (143–285 mg/kg bw/day during gestation through lactation) by the Expert Panel.

Studies with 2 isononyl alcohols, differing in degree of branching, demonstrated clinical signs and symptoms in pregnant rats at doses of 720 mg/kg bw/day and higher (37). Table and text discrepancies in dose values and reported effects at the higher dose levels were noted. Toxicity was more severe with type 1 isononyl alcohol, the alcohol that had a higher degree of branching. Maternal mortality was seen at the highest dose (1,440 mg/kg bw/day) with both alcohols and in the type 1 alcohol at 1,080 mg/kg bw/day. Fetal malformations and/or variations occurred at 1,440 mg/kg bw/day and at 1,080 mg/kg bw/day. Slight effects that may be associated with treatment were observed at 720 mg/kg bw/day. A dose of 144 mg/kg bw/day was without effect for both isononyl alcohols.

Table 9: Summary of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol & Study	*NOAEL (mg/kg bw/day)* *[Benchmark dose] ED_{05} in mg/kg bw/day*	*LOAEL (mg/kg bw/day) and Effects*		*Developmental Effects Observed at Higher Dose Levels*
		Maternal	*Developmental*	*Developmental*
Prenatal gavage study in Wistar rats. 10/group/study received 0, 40, 200, or 1,000 mg/kg bw/day on gd 6–15. Dams and pups examined in late gestation. DINP-1, DINP-2, and DINP-3 (35)	200 Maternal & Developmental	1,000 ↑Kidney and liver weights.	1,000 ↑Cervical and lumbar ribs-all. ↑Urogenital and skeletal malformation with DINP-3.	N/A
Prenatal gavage study in Sprague-Dawley rats. 25 per group received 0, 100, 500, or 1,000 mg/kg bw/day on gd 6–15. Dams and pups examined in late gestation. DINP-1 (31)	500 (Maternal) 100 *** (Developmental) [MLE(95%LCL): 193 (162) for lumbar ribs]	1,000 ↓Weight gain.	500 ↑ Fetuses with vertebral variations.	↑ Fetuses and litters with visceral variations (mainly dilated renal pelves). ↑ Fetuses and litters with lumbar ribs. ↑ Fetuses with cervical ribs.
Two generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% from 10 weeks prior to mating (0, 182–197, 356–397, and 696–802 mg/kg bw/day) through gestation (0, 143–146, 287–288, and 555–560 mg/kg bw/day and lactation 0, 254–285, 539–553, and 1,026–1,129 mg/kg bw/day during lactation)**. DINP-1 (36)*	None [250 (95% LCL) for decreased pup weight gain]	143−285 ↑ Mild histological liver changes in F_0 and F_1. ↑Kidney weight in F_0.	143−285 ↓ Weight gain on pnd 21in F_1. ↓ Weight gain on pnd 7 in F_2 females.	↓ Weight gain on pnd 0 (males), 7, 14, and 21 in F_1. ↓ Weight gain on pnd 4 (female), 7, 14, and 21 in F_2.

** Only maternal and developmental effects were listed in this table. Reproductive and male systemic effects are listed in Table 10.*
*** Range of doses for F_1 and F_2 dams.*
**** NOAEL selected by Expert Panel is lower than study author's selection*

5.1.3.1 Utility of Data to the CERHR Evaluation

There are adequate data available in rats to determine that prenatal oral exposure to DINP-1 results in developmental toxicity. The results of the Waterman et al. (31) and the Hellwig et al. (35) studies were remarkably consistent with respect to DINP-1. In both studies, exposure to DINP-1 resulted in increases in lumbar and cervical ribs. In addition, the effective dose levels were similar. Hellwig et al. (35) identified a LOAEL of 1,000 mg/kg bw/day and a NOAEL of 200 mg/kg bw/day with a sample size of 10/group. The Panel identified an effect level of 500 mg/kg bw/day from the Waterman et al. (31) study (sample size of 25/group) and 100 mg/kg bw/day level represented a NOAEL. In addition, Hellwig et al. (35) showed some similarities among the three DINPs in that each resulted in an increase in lumbar and cervical ribs. It is clear that the urinary and skeletal systems are target organs where developmental toxicity is observed. The data from the two-generation dietary study are sufficient to demonstrate an effect on postnatal growth, with a LOAEL of 143–285 mg/kg bw/day and no NOAEL. The reduced growth is consistent in both studies. Neither prenatal study extended dosing into the late gestation period which has been shown to be a critical window of development for other phthalates. In addition, the study designs did not allow for assessment of postnatal sexual maturation. The issue of late gestational exposure was addressed in a two-generation reproductive toxicity study reviewed in Section 5.1.4. Confidence in the isononyl alcohol study is limited due to table and text discrepancies in dose values and reported effects at the higher dose levels. The study is adequate to ascribe maternal and developmental toxicity at these higher doses and to assume the lowest dose was without effect.

