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* OCTYL PHTHALATE

OCTOBER, 2000 **NTP-CERHR-DNOP-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.
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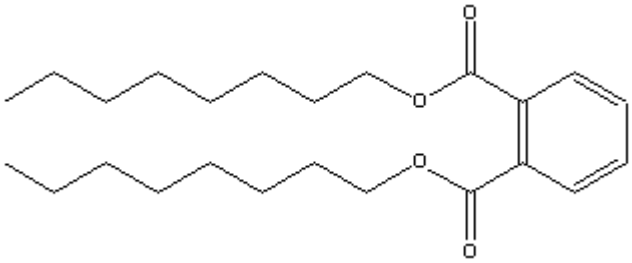
Di-n-Octyl Phthalate

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

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



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

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

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

Table 1: Physicochemical Properties of DnOP

Property	**Value**
Chemical Formula	$C_{24}H_{38}0_4$
Molecular Weight	390.54
Vapor Pressure	1.0×10^{-7} mmHg at 25 ^oC
Melting Point	-25 ^oC
Boiling Point	390 ^oC
Specific Gravity	0.978
Solubility in Water	Essentially insoluable (0.5 μg/L)
Log K_{ow}	8.06

(*2*)

1.2 Exposure and Usage

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

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of C6–10 phthalate substance. Commercial production of 50 million pounds of C6–10 phthalate in the United States in 1994 (*3*) equates to 10 million pounds of DnOP. C6–10 phthalate substance is used in PVC utilized in the manufacture of flooring and carpet tile, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys, vinyl gloves, garden hoses, weather stripping, flea collars, and shoes (*1*). DnOP-containing phthalate substances are also used in PVC intended for food applications such as seam cements, bottle cap liners, and conveyor belts.

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

General Population Exposure

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

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

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

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

Exposure to DnOP through air is also possible but expected to be minimal. Reported concentrations of DnOP in ambient air range from 0.06 to 0.94 ng/m^3. The highest reported concentration resulted in a calculated inhaled dose of 0.29 ng/kg bw/day for an adult (*10*). Reported concentrations in river water have ranged from 0.024 to 1 ppb. EPA estimates that DNOP levels in drinking water influents are less than 0.5 ppb (*10*). These levels are several orders of magnitude lower than levels found in food.

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

Medical Exposure

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

Occupational Exposure

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

The summary for Section 1 is located in Section 5.1.1.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

There were no human data located for Expert Panel review.

2.1.2 Experimental Animal Data

Acute Studies

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

Repeat-dose Toxicity

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

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

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

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

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

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

Systemic effects were studied in groups of young (~4–6 weeks old) Sprague-Dawley rats (*15*). Groups of (10/sex) were fed DnOP at dietary concentrations of 0, 5, 50, 500, or 5,000 ppm (males: 0, 0.4, 3.5, 36.8, or 350 mg/kg bw/day; females: 0, 0.4, 4.1, 40.8, or 403 mg/kg bw/day) for 13 weeks (Table WEB-1). Negative controls (10/sex) were fed basal diet and positive controls (10/sex) were fed 5,000 ppm DEHP

(males: 345 mg/kg bw/day; females: 411 mg/kg bw/day). Rats were observed daily, and body weights and food intake were measured weekly. At the end of the exposure period rats were killed and necropsied. Parameters evaluated included histopathology (reproductive organs preserved in Zenker's solution), hematology, blood chemistry, liver enzyme activity, peroxisome proliferation, and DnOP levels in tissues.

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

The DEHP-positive control group of rats, exposed to 345 (M) and 411(F) mg/kg bw/day, had effects in the liver and thyroid with respect to severity of lesions and biochemical changes that were similar to those observed in the high-dose DnOP group. However, DEHP also induced peroxisomal proliferation, seminiferous tubule atrophy, Sertoli cell vacuolation, and decreased sperm levels. Also, numerous additional biochemical changes were observed with the DEHP-exposed rats, such as increases in plasma levels of albumin and inorganic phosphate, total protein (in female rats), and hepatic aminopyrine-N-demethylase and analine hydroxylase. DEHP also produced significant hematological effects such as increased platelet counts in rats of both sexes, increased WBC, and decreased mean corpuscular volume and decreased hemoglobin in females.

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

2.2 Toxicokinetics

Phthalate Moiety

Absorption

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

Biotransformation

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

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

Distribution

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

Excretion

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

Side Chain -associated Toxicokinetics

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

2.3 Genetic Toxicity

Mixtures containing DnOP have not shown conclusive evidence of mutagenicity. Barber et al. (*22*) tested C6–10-phthalate, which contains approximately 20% DnOP, in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency that occurred in the presence and absence of S9 metabolic activation. The ACC (*1*) reviewed two studies of Di(n-octyl, n-decyl) phthalate, which contains DnOP as a component. Di(n-octyl, n-decyl) phthalate was reported to be negative in the Ames test and the Chinese hamster ovary cell/HPRT locus assay.

