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Provisional Peer-Reviewed Toxicity Values for

Di-*n*-octyl Phthalate
(CASRN 117-84-0)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR DI-*n*-OCTYL PHTHALATE (CASRN 117-84-0)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

Di-*n*-octyl phthalate (DNOP), CAS No. 117-84-0, is an odorless, colorless, oily liquid. A table of physicochemical properties for DNOP is provided below (see Table 1). DNOP has a low vapor pressure and, therefore, does not evaporate easily. DNOP is also characterized by its low solubility, high boiling point, and low melting point, indicating that DNOP is stable at room temperature and usually occurs as a liquid (ATSDR, 1997). The empirical formula for DNOP is $C_{24}H_{38}O_4$ (see Figure 1). Phthalate esters, as a class, are most often mixed with polyvinyl chloride (PVC) formulations for the production of flexible PVC materials. There are no known commercial sources of pure DNOP; it comprises approximately 20% of the C6-10 phthalate material, of which 50 million pounds were produced in the 1990s.

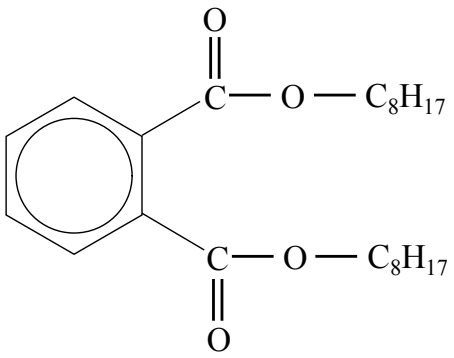

Figure 1. DNOP Structure

Table 1. Physicochemical Properties of DNOP (CASRN 117-84-0)[a]

Property (unit)	Value
Boiling point (ºC at 760 mmHg)	390º C
Melting point (ºC)	−25
Density (g/mL at 25°C)	0.978
Vapor pressure (mmHg at 25ºC)	1.44×10^{-4}
pH (unitless)	No data
Solubility in water (mg/L at 25ºC)	Essentially Insoluble (0.5 ug/L)
Relative vapor density (air = 1)	No data
Molecular weight (g/mol)	390.54
Log K_{ow}	8.06

[a]Source: ATSDR (1997).

The European Chemicals Agency (ECHA, 2010) and Agency for Toxic Substances and Disease Registry (ATSDR, 1997) confirm that there is confusion between the chemical name "di-*n*-octyl phthalate (DNOP)" and the more general term "di-octyl phthalate (DOP)," which is considered a synonym of bis(2-ethylhexyl)phthalate (DEHP). Studies occasionally use ambiguous phthalate nomenclature, which can lead to confusion regarding the identity of the chemical being described. Although studies sometimes use the term "di-octyl phthalate" to indicate di-*n*-octyl phthalate, ATSDR concluded that almost all references to "di-octyl phthalate" were in fact referring to DEHP (ATSDR, 1997).

In an evaluation of DNOP, ECHA (2010) included references that specifically used the chemical name "di-*n*-octyl phthalate." If a study used the term "di-octyl phthalate (DOP)," it was considered to refer to di-*n*-octyl phthalate if the CAS number (117-84-0) for di-*n*-octyl phthalate was provided. This rule is applied in this review in order to limit the possibility of including data on incorrect phthalate esters. In addition, several of the retrieved studies used the acronym "DOP" but also included the full chemical name, "di-*n*-octyl phthalate." In these instances, it was assumed that the chemical of interest was di-*n*-octyl phthalate, and these references are considered in this review.

