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 *n* HEXYL PHTHALATE

OCTOBER, 2000 **NTP-CERHR-DNHP-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.

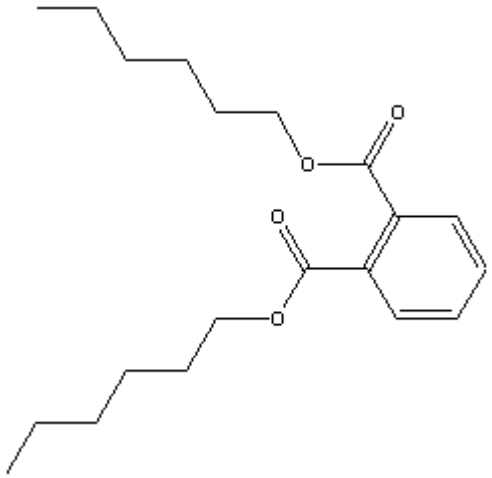
John Moore, DVM, DABT	Principal Scientist
Annette Iannucci, MS	Toxicologist
Ann Walker, MS, ELS	Information Specialist and Technical Editor

Di-n-Hexyl Phthalate

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

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



Di-n-hexyl phthalate (DnHP) (CAS Registry Number 84-75-3) is produced by reacting phthalic anhydride and normal hexyl alcohol in the presence of an acid catalyst (*1*). DnHP is often found as a minor component (less than 1%) of C6–10- phthalate mixtures; it may also be an isomer in mixtures of diisohexyl phthalates (DIHP) (CAS RN 68515-50-4) at levels of 25% or lower (*1*).

Synonyms: 84-75-3 — 1,2-Benzenedicarboxylic acid, dihexyl ester; dihexyl ester phthalic acid; di-n-hexyl phthalate; DnHP)

Table 1: Properties of DHP (isomer not clearly identified)

Property	**Value**
Chemical Formula	$C_{20}H_{30}0_4$
Molecular Weight	334.4
Vapor Pressure	5 x 10^{-6} mmHg at 25 ^oC
Melting Point	- 27.4 ^oC
Boiling Point	350 ^oC
Specific Gravity	1.011
Solubility in Water	Slight – 0.05 mg/L
Log K_{ow}	6.3

(*2*)

1.2 Exposure and Usage

Exposure to DnHP can occur from three sources: as a component of commercial diisohexyl phthalate (DIHP), where it may attain concentrations of up to 25%; by migration from consumer products where it has limited use; and, by its presence as a minor component (less than 1%) of commercial C6–10-phthalates. DnHP is used in the making of plastisols that are subsequently used in the manufacture of automobile parts (air filters, battery covers) and dip-molded products (tool handles, dishwasher baskets) (*3*). Commercial phthalate substances containing DnHP may be added to the PVC utilized in the manufacture of flooring, canvas tarps, and notebook covers (*1*). Substances containing DnHP may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations.

There is currently no information available on production volumes of DnHP, but production is stated to be "small" compared to other phthalates (*3*). Limited information is available for production volumes or consumption rates of C6–10-phthalate and DIHP. About 25,000 tons of C6–10-phthalate were produced in the United States in 1994 (*4*). The DnHP content would equal less than 250 tons. The annual consumption rate of DIHP in Europe was reported as less than 2,000 tons (*5*); therefore, DnHP consumption could be as high as 500 tons.

Release of DnHP to the environment can occur during the production of DnHP, C6–10-phthalates, or DIHP, and during the incorporation of the phthalates into plastic resins. Because DnHP, like other phthalates, is not bound to plastics, it can be released during the use or disposal of the product. Phthalates that are released to the environment can be deposited on or taken up by crops that are intended for human or livestock consumption, and thus, can enter the food supply.

Population Exposure

Adults. The general population is exposed to phthalates primarily through the oral and dermal routes. Based on data for other phthalates, the most likely source of human exposure to DnHP is dietary intake. DnHP may be found in food as a result of environmental uptake during cultivation or as a result of migration from processing equipment or packaging materials. In a survey of packaged fatty foods purchased from grocery stores in the UK, DHP (isomer not specified) was detected, but not quantified, in carcass meat, poultry, eggs, and milk (*6*). DHP (isomer not specified) was below limit of detection (0.01 mg/kg) in samples of household dust, and textiles. A level of 0.03 mg/kg was detected in flooring tile (7).

Infants and Children. DHP (isomer not specified) was detected but not quantified in 7 of 12 baby formulas from the UK (*7*). DHP levels in infant formula were not reported in an UK Ministry of Agricultural Fisheries and Food (MAFF) follow-up analysis (*8*). In a report by Pfordt and Bruns-Weller (*9*) in which the phthalate content of various household items was determined, DHP (isomer not specified) was below the limit of detection of 0.01 mg/kg in milk (breast and commercial), cream, nuts, baby food. Mouthing of toys is a potential source of oral phthalate exposure in children. There were no studies identified that documented the detection of DnHP-containing compounds in children's toys.