5.1.4 Reproductive Toxicity

Structural and functional reproductive effects were examined in one- and two-generation feeding studies in rats that included in utero exposure during the entire duration of pregnancy (36). In the one-generation dose range finding study, rats were administered dietary levels of 0, 0.5, 1.0, or 1.5% DINP and in the two-generation study, rats were administered dietary levels of 0, 0.2, 0.4, or 0.8% DINP. In the two-generation study, reproductive parameters including mating, fertility, and testicular histology were unaffected in both generations at the highest dose (0.8%; 665–779 and 696–802 mg/kg bw/day in males and females, respectively) and this dose was identified as the reproductive NOAEL. Developmental effects were observed, including decreased pup weight gain (most marked on pnd 21). The effects on pup weight gain are discussed in greater detail under Section 5.1.3. Histologic effects included mild hepatic eosinophilia in both sexes of parental rats in all dose groups of both generations and dilated renal pelves in F_1 parental males of the mid- and high-dose groups. The results of the study are consistent with the one-generation pilot study that was previously conducted. In the one-generation study, fertility was unaffected in male and female rats exposed to dietary DINP concentrations as high as 1.5% (966–1,676 and 1,114–1,694 mg/kg bw/day in males and females, respectively). The findings of these studies indicate that male and female rat fertility and structure of reproductive organs are unaffected by exposure to DINP at a maternal dose of 555–1,129 mg/kg bw/day during gestation and lactation, respectively, and adult exposure to concentrations as high as 1,676 mg/kg bw/day in males and 1,694 mg/kg bw/day in females.

Mode of Action

DINP exhibited no activity in an *in vitro* assay that measured binding of phthalates to rat uterine cytosolic estrogen receptors (40) and in an assay of estrogen-induced gene expression (46). The assays did not include the addition of esterases or lipases to metabolize the DINP to MINP. *In vivo* assays demonstrated that DINP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (40). There were no studies located on anti-androgenic activity. Thyroid and estrogen serum levels were unaffected in adult marmosets at doses as high as 2,500 mg/kg bw/day for 13 weeks (20).

Table 10: Summary of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies

Protocol & Study	***NOAEL (mg/kg bw/day)***	***LOAEL (mg/kg bw/day) and Effects***		***Reproductive Effects Observed at Higher Dose Levels***
		Repro	***Systemic***	
Two-generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% (Males: 0, 165–189, 331–379, and 665–779 mg/kg bw/day, Females: 0, 182–197, 356–397, and 696–802 mg/kg bw/day**) from 10 weeks prior to mating through gestation and lactation. DINP-1 (36)*	665–779 (M); 696–802 (F) (Reproductive) None (Systemic)	No effects on reproductive structure or function.	M: 165–189; F: 182–197 ↑ Mild liver effects in F_0 and F_1. ↑Kidney weight in F_0 females.	None

** Only effects in parental rats are listed. Developmental effects are listed in Table 9.*

*** Doses during the premating period-Combined for F_0 and F_1 rats.*

5.1.4.1 Utility of Data to the CERHR Evaluation

The data are sufficient to indicate that DINP exposures are not associated with detectable effects on reproductive function. The studies did demonstrate consistent effects on the liver (weight and histology) and kidney (weight). Given the constraints of the study design, the data demonstrate no likely reproductive toxicity at doses up to 779(M)–802(F) mg/kg bw/day in the two-generation study or at 1,676(M)–1,694(F) mg/kg bw/day in the one-generation study. However, the studies did not assess endpoints of reproductive development shown to be sensitive with other phthalates.

5.2 Integrated Evaluation

DINP is a complex substance of branched, predominantly C-9 isomers. There are no human data from which to assess the health effects associated with DINP exposure; studies of DINP toxicity are limited to laboratory animals. In the absence of human data and barring evidence to the contrary, it is assumed that the effects observed in laboratory animals are relevant to humans.

Based upon the physicochemical similarities between DINP and DEHP and on limited DINP monitoring data, general population exposures to DINP are expected to be lower than those to DEHP which are estimated at 3−30 μg/kg bw/day. It is reasonable to presume that humans would be exposed primarily through the oral route. Although data are scant, the ingestion of DINP through food does not appear to be common. Children may be exposed to higher levels of DINP (up to 10–100 fold higher) than adults because infants and small children mouth toys and other articles that contain DINP that can migrate into saliva and be swallowed. DINP is not used in medical devices, therefore intravenous exposure does not occur.

Dermal absorption of DINP is slow in rats. DINP administered orally to rats is metabolized by gut lipases to the monoester, which is rapidly absorbed. DINP and its metabolites are rapidly excreted in urine and feces, with no indication of accumulation in tissues with repeated (5×) daily doses. At low doses, approximately equal amounts of the DINP-derived material are excreted in urine and feces, with the urinary metabolites consisting of the monoester and its oxidation products, while the feces contain those metabolites plus the diester. There are no toxicokinetic studies in humans, but *in vitro* studies comparing the dermal uptake of other phthalates in human and rat skin suggest that dermal uptake of DINP in humans would be negligible.

Oral exposure to DINP has been shown to cause liver and kidney toxicity in adult rats and mice, but not in marmosets. The liver effects are generally consistent with those associated with peroxisome proliferation. Liver tumors have been noted in adult male rats exposed to 733 mg/kg bw/day, in female mice exposed to 336 mg/kg bw/day, and in male mice exposed to 742 mg/kg bw/day. Kidney tumors were noted in male rats, but these tumors are associated with a mechanism that is not believed relevant to humans (alpha-2-microglobulin). However, the increased incidence of nephropathy seen in female mice exposed to 1,888 mg/kg bw/day is not consistent with the alpha-2-microglobulin mechanism.