No reference dose (RfD), reference concentration (RfC), or cancer assessment for DNOP is included on the EPA IRIS database (U.S. EPA, 2010) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2009). No RfD or RfC values are reported in the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 2010). The Chemical Assessments and Related Activities (CARA) list includes a Health and Environmental Effects Profile (HEEP) for DNOP that declines to derive the potential carcinogenicity or noncancer toxicity values due to inadequate noncancer data on this chemical (U.S. EPA, 1987, 1994). The toxicity of DNOP following oral exposure has been reviewed by ATSDR (1997). ATSDR has determined that the liver is the target organ following acute oral exposure based on the reduction in ethoxycoumarin *O*-deethylase (ECOD) activity and increased relative liver weight in rats fed DNOP for 14 days. An acute oral minimal risk level (MRL) of 3 mg/kg-day is determined based on a LOAEL of 1000 mg/kg-day that is established in rats by Lake et al. (1986). For intermediate-duration exposures (exact duration not specified), ATSDR has determined an MRL of 0.4 mg/kg-day using a NOAEL of 40.8 mg/kg-day that is based on a statistically significant increase in hepatic ethoxyresorufin-*O*-deethylase (EROD) activity and histological changes that were observed in the livers of male and female rats (Poon et al., 1997). Thyroid toxicity was also observed at the doses specified by Poon et al. (1997). ATSDR did not derive a chronic MRL.

Neither the World Health Organization (WHO, 2010) nor The California Environmental Protection Agency (CalEPA, 2008, 2009) has derived toxicity values for exposure to DNOP. No occupational exposure limits for DNOP have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2010), the National Institute of Occupational Safety and Health (NIOSH, 2010), nor the Occupational Safety and Health Administration (OSHA, 2010).

The HEAST (U.S. EPA, 2010) does not report any toxicity values or an oral slope factor (OSF) for DNOP. The International Agency for Research on Cancer (IARC, 2010) has not reviewed the carcinogenic potential of DNOP. DNOP is not included in the *12th Report on Carcinogens* (NTP, 2011). CalEPA (2009) has not prepared a quantitative estimate of the carcinogenic potential for DNOP.

Literature searches were conducted on sources published from 1900 through September 2011 for studies relevant to the derivation of provisional toxicity values for di-*n*-octyl phthalate, CAS No. 117-84-0. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (U.S. EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were also searched for relevant health information: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant databases on DNOP and includes all potentially relevant and repeated short-term-, subchronic-, and chronic-duration studies. The principal studies are identified. The phrase, "statistical significance," as used throughout the document, indicates a p-value of <0.05.

Table 2. Summary of Potentially Relevant Data for DNOP (CASRN 117-84-0)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenic	ND							
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	0/49 with endometriosis, 38 female controls with other causes of infertility other than endometriosis, case-control study. A second fertile control group of 21was also used.	Concentrations of phthalates were measured by gas chromatographic analysis of blood.	Women with endometriosis had statistically significant higher blood concentrations of DNOP and other phthalate esters compared with controls; correlation between DNOP and endometriosis: $r = +0.57$ ($p < 0.0001$)	NDr	NDr	NDr	Reddy et al. (2006)	PR

Table 2. Summary of Potentially Relevant Data for DNOP (CASRN 117-84-0)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference	Notes[b]
Neurological	54/77 exposed (lived near plant that reprocessed used motor oil and chemical waste), 29/37 unexposed controls, adults (aged 15−65), matched cohort design, average exposure time 9.3 yr	Direct exposure not measured; 770-ppb DNOP measured in sludge; 960-ppb DNOP measured in sludge and soil. Exposure to water and air plumes from the facility may have occurred.	Exposed subjects had significantly impaired body balance (e.g., sway speed), reaction times, and cognitive and perceptual motor functions; increased signs of depression	NDr	NDr	NDr	Kilburn and Warshaw (1995)	PR
Carcinogenic	ND							
Animal								
			1. Oral (mg/kg-d)[a]					
Subchronic	5/0, F344 rat, diet, 7 d/wk, 4 wk	0, 100, 1000 (adjusted)	Significant increases in relative liver weights and peroxisomal beta-oxidation (PBOX) activities at 2 wk, but not 4 wk, and elevated periportal DNA synthesis at 2 and 4 wk observed in the 1000 mg/kg-d group	100	NDr	1000	Smith et al. (2000)	PR
	10/10, Sprague-Dawley rat, diet, 7 d/wk, 13 wk	**0, 0.4, 3.5, 36.8, 350.1 (males); 0, 0.4, 4.1, 40.8, 402.9 (females) (adjusted)**	**Mild to moderate cytoplasmic vacuolation in the liver at the highest dose (males and females); 3-fold (males) and 2-fold (females) increases in liver EROD activity at the highest dose.**	**36.8**	**NDr**	**350.1**	**Poon et al. (1997)**	**PR, PS**