Dermal contact with products containing DnHP is possible, but absorption through skin is unlikely. Studies in rats have demonstrated that DnHP is poorly absorbed through skin (*10*). An *in vitro* study conducted with other phthalates suggests that the DnHP absorption rate for human skin is lower than the absorption rate for rat skin (*11*). In studies with DEHP, Deisenger et al. (*12*) showed that absorption through skin from plasticized PVC film was significantly lower than that found from exposure to liquid DEHP.

The available data do not allow the estimation of DnHP exposures to the general population. However, a comparison of production volumes and consumption rates for DnHP-containing compounds versus those containing DEHP suggests that human exposure to DnHP is below the exposure value for DEHP, which was

estimated at 3–30 μg/kg bw/day by Doull et al. (*13*) (see also DEHP review). Exposures may be higher in children due to mouthing of DnHP-containing articles. Although vapor pressure and water solubility are higher than analogous values for DEHP, the Panel notes that physiochemcal properties are generally similar and support the assumption that DnHP human exposures will not exceed those of DEHP.

Medical Exposure

There are no known uses of DnHP or DnHP-containing compounds in medical devices.

Occupational Exposure

Workers may be exposed to DnHP primarily through inhalation and dermal contact. Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tankcars (*1*). Higher exposures to phthalates can occur during the production of flexible PVC because the processes are open and run at higher temperatures. According to the American Chemistry Council (ACC, formerly CMA) (*1*), phthalate levels in air are generally lowerr than 1 mg/m^3 and 2 mg/m^3 during the production of phthalates and flexible PVC, respectively. Four references in the scientific literature were cited in support of the ACC comment. Exposure levels were estimated by the ACC (*1*) using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 μg/kg bw/workday and 286 μg/kg bw/workday for workers employed in phthalate and flexible PVC manufacturing operations, respectively.

The summary for Section 1 is located in Section 5.1.1.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

No human data were located for Expert Panel Review.

2.1.2 Experimental Animal Data

Systemic effects following DnHP treatment for 3, 10, or 21 days were examined in 4-week-old Wistar rats (*14*). The effects were compared to those produced by approximately equal concentrations of DnOP, another straight-chain phthalate, and DEHP, a branched-chain phthalate (*14*). A group of 12 male rats was a fed a diet containing 20,000 ppm DnHP and a control group of 18 rats was fed the basal diet. Using actual food intake levels and rat body weights on the day of sacrifice, a DnHP dose of 1,824 mg/kg bw/day was calculated. Groups of 4 treated rats and 6 control rats were sacrificed and necropsied after 3, 10, or 21 days of treatment. Liver histopathology, enzyme activity, and peroxisome proliferation were examined. Levels of thyroid hormones in serum and thyroid histopathology were also examined (*15*).

DnHP treatment did not cause a change in body weight gain or food intake levels. DnHP treatment had no effect on testes weight or the gross appearance of testes, kidney, or pancreas (*14*). However, liver weight

was significantly increased following 21 days of DnHP treatment, with histology and chemistry changes observed at all 3 assessment times. Centrilobular necrosis and loss of glycogen were first observed at 3 days and centrilobular fatty accumulation was observed at 10 days of treatment. The effects became more pronounced with increasing duration of treatment. Examination by electron microscopy revealed proliferation and dilation of smooth endoplasmic reticuli and shortening of the microvilli in bile canaliculi at 3 days, the presence of lipid droplets within hepatocytes at 10 days, and possibly a small increase in lysosomes and peroxisomes at 3 and 21 days, respectively. The activity of the peroxisomal proliferation marker, cyanide-insensitive palmitoyl CoA oxidase, was significantly increased at levels approximately 2-fold greater than controls in rats only after 10 days of treatment. There was no change in total catalase activity, but catalase activity in the particulate fraction was significantly increased at 10 and 21 days of treatment. A significant decrease in glucose-6-phosphate activity at 21 days of treatment was the only other effect on liver enzymes. Effects of DnOP, DnHP, and DEHP on thyroid were studied in rats. Each phthalate was associated with a decrease in serum thyroxine (T4) levels. Inexplicably, the authors reported increased levels of serum triiodothyronine (T3) levels (See Table 2) and also concluded this parameter was essentially unaffected. Electron microscopic changes indicative of thyroid hyperactivity (increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage) were also observed (*15*).

In a comparison of the three tested phthalates, the effects induced by DnHP were similar to DnOP, but different from DEHP. DEHP treatment resulted in a more pronounced increase in liver weight and in increased mitotic activity. Less fat accumulated following treatment with DEHP, and when observed, the accumulation occurred in the midzonal and periportal zones rather than in the centrilobular region. Biochemical evidence of peroxisome proliferation (cyanide-insensitive palmitoyl CoA oxidation) occurred earlier with DEHP treatment (after 3 days of treatment) and was approximately 7-fold higher than it was following DnHP or DnOP treatment. Although DEHP was a stronger inducer of peroxisome proliferation, DnHP and DnOP also induced peroxisome proliferation following longer treatment periods. The data from Barber et al. (1986) provide further evidence of the limited potential for peroxisome proliferation for DnHP as compared to DEHP. Other effects suggesting liver damage were also observed following DnHP treatment. Thyroid effects were similar for all three phthalates.