The developmental studies available include examination of effects of prenatal exposure on prenatal development, as well as a limited assessment of postnatal developmental effects in one- and two-generation reproductive studies. The prenatal studies provide consistent results and are sufficient to establish that oral exposure to DINP causes fetal skeletal variations (lumbar and cervical ribs) and in some cases, urinary tract effects (hydroureter). The Panel was confident that 500 mg/kg bw/day was an effect level, and 100 mg/kg bw/day was a NOAEL. For one study, sponsors estimated a BMD for a 5% excess risk level of 193 mg/kg bw/day with a 95% lower confidence limit of 162 mg/kg bw/day. For the second developmental toxicity study, the Expert Panel identified a developmental NOAEL of 200 mg/kg bw/day. In addition, the results of the one- and two-generation dietary reproductive toxicity studies demonstrated a consistent reduction in mean pup body weights during lactation at doses as low as 143–285 mg/kg bw/day (doses during gestation and lactation)

and a NOAEL could not be identified. This effect level is similar to that obtained in the more robust prenatal study, although the effects are different and a similar mode of action is not assumed.

There is evidence that isononyl alcohol, a primary metabolite of DINP, is a developmental and maternal toxicant at high (~1,000 mg/kg) oral doses in rats. These doses appear to be greater than the doses of DINP that are associated with developmental toxicity, suggesting that effects at lower doses are probably associated with the monoester. The Panel does acknowledge there are no data to permit a judgment about an interactive effect between the alcohol and monoester metabolite.

Reproductive performance and histological effects on gonads and accessory sex organs were assessed in one- and two-generation dietary studies. Parental doses of up to 0.8% in feed (665–779 [M] and 696–802[F] mg/kg bw/day) did not affect fertility or sex organ histology in either the F_0 or F_1 male or female pups. A 13-week gavage study in adult marmosets resulted in no evidence of microscopic testicular changes at doses that did adversely affect body weight gain (2,500 mg/kg bw/day). Testicular lesions were not observed in prepubertal cynomolgus monkeys that were gavaged for 2 weeks with 500 mg/kg bw/day, reportedly the maximum dose that can be absorbed by the monkeys. Chronic 2-year studies in rats and mice gave no gross or histologic evidence of effects on testes or ovaries at doses that did cause liver and kidney effects and other clinical signs of toxicity. Thus, the data are sufficient to conclude that neither the reproductive organs nor fertility are affected by extended oral exposure to DINP. However, the Panel noted that some endpoints which are sensitive to other phthalates (i.e., preputial separation and nipple retention) were not evaluated in the two-generation study. The Panel is aware that additional data on reproductive tract development are being developed, but as yet only abstracts are available for review. The Panel also notes that the target organs in studies with adult rats, liver and kidney, are also target organs in developmental and multigeneration studies. This increases the Panel's confidence that these effects are real, and that different organ system susceptibilities between adults and young are unlikely.

5.3 Expert Panel Conclusions

DINP is used in toys, construction, and general consumer products. Although data are scant, exposure through food appears to be lower than for DEHP. Therefore, the Expert Panel believes that adult exposure to DINP will not exceed levels of 3–30 μg/kg bw/day, the estimates derived for DEHP. Exposures to DINP are likely to be below this level, but the Panel cannot quantitate how far below. Occupational exposures could occur through inhalation and dermal contact. Limited studies of occupational exposures suggest that inhalation exposure is below 1 mg/m^3 during production of DINP and below 2 mg/m^3 during production of PVC. Although estimates of dermal exposure are not available, the Expert Panel is confident that dermal exposure would not result in significant absorption into the body. Children could be exposed to DINP by eating contaminated food. However, DINP has not been detected in a limited survey of infant formula. By analogy, the Expert Panel believes that children's exposure to DINP via food will not exceed those levels estimated for DEHP. Additional exposure to children may occur due to mouthing of toys and other objects that contain DINP. Current models of non-dietary oral exposure predict that older infants and toddlers may incur exposures up to one order of magnitude higher than the upper limit of exposure expected for adults.

The toxicology database is sufficient to determine that oral maternal exposure to DINP can result in developmental toxicity to the conceptus. In rats, two prenatal developmental studies have shown effects on the developing skeletal system and kidney following oral exposure to DINP. The NOAELs for these studies were 100–200 mg/kg bw/day. In addition, developmental toxicity was noted in an oral two-generation reproductive toxicity study in rats. In this study, effects on pup growth were noted. These effects may be due to prenatal and/or lactational exposures to DINP. The LOAEL for the study was 143–285 mg/kg bw/day and a NOAEL was not identified. Based on the results of the toxicology studies, oral exposure to pregnant women and oral exposure to children should be examined. The Expert Panel has minimal concern for unborn children due to ambient maternal exposure to DINP. Based on estimates of exposure to DINP in toys and other objects that children may mouth, the Expert Panel has low concern for potential health effects in children. The Expert Panel cannot judge the potential health effects in unborn children following maternal occupational exposures due to the lack of toxicology data following inhalation exposures and the lack of occupational exposure information.

The oral prenatal developmental toxicity studies and the oral two-generation reproductive toxicity study have shown no effects on the reproductive system in rats. The NOAEL for reproductive toxicity is 665–779 (M) and 696–802 (F) mg/kg bw/day. The Expert Panel noted that some endpoints of reproductive development that have been shown to be sensitive with other phthalates were not assessed in the two-generation study of DINP, and therefore the Panel has only moderate confidence in the NOAEL. The Expert Panel has minimal concern about DINP resulting in reproductive toxicity in humans.

5.4 Critical Data Needs

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

Experimental studies.
Since some relevant endpoints (i.e., nipple retention) were lacking in many of the studies reviewed, uncertainties would be reduced if this additional information were gathered. The Expert Panel recommends a sequential approach for future studies that would focus on obtaining the most critical information first; subsequent studies would be dependent upon the results of the initial study. The Panel further recognized that data gathering should be an iterative process and that recommendations may change as initial tiers of data are gathered. The Expert Panel recommends that the following sequential steps be considered.