The summary for Section 2, including general toxicity, toxicokinetics, and genetic toxicity, is located in Section 5.1.2.

3.0 DEVELOPMENTAL TOXICITY

3.1 Human Data

There were no human data located for Expert Panel review.

3.2 Experimental Animal Toxicity

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

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

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

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

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

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data located for Expert Panel review.

4.2 Experimental Animal Toxicity

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

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

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

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

Mode of Action

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

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

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

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

The summary for Section 4 is located in Section 5.1.4.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

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

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

food. Dioctyl phthalate is approved for use as an indirect food additive in sealants used for food packaging (*4*).

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

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

5.1.1.1 Utility of Data to the CERHR Evaluation

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

5.1.2 General Biological and Toxicological Data

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

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

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

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

5.1.2.1 Utility of Data to the CERHR Evaluation

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

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

5.1.3 Developmental Toxicity

There are no data on the developmental toxicity of DnOP in humans. Two studies, where massive doses of DnOP were administered (4,890 and 9,780 mg/kg bw/day) to rats or mice by gavage or IP injection, suggest a potential for adverse prenatal effect or effect during the perinatal period expressed as death, growth retardation, and/or malformations (*23, 24*). However, litter size and pup weight and mortality were unaffected in a continuous breeding study where mice were exposed to dietary concentrations up to 7,500 mg/kg bw/day (*25, 26*). A primary metabolite, n-octanol, gave no sign of developmental toxicity at doses up to 1,300 mg/kg bw/day in rats (*27*). This dose caused severe intoxication and some deaths in the dams and prompts speculation whether the DnOP rat study that administered 4,890 mg/kg bw/day (*23*) led to severe maternal intoxication as well. The authors were silent on maternal effects. The limited study designs do not provide a basis for comparing consistency of response in the two species, nor do they allow meaningful assessment of dose-response relationships and determination of either LOAELs or NOAELs with any degree of confidence. The available studies do suggest a developmental toxicity response with gavage or IP administration with very high doses.

Table 2: 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 developmental toxicity study with IP exposure in Sprague-Dawley rats. 5 dams/group received 0, 4,890, or 9,780 mg/kg bw on gd 5, 10, and 15. Fetuses were evaluated on gd 20. (*23*)	Maternal: Not reported. Developmental: None.	Not reported.	4,890 ↑ Fetuses with external malformations. ↓ Fetal weight.	↑ Fetuses with external malformations. ↓ Fetal weight.

Prenatal gavage toxicity screening assay in CD-1 mice. 40 dams/group received 0 or 9,780 mg/kg bw/day on gd 6–13. Dams and pups evaluated on pnd 3 for litter size, survival, and body weight changes only. (*24*)	Maternal: Not reported. Developmental: None.	Not reported.	9,780 ↓ Litter size on pnd 0. ↓ Pup weight gain on pnd 1–3.	No higher doses.
Continuous breeding study in CD-1 mice. 20 dams/group received DnOP in feed at 0, 1,800, 3,600, or 7,500 mg/kg bw/day throughout mating and gestation. (*26*) (*25*)	Maternal: * Developmental: 7,500	*	No effects on live pups/litter or pup weight.	

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

5.1.3.1 Utility of Data to the CERHR Evaluation

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

5.1.4 Reproductive Toxicity

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

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

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

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

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

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

5.1.4.1 Utility of Data to the CERHR Evaluation

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

5.2 Integrated Evaluation

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

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

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

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

5.3 Expert Panel Conclusions

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

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

There is a single dietary exposure multigeneration study on DnOP in mice which was negative at exposures of 7,500 mg/kg bw/day (*26*). This lack of effect is loosely corroborated by the dietary study in rats (*15*) which found no histologic effects on reproductive organs after sub-chronic exposure to concentrations as large as 350 or 403 mg/kg bw/day for males and females, respectively. In addition, no testicular lesions were observed in young male rats that were gavage dosed with 2,800 mg/kg bw/day for 4 days. Since there are no adverse reproductive effects in either study, no LOAEL can be estimated. The reproductive toxicity NOAEL in mice is 7,500 mg/kg bw/day and in rats is 350 mg/kg bw/day.

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

5.4 Critical Data Needs

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

6.0 REFERENCES

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7.0 WEB TABLES