Table 2: Summary of Changes in the Livers of Rats Administered Diets Containing 2% w/w DEHP, DnOP, or DnHP

EFFECT	Treatment		
	DEHP	DnHP	DnOP
Liver Morphology			
Hepatomegaly	+++	+(Late)	+(Late)
Centrilobular loss glycogen	+	+	++
Centrilobular necrosis	–	++	++
Peroxisome proliferation	+++	+(Late)	+(Late)
Smooth endoplasmic proliferation	++	+	+
Increase of inner mitochondrial matrix	++	–	–
Initial burst of mitosis	++	–	–
Liver Biochemistry (day 21)			
Cyanide-insensitive palmitoyl CoA oxidation day 10	↑↑↑↑[d]	↑	↑
α–Glycerophosphate dehydrogenase day 21	↑↑	–	–
G-6-Phosphate day 21	↓↓↓	↓	↓
Succinate dehydrogenase μmol/min/g protein			

day 21	–	–	↓
Catalase			
day 10	↓	↑↑	↑↑
Thyroid function			
Serum triiodothyronine (T_3) day 21	140%[c]	183%	133%
Serum thyroxine (T_4) day 21	64%	58%	76%

Adapted from (*14*)
[a] (+) denotes the degree of change seen when compared to age-matched controls.
[b](–) = absence of the lesion or effect.
[c] Expressed as % of control value.
[d] Multiple arrows denote more extreme effect.
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

2.2 Toxicokinetics

Phthalate Moiety

Absorption

No inhalation or oral toxicokinetic data have been reported for DnHP

Rodents: Dermal
Dermal absorption of DnHP has been studied along with a series of phthalates in the rat (*10*). Hair from a skin area (1.3 cm in diameter) on the back of male F344 rats was clipped, the ^{14}C-phthalate diester was applied in a dose of 157 μmol/kg, and the area of application was covered with a perforated cap. The rats were restrained and housed for 7 days in a metabolic cage that allowed separate collection of urine and feces. Urine and feces were collected every 24 hours, and the amount of ^{14}C excreted was taken as an index of the percutaneous absorption. At 24 hours, diethyl phthalate showed the greatest excretion (26%). As the length of the alkyl side chain increased, the amount of ^{14}C excreted in the first 24 hours decreased significantly. The cumulative percentage dose excreted in 7 days was greatest for diethyl, dibutyl, and diisobutyl phthalate, about 50–60% of the applied ^{14}C; and intermediate (20–40%) for dimethyl, benzyl butyl, and dihexyl phthalate (DnHP excretion was approximately 18%). Urine was the major route of excretion of all phthalate diesters except for diisodecyl phthalate. This compound was poorly absorbed and showed almost no urinary excretion. After 7 days, the percentage dose for each phthalate that remained in the body was minimal and showed no specific tissue distribution. Most of the unexcreted dose remained in the area of application. These data show that the structure of the phthalate diester determines the degree of dermal absorption. Absorption maximized with diethyl phthalate and then decreased significantly as the alkyl side chain length increased. Urine was the principal route of excretion, and there was no evidence of accumulation in any tissue that was examined. Deisenger et al. (*12*) in studies with ^{14}C-labeled DEHP showed that absorption through skin from plasticized PVC film was significantly lower than that found from exposure to liquid DEHP.

Biotransformation

No oral or inhalation toxicokinetic data have been reported for DnHP. However, as other phthalates are converted to monoesters and alcohol and rapidly excreted, it is anticipated that dihexyl phthalate would behave in the same way.

Distribution

Dermally absorbed DnHP was widely distributed throughout the body with no tissue containing >0.6% of the applied dose. There was no evidence for accumulation in any tissue.

Excretion

The major route of excretion of dermally absorbed DnHP was via the urine (*10*).

Side Chain-associated Toxicokinetics

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

2.3 Genetic Toxicity

In genetic toxicity tests, DnHP was inactive in the Salmonella/mammalian microsome mutagenicity assay with and without activation by rat and hamster S9 metabolic systems (*16*). According to a review conducted by the ACC (*1*), DnHP also tested negative in two bacterial assays. Barber et al. (*17*) tested C6–10-phthalate, which contains minor amounts of DnHP, in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency that occurred in the presence and absence of S9 metabolic activation.

The ACC (*1*) has reported that DIHP (which may contain up to 25% DnHP) was inactive in a mouse micronucleus test that was conducted by Exxon Biomedical Sciences Inc in 1996.

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

No human data were located for Expert Panel Review.

3.2 Experimental Animal Toxicity

DnHP (CAS No. 84-75-3) was evaluated in the Chernoff-Kavlock screening assay (*18*) (Table Web 1). CD-1 mice (48–50 dams/group) were gavaged on gd 6–13 with 9,900 mg/kg bw/day (undiluted chemical, 10 mL/kg/day) or corn oil. According to the standard protocol, dams are allowed to litter and a postnatal evaluation is conducted. However, there were no live litters (0/34). One exposed dam died. Body weight changes in dams could not be evaluated due to complete litter loss.