1) Conduct a perinatal developmental study in orally exposed rats that addresses 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 exposed through development. Although a two-generation reproductive toxicity study has evaluated some relevant endpoints, the recommended study would provide greater certainty about the lack of such effects with DINP. If DINP does affect these endpoints and the effective dose levels are of possible human health concern, then the Expert Panel recommends that the following study (2) be conducted.

2) Conduct a perinatal developmental study with oral exposure in a non-rodent species. There are species differences in the developmental toxicity associated with other phthalates. The developmental effects of DINP have only been examined in the rat. Therefore, there is some uncertainty whether other species would exhibit similar responses and whether the rat is an appropriate model for assessing potential human risk.

Human exposure.
Human exposure to DINP has not been well studied; there are no reports of levels in biological materials (blood, urine, etc.), and the environmental data consist primarily of estimates.

Patterns of use, expected environmental levels, and vulnerability of exposed population groups should dictate decisions about measuring DINP in environmental media. For example, determining DINP exposures in young children is of highest priority, based on the use patterns and vulnerability described above. Workers producing PVC products are a second priority.

Collection of biological samples de novo should be accompanied by environmental measurements to provide information on exposure sources. Existing biological samples should be utilized where available if they can provide useful information about exposure.

Although information about exposure of young children is a critical data need, manufacturers of children's toys should be polled to determine if their products will continue to contain DINP in the future. If so, an estimate of the DINP content should be made by the manufacturer and confirmed by independent studies. Salivary extraction of DINP and better estimates of mouthing behavior, especially within the potentially highest risk group of 3–12 month-old children, using data from more children, should be carried out.

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7.0 TABLES

Table 7-1: DINP, General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Body Weight*	*Organ Weight***	*Liver Effects*	*Hematology*	*Other*
Fischer 344 Rat BIBRA 1985 (1)	Subchronic study : 21 days. Rats (~41–43 days old) were fed diets with 0, 0.6, 1.2, or 2.5% DINP and then sacrificed and necropsied. Peroxisome proliferation was studied by electron microscopy examination of liver and measurement of peroxisomal enzyme activity.	5	0					
		5	639(M) 607(F)	NE	↑Li, Ki	↑11-OH(M) and 12-OH(M). ↑Protein.	NA	↓Serum Ch, Tg(M).
		5	1,192(M) 1,193(F)	↓	↑Li, Ki	↑PCoA(M), 11-OH and 12-OH(M). ↑Protein. Histological changes.	NA	↓Serum Ch, Tg(M). ↑Serum Tg(F).
		5	2,195(M) 2,289(F)	↓	↑Li, Ki, Te	↑PCoA, 11-OH and 12-OH. ↑Peroxisomes. ↑Protein. Histological changes.	NA	↓Serum Ch, Tg(M). ↑Serum Tg(F). No dose-related testicular lesions.
	↑A group of positive control rats was administered DEHP at 1.2%.	5	*1,084(M)* *1,063(F)*	↓	↑Li, Ki	↑PCoA, 11-OH and 12-OH. ↑Peroxisomes. ↑Protein.	*NA*	↓Serum Ch, Tg(M). No dose-related testicular lesions.

*Dose measured in mg/kg bw/day.
**Organ to body weight ratio.
↑=Statistically Significant Increase
↓=Statistically Significant Decrease
NA=Not Analyzed
NE=No Effects
F=Female
M=Male
Te=Testes
Li=Liver
Ki=Kidney
11-OH=11-Hydroxylase
PCoA=Palmitoyl-CoA Oxidas
12-OH=12-Hydroxylase
Tg=Triglyceride
Ch=Cholesterol

Table 7-2: DINP, General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Body Weight*	*Organ Weight***	*Liver Effects*	*Hematology*	*Other*
Fischer 344 Rat Lington et al. 1997 (2)	Chronic study : 2 years. 6-week-old rats were fed diets with 0, 0.03, 0.3, and 0.6% DINP-1 for 2 years. 10 rats/sex/group were sacrificed and necropsied at 6, 12, and 18 months and the rest were killed and necropsied at the end of the study. Hematology, serum chemistry, and urinalysis were evaluated every 6 months. Peroxisome proliferation was examined microscopically in 2 rats/sex/group at 24 months.	110	0					
		110	15(M) 18(F)	NE	NE	NE	NE	NOAEL
		110	152(M) 184(F)	↓(M; 18–24 mo)	↑Li, Ki (6–24 mo)	Hepatic lesions (24 mo) ↑SGOT (M; 6–12 mo) ↑SGPT (M; 24 mo) ↑MNCL.	NE	↓Survival (F).
		110	307(M) 375(F)	↓(M; 12–24 mo)	↑Li, Ki (6–24 mo) ↑Sp (24 mo) ↑Ad (24 mo)	Hepatocyte enlargement (6–24 mo) and lesions (24 mo) ↑SGOT (M; 6–18 mo) ↑SGPT(M; 6 &18 mo) ↑MNCL. No evidence of peroxisomal proliferation	↓RBC, Hb, Hct (M; 24 mo)	↓Survival (F). ↑Urine volume (M; 6–24 mo) ↑Urine K and glucose (M; 6–18 mo) No evidence of testicular damage.

