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

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT

on

DI ISONONYL PHTHALATE

OCTOBER, 2000 **NTP-CERHR-DINP-00**

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
Telephone: 703-838-9440

A Report of the CERHR Phthalates Expert Panel:

Name	Affiliation
Robert Kavlock, PhD (Chair)	National Health and Environmental Effects Research Laboratory/USEPA, Research Triangle Park, NC
Kim Boekelheide, MD, PhD	Brown University, Providence, RI
Robert Chapin, PhD	NIEHS, Research Triangle Park, NC
Michael Cunningham, PhD	NIEHS, Research Triangle Park, NC
Elaine Faustman, PhD	University of Washington, Seattle, WA
Paul Foster, PhD	Chemical Industry Institute of Toxicology, Research Triangle Park, NC
Mari Golub, PhD	California Environmental Protection Agency, Sacramento, CA
Rogene Henderson, PhD	Lovelace Respiratory Research Institute, Albuquerque, NM
Irwin Hinberg, PhD	Health Canada, Ottawa, Ontario, Canada
Ruth Little, ScD	NIEHS, Research Triangle Park, NC
Jennifer Seed, PhD	Office of Toxic Substances/USEPA, Washington, DC
Katherine Shea, MD, MPH	Duke University, Durham, NC
Sonia Tabacova, MD, PhD	Food and Drug Administration, Rockville, MD
Rochelle Tyl, PhD, DABT	Research Triangle Institute, Research Triangle Park, NC
Paige Williams, PhD	Harvard University, Boston, MA
Timothy Zacharewski, PhD	Michigan State University, East Lansing, MI

With the Support of CERHR Staff:

NTP/NIEHS

Michael Shelby, PhD	Director, CERHR
Christopher Portier, PhD	Acting Associate Director, NTP
Gloria Jahnke, DVM	Technical Consultant
Lynn Goldman, MD	Technical Consultant

Sciences International, Inc.

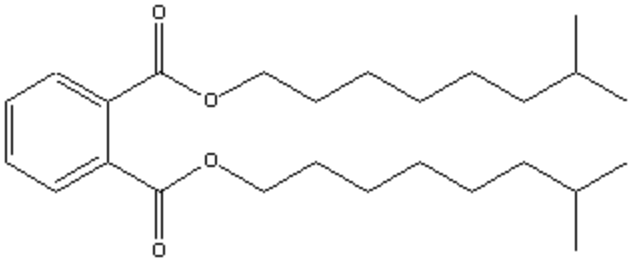
John Moore, DVM, DABT	Principal Scientist
Annette Iannucci, MS	Toxicologist
Ann Walker, MS, ELS	Information Specialist and Technical Editor

Diisononyl Phthalate

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 ^oC
Boiling Point	370 ^oC
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/10cm^2/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 cm^2 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 pacifiers. 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 WEB-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 WEB-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 WEB-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 B6C3F$_1$/CrlBR mice (*19*) (Table WEB-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 WEB-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 hepatocyte 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 WEB-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)

*≤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		0.06	0.10	0.05*
Sig. using nested analysis		0.18	0.14	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 conclude 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 BMD_{05} (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 WEB-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 F_0 and F_1 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 F_1 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 F_2 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 WEB-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.

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.

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*).

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 14^{th} (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.

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.

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.

5.1.4 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.

6.0 REFERENCES

1. CMA. Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. FR Doc. 99-9484. Washington, DC: Chemical Manufacturers Association, 1999.
2. American Chemistry Council Phthalate Esters Panel. Comments on Draft CERHR Evaluation of Di-Isononyl Phthalate (DINP):ACC, 2000.
3. Staples CA, Peterson DR, Parkerton TF, Adams WJ. The environmental fate of phthalate esters: A literature review. Chemosphere 35:667-749(1997).
4. MAFF. Phthalates in infant formulae. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;7p.
5. MAFF. Phthalates in infant formulae. Food surveillance information sheet 168: Joint Food Safety and Standards Group, 1998.
6. MAFF. Phthalates in food. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;9p.
7. CPSC. The risk of chronic toxicity associated with exposure to diisononyl phthalate (DINP) in children's products. Bethesda, MD, 1998.
8. Rastogi SC. Gas chromatographic analysis of phthalate esters in plastic toys. Chromatographia 47:724-726(1998).
9. Marin ML, Lopez J, Sanchez J, Vilaplana J, Jimenez A. Analysis of potentially toxic phthalate plasticizers used in toy manufacturing. Bull Environ Contam Toxicol 60:68-73(1998).
10. Toy Manufacturers of America I-T. Voluntary standard specification for the reduction of DEHP (di[2-ethylhexyl]phthalate) in PVC pacifiers and teethers.: Toy Manufacturers of America, Inc., 1986.
11. Canada H. Updated. Risk assessment on diisononyl phthalate in vinyl children's products. Ottawa, Ontario: Consumer Products Division, Product Safety Bureau, Environmental Health Directorate, Health Protection Branch, 1998.
12. RIVM, Milieu RVVE. Phthalate release from soft PVC baby toys RIVM report 613320 002: National Institute of Public Health and the Environment, 1998.
13. Fiala F, Steiner I, Kubesch K. Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles. (2000).
14. Juberg DR, Thompson KM, Alfano K, Coughlin RJ. An observational study of object mouthing behavior by young children. Rochester, New York: International Center for Toxicology and Medicine, Submitted to journal.
15. Doull J, Cattley R, Elcombe C, Lake B, Swenberg J, Wilkinson C, Williams G. Expert panel report on DEHP.: U.S. Environmental Protection Agency, 1998.
16. BIBRA BIBRA-. A 21-day feeding study of di-isononyl phthalate. Report No. 0495/6/85.: Chemical Manufacturers Association, 1985.
17. Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA. Chronic toxicity a carcinogenic evaluation of diisononyl phthalate in rats. Fundam Appl Toxicol 36:79-89(1997).
18. Moore MRCL. Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Covance 2598-104 Volume 1 of 5. Vienna, VA: Aristech Chemical Corporation, 1998.
19. Moore MRCL. Oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Covance 2598-105 Volume 1 of 6. Vienna, VA: Aristech Chemical Corporation Performing Laboratory, 1998.