Nine other phthalates were evaluated in the Chernoff-Kavlock screening assay and the authors concluded that "dramatically positive results were seen with the diesters having intermediate chain lengths: n-butyl, i-

butyl, and n-hexyl. The shorter (methyl and ethyl) and longer (n-octyl and i-decyl) diesters were generally negative, although litter size and neonatal weight gain were both reduced in the di (n-octyl) phthalate group relative to its concurrent control" (*18*).

A limited number of developmental effects were observed in a continuous breeding study that exposed CD-1 mice to dietary DnHP concentrations of 0, 0.3, 0.6, or 1.2% (0, 380, 800, or 1,670 mg/kg bw/day) (*19, 20*). Complete details of this study are included in Section 4. Developmental effects could not be evaluated at the top two doses due to either very high rates of infertility or complete infertility. The number of live pups/litter was reduced in the 380 mg/kg bw/day group (n= 3 versus 12 in control group).

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 Data

Two studies were reviewed in the evaluation of the reproductive toxicity of DnHP. None of the studies available are considered definitive and no multigeneration reproduction study has been published for this phthalate ester. Only one study measured effects of the agent on reproductive function in the mouse. The other has shown subacute effects of DnHP on testicular weight and morphology at high dose levels in the rat.

The key study for the assessment of the reproductive toxicity of DnHP is reported by Lamb et al. (*21*) and Reel et al. (*20*). In Lamb et al. (*21*) (Table WEB-2), DnHP was one of four phthalate esters compared using the continuous breeding protocol in mice (See DBP monograph). Twenty pairs of male and female CD-1 mice (40 pairs in control group) were dosed with DnHP for 7 days prior to and during a 98-day cohabitation period. The doses were 0, 0.3, 0.6, or 1.2% w/w in the diet. Intake levels in mg/kg bw/day were not reported in the original study by Reel et al. (*20*) or in the summary by Lamb et al. (*21*). However, intakes were estimated in other summaries of RACB studies. Morrissey et al. (*22*) estimated intake levels of 0, 430, 880, or 1,870 mg/kg bw/day and Chapin and Sloane (*23*) estimated intake levels of 0, 380, 800, or 1,670 mg/kg bw/day. The Expert Panel noted that the differences in estimated doses were small and biologically insignificant considering the inherent variability in such estimates. This variability reflects dramatic changes in animal weight as the animal matures, and therefore alters the amount of chemical consumed per unit of body weight. For consistency, the values of Chapin and Sloane will be used throughout this monograph. Litters were examined and removed. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair, number of live pups per litter, pup weight, and offspring survival. Organs were collected for histological evaluation and testes were preserved in Bouin's solution. DnHP exposure resulted in a dose-related reduction in the proportion of pairs able to produce even a single litter during the continuous breeding phase. No litters were produced at the high dose (1,670 mg/kg bw/day), 1 litter in the mid-dose group (800 mg/kg bw/day), 14 of 17 pairs had litters in the low-dose group (380 mg/kg bw/day), compared to all pairs having litters in the control group. The numbers of litters per pair, the number of live pups per litter, and the proportion of pups born alive were also

significantly affected by DnHP exposure. Significant effects occurred at the lowest dose level with clear adverse effects seen in the absence of any body weight effects.

A crossover mating trial was performed between the high-dose males and control females. There was a significant decrease in detected matings (56%) compared to controls (90%), and only 1 of 18 treated males sired a litter. When the high-dose females were mated with control males, there was no decrease in copulatory plugs, but none of the females became pregnant. Only the control and high-dose DnHP groups were necropsied. Sperm assessment showed a significant decrease in sperm number (7% of control) and motility (22% of control) parameters. Only 3 of 18 males had sufficient numbers of sperm to allow assessment of abnormal forms; incidence in these 3 was diminished in number compared to control. There were significant decreases in the relative weights of the epididymis, testis, and seminal vesicle. There was extensive atrophy of the seminiferous epithelium with mature sperm markedly diminished in the epididymis. No treatment-related microscopic lesions were detected in the ovaries, uterus, or vagina of the female mice. For females, liver to body weight ratio was significantly increased (31%) and uterine weight significantly decreased (31%). Body and relative kidney/adrenal weights were significantly decreased and liver to body weight ratio was significantly increased in both males and females of the high-dose group, but histological changes were not noted. A second generation was not evaluated.

In a short-term study (*24*) which employed a single dose level of DnHP (2.4 g/kg bw/day) given by gavage in corn oil to a group of 12 pubertal male Sprague Dawley rats (4-weeks-old) for 4 days, marked effects on testis weight (65% of control value) were noted in the absence of body weight effects. Histologic examination of formalin-preserved testes revealed a marked seminiferous tubular atrophy with the majority of tubules showing few spermatogonia and Sertoli cells, but normal Leydig cell morphology.