*Dose measured in mg/kg bw/day
**Organ to body weight ratio
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

NE=No Effects
F=Female
M=Male
Mo=Month

Ad=Adrenal
Ki=Kidney
Li=Liver
Sp=Spleen

SGOT=Serum Glutamic Oxaloacetic Transaminase
SGPT=Serum Glutamic Pyruvic Transaminase
MNCL=Mononuclear Cell Leukemia
RBC=Red Blood Cell
Hb=Hemoglobin
K=Potassium

Table 7-3: DINP, General Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose*	*Body Weight*	*Organ Weight***	*Liver Effects*	*Hematology*	*Other*
F344 Rats Moore 1998 (3)	Chronic study : 2 years. 6-week old rats were fed diets with 0, 500, 1,500, 6,000, or 12,000 DINP. 5 rats/sex/dose were killed on weeks 1, 2, and 13; 15 rats/sex/dose were killed at 79 weeks; and 55 rats/sex/group at 104–106 weeks. Clinical evaluations (hematology, serum chemistry, and urinalysis) were conducted every 26 weeks. Peroxisome proliferation was examined in 5 rats/sex only in the controls and high dose group during weeks 1, 2, and 13 and in 3–5 rat/sex in the control, 359–442 mg/kg bw/day group, and high-dose group at week 104.	85	0					
		70	29.2(M) 36.4(F)	NE	NE	NE	NE	NE
		70	88.3(M) 109(F)	NE	NE	NE	NE	NOAEL
		85	359(M) 442(F)	NE	↑Ki (wk 79-104) ↑Li (wk 1-104)	↑PCoA (F, wk 104) ↑ASAT, ALAT (wk 52, 78, 104)	↑Anemia	Kidney lesions (M, wk 79–104) ↑ Serum urea (wk 26–104) MNCL (wk 104)
		85	733(M) 885(F)	↓ (wk 9–104)	↑Ki (wk 79-104) ↑Li (wk 1-104)	↑PCoA (wk 1–104) Lesions (wk 2–104) Neoplasia (M, wk 79–104) ↑ASAT, ALAT (wk 52, 78, 104) ↓ASAT, ALAT (F, wk 26)	↑Anemia	↓Survival (M) ↑Serum urea (wk 26–104) ↑Urine vol with ↓Cl, Ca, K, Cre (M, wk 104) Kidney lesions (wk 79–104) Kidney neoplasm (M, wk 104) MNCL (wk 104) No testicular effects
	A group of 55 rats/sex was exposed to the high dose for 78 weeks and sacrificed at 105–106 weeks to study recovery effects.*	55	637(M) 774(F)	↓(F)	↑Ki(F)		NE	↑Urine vol with ↓ Cre(M) MNCL (wk 104) Kidney lesions and neoplasm (M)

*Only effects observed by week 104 listed
**Organ to body weight ratio
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

NE=No Effects
F=Female
M=Male

Ki=Kidney
Li=Liver
wk=Week
vol=Volume

ALAT=Alanine aminotransferase
ASAT=Aspartate aminotransferase
MNCL=Mononuclear Cell Leukemia
PCoA=Palmitoyl-CoA Oxidase

Ca=Calcium
Cl=Chloride
Cre=Creatinine
K=Potassium

Table 7-4: DINP, General Toxicity, Mice

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Body Weight*	*Organ Weight****	*Liver Effects*	*Hematology*	*Other*
B6C3F$_1$/ CrlBR mice Moore 1998 (4)	Chronic study: 2 years. 6-week-old mice were fed diets with 0, 500, 1,500, 4,000, and 8,000 ppm DINP. 15 mice/dose/ sex were evaluated and sacrificed at 79 weeks and 55 mice sex/group at 105–106 weeks. Clinical evaluations (hematology, serum chemistry, and urinalysis) were conducted every 26 weeks. Peroxisome proliferation was examined in 5 mice/sex in the highest dose group and controls during the midpoint and end of study.	70	0					
		70	90.3(M) 112(F)	NE	NE	NE	NE	NOAEL (F)
		70	276(M) 336(F)	NE	NE	↑Neoplasia (F)	NE	NOAEL (M)
		70	742(M) 910(F)	↓ (wk 1–104)	↓Ki(M), ↑Li(M) (wk 79-104)	↑Neoplasia (M)	NE	NE
		70	1,560(M) 1,888(F)	↓ (wk 1–104)	↓Ki (M), ↑Li (wk 79-104)	↑Neoplasia and non-neoplastic changes ↑Serum ASAT, ALAT (M) ↑PCoA (wk 79–104)	↓WBC (wk 26–98)	↓Survival (M) ↑Nephropathy(F) ↑Serum protein (M, week 104) ↑Urinary vol with ↓Na, Cl, K (week 52–104) No effects on testicular histology
	A group of 55 mice/sex was exposed to the high dose for 78 weeks and sacrificed at 105–106 weeks to study recovery effects.**	55	1,377(M) 1,581(F)	↓(M)	↓Ki(M)	↑Neoplasia	NE	NE

*Dose measured in mg/kg bw/day.
**Only effects observed by week 104 listed
***Organ to body weight ratio
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

NE=No Effects
F=Female
M=Male
wk=Week
vol=Volume

Ki=Kidney
Li=Liver
WBC=White Blood Cell

ALAT=Alanine aminotransferase
ASAT=Aspartate aminotransferase
PCoA=Palmitoyl-CoA Oxidase