20. Hall M, Matthews A, Webley L, Harling R. Effects of dii-isononyl phthalate (DINP) on peroxisomal markers in the marmoset - DINP is not a peroxisome proliferator. The Journal of Toxicological Sciences 24:237-244(1999).
21. Pugh G, Isenberg JS, Kamendulis LM, Ackley DC, Clare LJ, Brown R, Lington AW, Smith JH, Klaunig JE. Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. Toxicol Sci 56:181-188(2000).
22. Short RD, Robinson EC, Lington AW, Chin EC. Metabolic and peroxisome proliferation studies with di (2-ethylhexyl) phthalate in rats and monkeys. Toxicol Ind Health 3:185-194(1987).
23. Caldwell DJ. Review of mononuclear cell leukemia (MNCL) in F-344 rat bioassays and its significance to human cancer risk: A case study using alkyl phthalates. Accepted for publication in Regulatory Toxicology and Pharmacology(1999).
24. Midwest Research Institute M. Dermal disposition of 14C-diisononyl phthalate in rats 35320. Kansas City, MI: Exxon Corporation, Medical Department, Research and Environmental Health, P.O. Box 235, East Millstone, NJ, 1983.
25. Scott RC, Dugard PH, Ramsey JD, Rhodes C. In vitro absorption of some o-phthalate diesters through human and rat skin. Environ Health Perspect 74:223-227(1987).
26. Deisinger PJ, Perry LG, Guest D. In vivo percutaneous absorption of DEHP from DEHP-plasticized polyvinyl chloride film in male Fischer 344 rats. Food Chem Toxicol 36:521-527(1998).
27. Hazelton HL-L. Metabolism study of 14c phthalate ester in rats - Final Report 32563. Vienna, VA: Esso Research and Engineering Company, 1972.
28. Midwest Research Institute M. Single and repeated oral dose pharmacokinetics of 14 C-labeled diisononyl phthalate with cover letter. MRI project No. 7282-B. Kansas City, MI: Exxon Corporation, Medical Department, Research and Environmental Health, P.O. Box 235, East Millstone, NJ, 1983.
29. OECD. Risk assessment - 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters C9-rich and di-"isononyl"phthalate CAS No.: 68515-48-0 and CAS No.: 28553-12-0: EINECS-No.: 271-090-9 and EINECS-No.: 249-079-5, 1998.
30. Barber E, Cifone M, Rundell J, Przygoda R, Astill B, Moran E, Mulholland A, Robinson E, Schneider B. Results of the L5178Y mouse lymphoma assay and the Balb/3t3 cell invitro transformation assay for eight phthalate esters. J Appl Toxicol 20:69-80(2000).
31. Waterman SJ, Ambroso JL, Keller LH, Trimmer GW, Nikiforov AI, Harris SB. Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reprod Toxicol 13:1-6(1999).
32. Ryan L. The use of generalized estimating equations for risk assessment in developmental toxicity. Risk Analysis 12:439-447(1992).
33. McKee R. Personal communication to Jack Moore, 2000.
34. Chen JJ. Dose-response modeling of growth for develomental toxicity. Environmetrics 7:135-144(1996).
35. Hellwig J, Freudenberger H, Jackh R. Differential prenatal toxicity of branched phthalate esters in rats. Food Chem Toxicol 35:501-512(1997).
36. Waterman SJ, Keller LH, Trimmer GW, Freeman JJ, Nikiforov AI, Harris SB, Nicolich MJ, McKee RH. Two-generation reproduction study in rats given di-isononyl phthalate in the diet. Reprod Toxicol 14:21-36(2000).
37. Hellwig J, Jackh R. Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats. Food Chem Toxicol 35:489-500(1997).
38. Ostby J, Price M, Furr J, Lambright C, Hotchkiss A, Parks l, Gray Jr LE. 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 or Reproductive Biology Jan 29, 2000(2000).
39. Gray LE, Ostby J, Furr J, Price M, Veeramachaneni DNR, Parks L. Perinatal exposure to the phthalates in DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. Toxicol. Sci. (in press)(2000).

40. Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR, Matthews JB. Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. Toxicol Sci 46:282-293(1998).
41. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582-587(1995).
42. Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W. Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environ Health Perspect 106:551-7(1998).
43. Nakai M, Tabira Y, Asa D, Yakabe Y, Shimyozu T, Noguchi M, Takatsuki M, Shimohigashi Y. Binding characteristics of dialkyl phthalates for the estrogen receptor. Biochemical and Biophysical Research Communications 254:311-314(1999).
44. Petit F, Le Goff P, Cravedi J-P, Valotaire Y, Pakdel F. Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and troup hepatocyte cultures. Journal of Molecular Endocrinology 19:321-335(1997).
45. Coldham NG, Dave M, Sivapathasundaram S, McDonnell DP, Connor C, Sauer MJ. Evaluation of a recombinant yeast cell estrogen screening assay. Environ Health Perspect 105:734-742(1997).
46. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect 1997 105:802-811(1997).
47. Scott RC, Dugard PH, Ramsey JD, Rhodes C. In vitro absorption of some o-phthalate diesters through human and rat skin. Environ Health Perspect 74:223-227(1987).

7.0 WEB TABLES