Mode of Action

DnHP has been studied in an *in vitro* assay in order determine the mechanism of testicular toxicity. Incubation of Sertoli and germ cell cultures with 1, 10, or 100 μM DnHP resulted in a dose-related detachment of germ cells from the Sertoli cell monolayer (*25*). The detached germ cells were viable and structurally normal, but changes were observed in the morphology of the Sertoli cells. The findings suggest that germ cell loss following *in vivo* exposure to DnHP is a secondary effect resulting from toxic insult to Sertoli cells.

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 (*26*), rainbow trout hepatic cytosol (*27*), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (*28, 29*) and rainbow trout ERs expressed in yeast (*30*). Triated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. The estrogenic activity of DIHP has been examined using a battery of short-term *in vitro* and *in vivo* assays. DIHP did compete with tritiated estradiol for binding to the rat uterine cytosolic estrogen receptor (*26*). DIHP, in contrast to the positive control estradiol, did not significantly induce an *in vivo* vaginal cornification response or an increase in uterine weight at any of the concentrations tested (20, 200, and 2,000 mg/kg) over the course of a 5-day experiment using immature and adult ovariectomized Sprague Dawley rats (*26*). DnHP is a constituent of DIHP and may reach concentrations of 25% of the complex substance.

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

A limited quantity of DnHP is produced for commercial use in automotive parts and dip molded products such as dishwasher baskets and tool handles (*3*). It is a component of other phthalate mixtures and may constitute up to 25% of commercial diisohexyl phthalates (DIHP). The compound is also present in C6–10-phthalate substances at lower than 1% concentration. Assuming equivalent production and usage of C6–10-phthalates and DIHP in the US and Europe, the aggregate annual production of DnHP in these two products could be as much as 750 tons. Phthalates containing DnHP may be used in PVC used to manufacture flooring, canvas tarps, and notebook covers (*1*). Such phthalates may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations.

DHP (isomer not specified) was detected but not quantified in 7 of 12 baby formulas from the UK (*7*). DHP levels in infant formula were not reported in a MAFF follow-up analysis (*8*). In a survey of packaged fatty foods purchased from grocery stores in the UK, DHP (isomer not specified) was detected, but not quantified, in carcass meat, poultry, eggs, and milk (*6*). In a German Survey (*9*), the DHP level (isomer not specified) was below the limit of detection of 0.01 mg/kg in milk (breast and commercial), cream, nuts, baby food. Based on production volumes and consumption rates of DnHP-containing compounds versus DEHP, human exposure to DnHP is likely lower than exposure to DEHP, which was estimated at 3–30 μg/kg bw/day by Doull et al. (*13*). Exposures may be higher in children due to mouthing of DnHP-containing articles. Variations in food exposure estimates are possible due to inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical location. In occupational settings, exposure is thought to be highest in workers at flexible PVC manufacturing facilities. Based on general levels of phthalates reported, the ACC (*1*) estimated an exposure level of 286 μg/kg bw/workday for production of phthalate-containing PVC pipe. Absorption from dermal exposure is expected to be low; there are no data for absorption through inhalation.

5.1.1.1 Utility of Data to the CERHR Evaluation

There is very limited information on exposure and exposure pathways to DnHP in humans. Such estimates are complicated as DnHP is rarely produced directly for commercial use, but is a component (up to 25%) in commercial diisohexyl phthalates (DIHP) and at less than 1% in C6–10- phthalate substances. C6–10-Phthalates and DIHP are used in a variety of consumer products. DnHP has been detected in environmental samples (air, water, and soil); however, quantitative estimates for exposure are limited.

5.1.2 General Biological and Toxicological Data

Human data were not found for the categories presented in this section.

General Toxicity. General toxicity information for DnHP is limited but is available from a repeated-dose dietary study in which 4 Wistar rats (4 weeks old) were exposed to 1 high dose (1,824 mg/kg bw/day) for 3, 10, or 21 days (*14*, *15*). The liver was identified as the principal target organ and effects observed included necrosis, fatty accumulation, and glycogen loss. DnHP was found to be a weak peroxisome proliferator as evidenced by both morphological and biochemical enzyme profiles compared to DEHP as effects occurred

at later time points and to a lesser extent than with DEHP. Microscopic changes suggested thyroid hyperactivity.

Toxicokinetics. No oral or inhalation toxicokinetic data have been reported for DnHP. DnHP is slowly absorbed dermally in rats with approximately 18% of ^{14}C excreted in urine within 7 days. After 7 days, the percentage that remained in the body was minimal based on a study by Elsisi et al. (*10*), and showed no specific tissue distribution. It is assumed from research on structurally-related phthalates that DnHP is rapidly absorbed as the monoester from the gut following oral exposure.

Genetic Toxicity. DnHP has tested negative in the Salmonella and two other bacterial assays and in a mouse micronucleus test (*1, 16*). C6–10- Phthalate mixture was considered equivocal in the mouse lymphoma mutation assay due to a non-dose related increase in mutations in the presence and absence of metabolic activation, but tested negative in the Balb/3T3 cell transformation assay (*17*). DIHP was inactive in the mouse micronucleus test (*1*).