Cl=Chloride
K=Potassium
Na=Sodium

Table 7-5: DINP, General Toxicity, Marmosets

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Body Weight*	*Organ Weight*	*Histopathology*	*Hematology*	*Chemistry*	*Other*
Marmoset Hall et al. 1999 (5)	Subchronic study: 13 Weeks. Male and female marmosets (16–25 months old) were gavaged with DINP in 1% methylcellulose and 0.5% Tween. Clofibrate was used as a positive control. Parameters evaluated at sacrifice included estradiol and testosterone concentrations, biochemical evidence of peroxisomal proliferation, and organ weights and histopathology.	1–2	0						
		2	100	NE	NE	NE	NE	NE	
		2	500	NE	NE	NE	NE	NE	
		2	2,500	decrease[a]	NE	NE	NE	No change in PCoA	Ungroomed coat and reddening of the skin. Thin appearance, hunched posture and reduced activity in one male.[a]
		1–2	500 Clofibrate	decrease[a]	↑ Ki to body weight ratio[a]	NE	↑ Anemia	↑ PCoA ↑ 11-OH	

[a]Dose measured in mg/kg bw/day.
*Efects were not statistically significant
↑=Statistically Significant Increase
NE=No Effects
11-OH = Lauric acid 11-hydroxylase activity
PCoA=Palmitoyl-CoA Oxidase activity

Table 7-6: DINP Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Number*[b]	*Dose*[d]	*Maternal Effects*	*Fetal Effects*
Wistar Rat Hellwig et al. 1997 (7)	Prenatal developmental toxicity study. Three types of DINP[a] manufactured by different processes were administered in oil by gavage on gd 6–15. Dams were weighed on gd 0, 6, 10, 15, and 20 and sacrificed on gd 20. Maternal uteri were weighed, corpora lutea were counted and implantation sites examined. Fetuses were weighed and examined for gross external malformations. Half of the fetuses were examined for visceral malformations and the other half for skeletal malformations.	9	0		
		9–10	40	↑Kidney to bodyweight ratio in DINP-2. ↓Implantation sites/ litter in DINP-2.	↓Live fetuses/litter in DINP-2.
		8–10	200	NE	NE
		9–10	1,000	↑Kidney to bodyweight ratio in DINP-1. ↑Liver to bodyweight ratio in DINP-3.	↑Fetuses/litter with cervical ribs in: DINP-1: 11 fetuses/5 litters vs 0 fetuses DINP-2: 4 fetuses/4 litters vs 0 fetuses DINP-3: 12 fetuses/7 litters vs 0 fetuses. ↑Fetuses/litter with lumbar ribs in: DINP-1: 37 fetuses/10 litters vs 0 fetuses DINP-2: 10 fetuses/5 litters vs 0 fetuses DINP-3: 34 fetuses/8 litters vs 0 fetuses. ↑Fetuses/litter with hydroureter in DINP-3: 2 fetuses/8 litters vs 4 fetuses/3 litters. ↑Fetuses/litter with malformations in: DINP-3 (7.3 vs 4.3%).[c] ↑ Dilated renal pelves in DINP-1,2,3.

[a] (1) CAS RN: 68515-48-0; (2) CAS RN: 28553-12-0; (3) CAS RN: 28553-12-0 (2 and 3 by different manufacturing process).
[b] Number of litters examined per type of DINP.
[c] Skeletal and visceral malformations (humorous, femur, kidney, and ureter); not statistically significant.
[d] Dose measured in mg/kg bw/day.
↑= Statistically Significant Increase
↓= Statistically Significant Decrease
NE= No Effect

Table 7-7: DINP, Developmental Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Number**	*Dose***	*Maternal Effects*	*Fetal Effects*
Sprague-Dawley Rat Waterman et al. 1999 (8)	Prenatal developmental toxicity study. DINP-1 administered in oil by gavage on gd 6–15. Sacrificed on gd 21. Dams weighed on gd 0, 6, 9, 12, 15, 18, and 21. Maternal uterus and ovaries were weighed, corpora lutea were counted and implantation sites examined. Fetuses were weighed, sexed, and examined for gross external malformations. Half of the fetuses were examined for visceral malformations and the other half for skeletal malformations.	24	0		
		25	100	NE	↑ % Fetuses with dilated renal pelves (3.7 vs 0%).
		24	500	NOAEL	↑ % Fetuses with dilated renal pelves (4 vs 0%). ↑ % Fetuses with lumbar ribs (19 vs 4%).
		23	1,000	↓ Weight gain (transient). ↓ Food intake (transient).	↑% Litters with dilated renal pelves (26 vs 0%). ↑ % Fetuses with dilated renal pelves (4.5 vs 0%). ↑ % Litters with lumbar ribs (78 vs 25%). ↑ % Fetuses with lumbar ribs (35 vs 4%). ↑ % Fetuses with cervical ribs (6 vs 2%).

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

Table 7-8: DINP, Reproductive Toxicity, Rats

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Effects*
CD Rats Waterman et al. 2000 (9)	Two generation reproductive toxicity study. DINP administered in feed for 10 weeks prior to mating at 0, 0.2, 0.4, or 0.8%. Males treated until delivery of last litter and females through gestation to lactation. Breeding pairs housed together for 3 weeks; body weight and food intake was measured weekly. One male and female from each litter reared to adulthood and remaining pups were examined and discarded.	30	0	
		30	165/182/146/254	↑Kidney weight in F_0 females. ↓Weight gain in F_1 pups.
		30	331/356/287/539	↑Liver weight in F_0 females. ↑Kidney weight in F_0 males and females. ↓Weight gain in F_1 pups.
		30	665/696/555/1,026	↓Weight gain in F_0 females (pnd 14, 21). ↑Liver weight in F_0 males and females. ↑Kidney weight in F_0 males and females. ↓Left ovary weight in F_0 females. ↓Weight gain in F_1 pups. No effect on weights of male reproductive organs, testicular histology, or litter size, mating, offspring survival, and sex ratio.
	One male and female F_1 rat/litter continued to receive the same doses as parental rats and were then mated within dose groups during adulthood.	30	189/197/143/285	NE
		30	379/397/288/553	↓F_2 pup weight gain during lactation.
		30	779/802/560/1,129	↓Body weight in F_1. ↑Liver weight in F_1 females and kidney weight in F_1 males. ↓F_2 pup weight gain during lactation. No effects on mating, fertility, litter size, pup weight, survival, or sex ratio, sex organ weights or testicular histology.

*Doses (in mg/kg bw/day) in males during premating/females during premating/females during gestation/females during lactation.
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

Table 7-9: DINP, General Toxicity, Monkeys

Species, Strain, and Source	*Experimental Regimen*	*Animal Number/ Sex*	*Dose**	*Body Weight*	*Organ Weight*	*Histopathology*	*Hematology*	*Chemistry*	*Other*
Cynomolgus monkey Pugh et al. 2000 (6)	Subacute study: 2 weeks. 2-year-old males were gavaged with DINP in 0.5% methylcellulose for 2 weeks and then sacrificed and necropsied.	4	0						
		4	500	NE	No effects on organ to body-weight ratios	No testicular or hepatic lesions.	↑Neutrophils ↓Lymphocytes	NE	No peroxisome proliferation No effects on gap junctional intracellular communication

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

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

Center For The Evaluation Of Risks To Human Reproduction

PUBLIC COMMENTS ON THE PHTHALATES EXPERT PANEL REPORTS

Appendix III

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December 11, 2000

Michael D. Shelby, Ph.D.
Director, CERHR
National Toxicology Program B3-09
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Dear Dr. Shelby:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sincerely,

James S. Benson
Executive Vice President
Technology & Regulatory Affairs

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

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

Attachment 1

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

Type of Study: GLP

Table 1. Study Design

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

*Dose had to be decreased to 600 mg/kg

Total Number of Animals: 112 pups

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

Observations: Daily

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

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

Sperm Count: At 90 day

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

Necropsy: Gross observations

Clinical Pathology: None

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

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

DEC - 7 2000

December 1, 2000

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council *Good Chemistry Makes It Possible*

Ms. Kate Rawson
Editor, The Rose Sheet
5550 Friendship Blvd., Suite One
Chevy Chase, MD 20815-7278

Dear Sir/Madam:

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

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

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

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

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

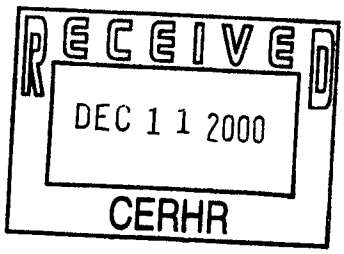
In summary, independent scientists, international government bodies and phthalate producers have conducted extensive studies about the safety, health and environmental effects of phthalates. This substantial body of scientific data does not present credible evidence that people are harmed by phthalates.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

Sincerely yours,

Courtney M. Price/HCS

Courtney M. Price
Vice President, CHEMSTAR

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



December 11, 2000

Michael D. Shelby, Ph.D
Director, CERHR
NIEHS/NTP B3-09
111 Alexander Drive, Bldg. 101
P.O. Box 12233
Research Triangle Park, NC 27709-2233

Re: Evaluations of Seven Phthalate Esters

Dear Dr. Shelby:

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

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

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

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

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

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

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

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

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

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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:

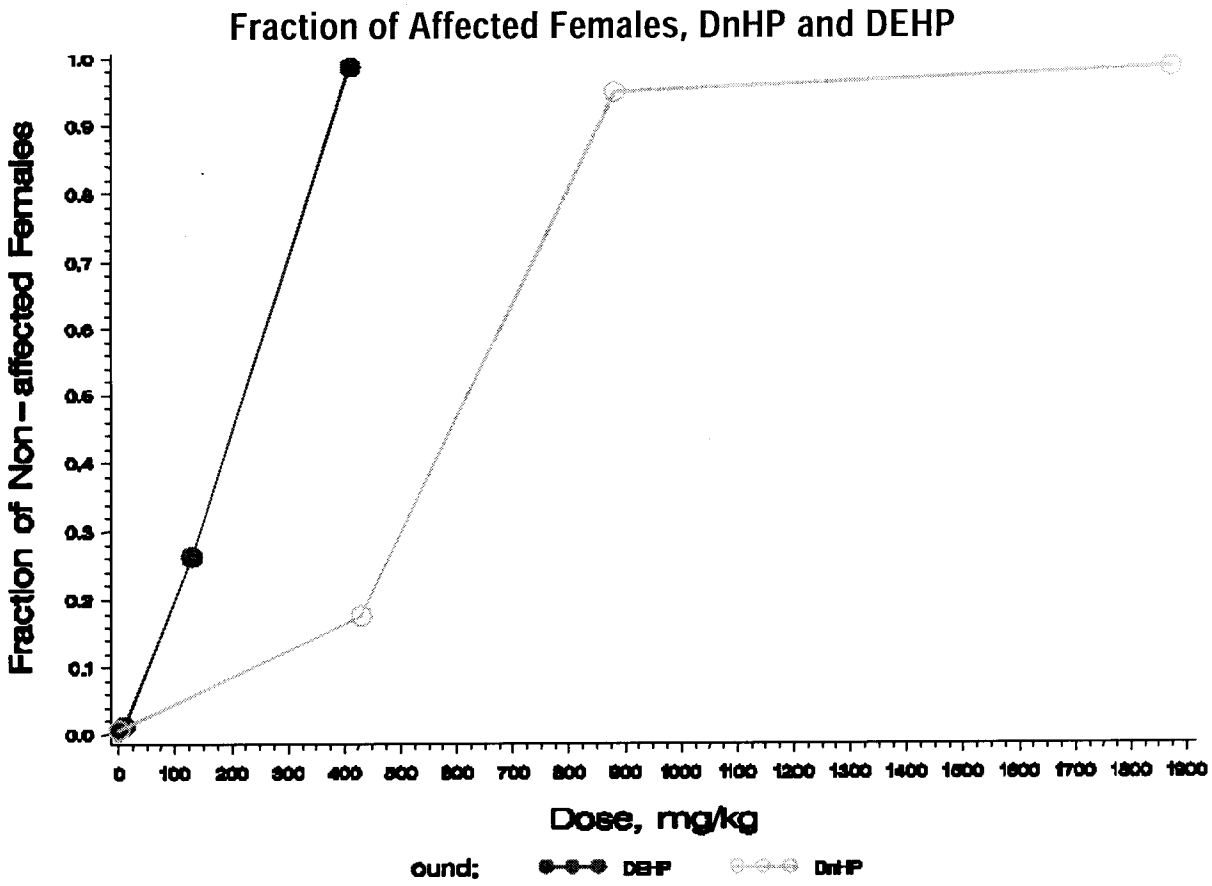
Compound	Dose (mg/kg)	Number Affected	Sample Size	Fraction Affected
DnHP	0	0	39	0.0
	430	3	17	0.18
	880	18	19	0.95
	1870	16	16	1.0
DEHP	0	0	40	0.0
	10	0	20	0.0
	130	5	19	0.26
	410	18	18	1.0