5.1.2.1 Utility of Data to the CERHR Evaluation

There is sufficient data available from a dietary study in rats to show that DnHP can cause liver and thyroid toxicity (1,824 mg/kg bw/day; for 3, 10, or 21 days of exposure). Signs of liver necrosis and slight peroxisome proliferative changes (histologic and biochemical) were observed. In these same studies, testis weight and gross appearance were unaffected.

Limited dermal toxicokinetic information for DnHP in rodents suggests dermal absorption with renal excretion in 7 days. Kinetic information from structurally-related phthalates suggests that DnHP is rapidly absorbed from the gut as the monoester and as n-hexanol after oral exposure.

5.1.3 Developmental Toxicity

No human data were located for Expert Panel review.

Data for DnHP are limited to 1 screening assay (*31*) in which a massive oral dose (9,900 mg/kg bw/day) was administered to 48 mice on gd 6–13 (*18*). None of the 34 pregnant dams gave birth to a live litter. These positive results (pregnancy loss) in a screening assay are of relevance to the Panel's evaluation. A reduction in live pups per litter was observed in CD-1 mice exposed to the lowest dose of DnHP (380 mg/kg bw/day) in a reproductive toxicity assay (*20, 23*).

Table 3: Summary of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects		Developmental Effects Observed at Higher Dose Levels
		Maternal	Developmental	Developmental
Prenatal gavage toxicity screening assay in CD-1 mice. 48–50 dams/group received 0 or 9,990 mg/kg bw/day on gd 6–13. Postnatal evaluation conducted. (*18*)	Developmental: None Maternal: Not reported.	Could not be evaluated.	9,990 No live pups delivered.	No higher doses.

Continuous breeding study in CD-1 mice. 20 dams/group received DnHP in feed at 0, 380, 800, or 1,670 mg/kg bw/day throughout mating and gestation. (*20, 21*)	Maternal: * Developmental: None	*	380 ↑ Pup mortality	Cannot be evaluated due to infertility in parents.

* Only developmental effects reported in this table. See Table 4 in Section in 5.1.4 for a description of effects in parental mice.

5.1.3.1 Utility of Data to the CERHR Evaluation

The data from one screening level study in mice administered one dose level, 9,900 mg/kg bw/day on gd 6–13, are sufficient to indicate that DnHP is a developmental toxicant (loss of all litters) at high doses in mice. Pup mortality at 380 mg/kg was also observed in a breeding study. These data provide evidence for hazard identification, but do not provide dose-response information and are therefore inadequate for determination of LOAELs or NOAELs.

5.1.4 Reproductive Toxicity

No human data were located for Expert Panel review.

Reproductive studies for DnHP include a continuous breeding study in mice (*20, 21*) and a 4-day exposure study in rats (*24*). Testicular weights were also measured in a 21-day subchronic exposure study in Wistar rats (*14*).

In the one-generation study, male and female mice were exposed to 0, 0.3, 0.6, or 1.2% DnHP in the diet (~0, 380, 800, or 1,670 mg/kg bw/day) throughout a 98-day breeding period (*20, 21, 23*). A NOAEL was not identified because reproductive effects were observed at all dose levels. Fertility was reduced in all treated groups in a dose-related manner, with severe reduction at doses of 800 mg/kg bw/day and higher, and complete infertility at the highest dose (1,670 mg/kg bw/day). The number of litters produced and pup survival were reduced in the lowest dose group (380 mg/kg bw/day). Mating of high-dose animals to control animals demonstrated that both males and females were affected. Testicular atrophy and reduced sperm counts were demonstrated in the high-dose males. The high-dose group was infertile, the middle-dose and the low-dose groups were subfertile. Thus, a NOAEL was not achieved. A LOAEL of 380 mg/kg bw/day was assessed based on fewer litters and decreased numbers of pups per litter. These mid- and low-dose groups were not evaluated at necropsy, and the lack of a thorough assessment of an unaffected group leads the Panel to state that while confidence in the quality of the study is high, the Panel's confidence is moderate-to-low that these doses correctly represent the LOAEL.

Results of the mouse study are supported by a subacute study in which testicular atrophy was observed in 4-week-old Sprague Dawley rats gavaged with 2,400 mg/kg bw/day for 4 days (*24*). This study was designed to compare different phthalate esters, rather than to provide dose-response information and is of limited utility for risk assessment. It does show that DnHP is a reproductive toxicant at high doses in the young male rat. However, testicular weights were unaffected in 4-week-old Wistar rats fed 1,824 mg/kg bw/day through the diet for 21 days (*14*). The evidence indicates that at oral doses of 380 mg/kg bw/day and higher, DnHP is a reproductive toxicant to male and female mice and male rats. Findings of an *in vitro* assay suggested that testicular toxicity may result in part from primary damage to the Sertoli cells that ultimately leads to the detachment of germ cells (*25*).

<u>Mode of Action</u>

An isomeric mixture of dihexyl phthalates exhibited weak activity in an *in vitro* assay that measured binding of phthalates to rat uterine estrogen receptors (*26*). *In vivo* assays demonstrated that an isomeric mixture of dihexyl phthalates does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*26*). The findings suggest that for compounds that may contain DnHP as a component, toxicity is not mediated through estrogenic activity.

Table 4: Summary of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies

Protocol & Study	Reproductive NOAEL (mg/kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and Effects	Systemic LOAEL (mg/kg bw/day) and Effects	Reproductive Effects Observed at Higher Dose Levels
Continuous breeding and crossover mating study in CD-1 mice. 20 pairs of mice were fed diets with DnHP (0, 380, 800, and 1,670 mg/kg bw/day) for 7 days prior to mating and during a continuous 98-day mating period.* (*20, 21*)	Reproductive: None Systemic: Not identified due to limited examination of lower dose groups.	380 ↓ Fertility. ↑ Pup mortality	1,670 ↓ Body weight. ↑ Liver weight.	Severe to complete infertility in males and females. ↓ Mating in males. ↓ Sperm count and motility. ↓ Male reproductive organ weights.

* Developmental effects are reported in the Table 3 in Section 5.1.3.
↑=Statistically Significant Increase
↓=Statistically Significant Decrease

5.1.4.1 Utility of Data to the CERHR Evaluation

The two rat studies and one mouse study are sufficient to indicate that DnHP is a reproductive toxicant to male and female mice and to male rats. The testis is the target organ and damage to the Sertoli cells may be the primary lesion. The mouse study indicates reduced fertility at 800 mg/kg bw/day and infertility at 1,670 mg/kg bw/day, with reduced numbers of litters and a reduced postnatal survival at 380 mg/kg bw/day (the low dose). No NOAEL was identified in this study. In the short-term (4-day and 21-day) studies in male rats, testicular atrophy occurred at gavage doses of 2,400 mg/kg bw/day for 4 days, but no effects were noted on testis weight after 21 days at 1,824 mg/kg bw/day in the diet. Consistent male testicular effects were therefore observed in two species. These data do not allow confidence in the assignment of NOAELs and LOAELs for reproductive toxicity in animal models.

5.2 Integrated Evaluation

DnHP is a component in mixtures of C6–10-phthalates (<1%) and diisohexyl phthalates (up to 25%). While some exposure to humans occurs through contact with consumer products, the level is expected to be low. There are no data that document the use of the phthalate mixtures in medical devices. As with all phthalates, dietary intake is expected to be the primary route of exposure; absorption through skin contact is assumed to be negligible. Potential sources of phthalates in foods include migration from packaging materials and general environmental contamination. There are no data on the toxicokinetics of DnHP following oral exposure. However, based on data from other phthalates, it is quite plausible to predict that orally administered DnHP would be converted to the monoester by intestinal enzymes and then rapidly absorbed and excreted.

There are no human data from which to assess the health hazards associated with DnHP exposure. Studies of DnHP toxicity are limited to rats and mice. In the absence of human data, it is assumed that the effects observed in rodents are relevant to humans.

Limited general toxicity data indicate that the liver is the target organ for adverse effect after exposure to relatively high doses. Thyroid function may also be affected. Such data are generally consistent with effects observed with related phthalates. Limited studies with mixtures that contain DnHP provide little evidence of estrogenic activity.

In a screening protocol design in mice, complete litter loss was observed at a massive oral dose (9,900 mg/kg bw/day). This study is sufficient to determine that DnHP is a developmental toxicant in animals following high exposures; however, it is not sufficient to set a NOAEL. Since only one dose was tested, there is no information on the shape of the dose-response curve. Evaluation of maternal toxicity was limited to body weight changes which could not be assessed due to complete litter loss.

The reduced litter survival observed in a breeding study in mice confirms effects on litter survival and also indicates reduced offspring survival at 380 mg/kg bw/day. A NOAEL was not identified. Maternal toxicity was evaluated in high-dose animals (exposed to 1,670 mg/kg bw/day) and included decreased body weight and increased liver weight.

Experimental animal data are adequate to establish that DnHP is a reproductive toxicant in rodents and to identify the male reproductive system as a target of toxicity. A multiple-oral dose study in mice demonstrated that DnHP treatment induced adverse reproductive effects at the lowest dose tested (380 mg/kg bw/day); the NOAEL is therefore unknown. Infertility occurred in both males and females. Unfortunately, the study did not examine reproductive function in the F_1 offspring. The rodent data are of assumed relevance for humans, but are inadequate to determine dose-response relationships for risk evaluation in humans.

5.3 Expert Panel Conclusions

There is very limited information on exposure and exposure pathways for DnHP in humans; thus, the Expert Panel has low confidence in the completeness of the database upon which estimates are made. Such estimates are complicated as DnHP is rarely produced directly for commercial use, but is a component (up to 25%) in commercial diisohexyl phthalates (DIHP) and at less than 1% in C6–10-phthalates. Both C6–10-phthalates and DIHP are used in a variety of consumer products. Based on these inadequate data, the Expert Panel believed that human exposures to DnHP are likely lower than those to DEHP; however, how much lower was difficult to ascertain. To allow for integrated evaluation of exposure and toxicity information, the Expert Panel made a conservative estimate (i.e., an overestimation) of general population exposure by assuming that DnHP would be at or below the DEHP exposure level of 3–30 µg/kg/day (*32*). A possible exception may be non-dietary sources in children. A similar approach for estimating occupational phthalate exposures in flexible PVC production was done by assuming analogy to measured DEHP levels (286 µg/kg bw/workday).