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

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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

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.

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

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

B. Fetal Based Data

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

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

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

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

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

III. Conclusion

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

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ATTACHMENT 7

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

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

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

General Comment

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

Specific Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

April 11, 2001

American Chemistry Council *Good Chemistry Makes It Possible*

Michael D. Shelby, Ph.D.
Director, CERHR
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John A. Moore, D.V.M.
Principal Investigator, CERHR
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18000 Diagonal Road
Alexandria, VA 22314

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).

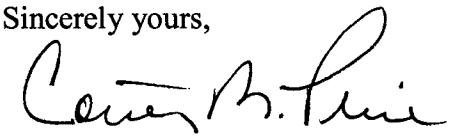
bulbocavernosus muscles in the 1500 mg/kg/day group. Weights of testes, ventral prostate, epididymis and bulbourethral glands were unaffected.

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,



Courtney M. Price
Vice-President, CHEMSTAR

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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.

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

JAN 0 9 2001

Dear Dr. Shelby,

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

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

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

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

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

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

Sincerely,

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

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Toll Free: (877) 817-1988
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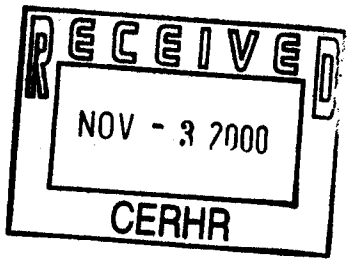

October 30, 2000

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

Dear Dr. Shelby:

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

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

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

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

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

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

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

Thank you very much for your attention to this matter.

Sincerely,

Richard Wiles
Vice President for Research

Jane Houlihan
Senior Analyst

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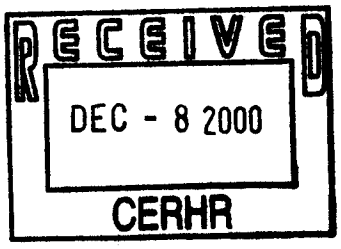

December 7, 2000

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

Dear Dr. Shelby:

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

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

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

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

Sincerely,

Richard Wiles
Vice President for Research

Jane Houlihan
Senior Analyst

Attachment

References

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

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

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

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)

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

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

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

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

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

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

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

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

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

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

a

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

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

References

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

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

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HEALTH CARE WITHOUT HARM

THE CAMPAIGN FOR ENVIRONMENTALLY RESPONSIBLE HEALTH CARE

December 8, 2000

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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.

HCWH AT THE CENTER FOR HEALTH, ENVIRONMENT AND JUSTICE
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Of course, the extent to which DEHP from the tube is actually absorbed systemically is another question and was not examined in this study. Latini was prompted to study this question because of a hypothesized connection between DEHP exposure and bronchopulmonary dysplasia.

Animal models:

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

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

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

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

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

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

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

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

Biotransformation:

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

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

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[10] Abbott D, Hearn J. Physical, hormonal, and behavioral aspects of sexual development in the marmoset monkey, Callithrix jacchus. J Reprod Fertil 53(1):155-166, 1978.
[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:

Mrs. Beverly Smith

21 Rolling Hill Dr.

Fairport, N.Y. 14450

ECE

OCT 19 2000

LAB OF

RE: 60 day public comment period on phthalates.

I read the article in Science News of Sept. 2, 2000 - page 152-154 on phthalates with much interest. My nephew was born with pyloric stenosis and an inguinal hernia which made it necessary for him to have intravenous feeding and blood transfusions during surgery. Now an adult desiring marriage and children he has only one incompletely developed testicle.

Appendix III

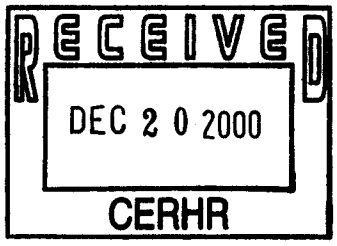
His sterility fits the pattern described in this article.

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

Sincerely,

Beverly Smith

Kemikalieinspektionen



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