With regard to developmental toxicity, the database is insufficient to fully characterize the potential hazard. However, the limited oral developmental toxicity data available (screening level assessment in the mouse) are sufficient to indicate that DnHP is a developmental toxicant at high doses (9,900 mg/kg bw/day). These data were inadequate for determining a NOAEL or LOAEL because only one dose was tested.

The data are sufficient to indicate that DnHP is a reproductive toxicant in both sexes of two rodent species following oral exposure. They are insufficient to identify a NOAEL. Adverse effects were identified at 380 mg/kg bw/day, the lowest dose tested.

Considering the inadequate quantitative information from the experimental animal studies and the inadequate human exposure data, the Panel concluded there is insufficient information to ascertain the potential for risk to human reproduction.

5.4 Critical Data Needs

Although there is little or no commercial production or use of pure DnHP, it was reviewed to determine structure-activity relationships with other phthalates. DnHP may be present in C6–10-phthalate mixtures (less than 1%) and in diisohexyl phthalate mixtures (up to 25%). Therefore, the public may be better served by focusing on data needs for the diisohexyl phthalate mixtures instead of pure DnHP.

6.0 REFERENCES

1. CMA. Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. FR Doc. 99-9484. Washington, DC: Chemical Manufacturers Association, 1999.
2. Staples CA, Peterson DR, Parkerton TF, Adams WJ. The environmental fate of phthalate esters: A literature review. Chemosphere 35:667-749(1997).
3. ACC. Personal communication, 2000.
4. ACC. Meeting with Aristech Chemical Company, 1999.
5. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect 1997 105:802-811(1997).
6. MAFF. Phthalates in food. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;9p.
7. MAFF. Phthalates in infant formulae. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;7p.
8. MAFF. Food surveillance information sheet - Phthalates in infant formulae - follow-up survey. Joint Food Safety and Standards Group, vol 1999:MAFF - UK, 1998;13p.
9. Pfordt J, Bruns-Weller E. Die phthalsaureester als eine gruppe von umwelt-chemikalien mit endokrienem potential: Niedersachsisches Ministerium fur Ernahrung, Landwirtschaft und Forsten, 1999.
10. Elsisi AE, Carter DE, Sipes IG. Dermal absorption of phthalate diesters in rats. Fundam Appl Toxicol 12:70-77(1989).
11. 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).
12. 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).
13. 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.
14. Mann AH, Price SC, Mitchell FE, Grasso P, Hinton RH, Bridges JW. Comparison of the short-term effects of di(2-ethylhexyl) phthalate, and di (n-octyl) phthalate in rats. Toxicol Appl Pharmacol 77:116-132(1985).
15. Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P, Bridges JW. Effects of phthalic acid esters on the liver and thyroid. Environ Health Perspect 70:195-210(1986).
16. Zeiger E, Haworth S, Mortelmans K, Speck W. Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in Salmonella. Environ Mutagen 7:213-232(1985).
17. 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 *in vitro* transformation assay for eight phthalate esters. J Appl Toxicol 20:69-80(2000).
18. Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogen Carcinogen Mutagen 7:29-48(1987).
19. Chapin RE, Sloan RA, Haseman JK. The relationships among reproductive endpoints in Swiss mice, using the reproductive assessment by continuous breeding database. Fundam Appl Toxicol 38:129-142(1997).
20. Reel JR, Lawton AD, Myers CB. Di-N-Hexyl Phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed. NTP-85-187. NTIS#PB85-249332: National Toxicology Program, National Institute of Environmental Health Sciences, 1985.
21. Lamb JC, IV. Reproductive effects of four phthalic acid esters in the mouse. Toxicol Appl Pharmacol 88:255-269(1987).

22. Morrissey RE, Lamb JC, IV, Morris RW, Chapin RE, Gulati DK, Heindel JJ. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundam Appl Toxicol 13:747-777(1989).
23. Chapin RE, Sloane RA. Reproductive assessment by continuous breeding: Evolving study design and summaries of ninety studies. Environmental Health Perspectives 105:199(1997).
24. Foster PMD, Thomas LV, Cook MW, Gangolli SD. Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. Toxicol Appl Pharmacol 54:392-398(1980).
25. Gray TJ, Gangolli SD. Aspects of the testicular toxicity of phthalate esters. Environ Health Perspect 65:229-235(1986).
26. 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).
27. 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).
28. 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).
29. 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).
30. 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).
31. Chernoff N, Kavlock RJ. An in vivo teratology screen utilizing pregnant mice. J Toxicol Environ Health 10:541-550(1982).
32. Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, Williams G, van Gemert M. A cancer risk assessment of di(2-ethylhexyl)phthalate: Application of the new U.S. EPA Risk Assessment Guidelines. Regulatory Toxicology and Pharmacology 29:327-357(1999).

7.0 WEB TABLES