Table 2. Summary of Potentially Relevant Data for DNOP (CASRN 117-84-0)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference	Notes[b]
Subchronic	5/0, B6C3F$_1$ mouse, diet, 7 d/wk, 4 wk	0, 90, 1804 (adjusted)	Elevated levels of peroxisomal beta-oxidation activity (PBOX) at 4 wk in animals administered 90 mg/kg-d; elevated levels of PBOX at 2 and 4 wk at 1804 mg/kg-d; effects were not considered adverse	NDr	NDr	90	Smith et al. (2000)	PR
Chronic	Unspecified number, males, F344 rat, diet, unspecified frequency, 65 wk	0, 789.5 (adjusted)	Increased *N*-acetyl-β-glucosaminidase, β-galactosidase, α-mannosidase, aryl sulfatase, cathepsin D, and β-glucuronidase levels	NDr[d]	NDr	NDr[d]	Carter et al. (1989) (abstract only)	PR
	12−18/0, F344 rat, diet, 7 d/wk, 60−65 wk	0, 395, 789.5 (initiated with 30-mg/kg diethylnitrosamine (DEN) (adjusted)	No noncancer effects reported	NDr[d]	NDr	NDr[d]	DeAngelo et al. (1989) [conference proceeding]	PR
Reproductive	10/10, CD-1 mouse, diet, 7 d/wk, 7 d prior to and for 98 d of cohabitation	F0: 0, 1820, 3620, 7460 (adjusted)[c] F1: 0, 8640 (adjusted)[c]	F0: No effects on reproductive or clinical parameters in any animals at any dose F1: Highest dose of DNOP resulted in significant increases in liver and seminal vesicle weights in males and kidney and liver weights in females; no effects on reproductive indices or pup outcomes	F0: 7460[e] F1: NDr	NDr	F0: NDr F1: 8640	Heindel et al. (1989); Morrisey et al. (1989). Heidel et al. (1989) is the original report, Morrisey et al. (1989) is a meta-analysis of 48 RACB reproductive studies including the Heindel et al. (1989) study	PR

Table 2. Summary of Potentially Relevant Data for DNOP (CASRN 117-84-0)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference	Notes[b]
Reproductive	F0: 20/20 (treated), 40/40 (control), CD-1 mouse, diet, 7 d/wk, 18 wk F1: 20/20, CD-1 mouse, diet, 7 d/wk, 16 wk	F0 males: 0, 1820, 3620, 7460 (adjusted) F0 females: 0, 1699, 3411, 7120 (adjusted) F1 males: 0, 8101 (adjusted) F1 females: 0, 9438 (adjusted)	F0: No significant effects on reproductive or clinical parameters examined F1: Significant increases in absolute and relative kidney and liver weights in males and females; significant decrease in seminal vesicle weight in F1 males	F0: 7120[e] F1: NDr		F0: NDr F1: 8101	NTP (1985). This study reports the same data as Heinel et al. (1989) and Morrisey et al. (1989)	PR
	SD rats, 7 d per wk by gavage, 500 mg/kg-d, 4 wk. Number of animals not reported.	500 mg/kg-d	Significant changes in sperm counts and sperm motility	NDr	NDr	500	Kwack et al. (2009)	PR
Carcinogenic	Unspecified number, males, F344 rat, diet, unspecified frequency, 65 wk	0, 214	Increase in observed liver nodules	NDr[d]	NDr	NDr[d]	Carter et al. (1989) (abstract only)	PR
	12−18/0, F344 rat, diet, 7 d/wk, 60−65 wk	0, 107, 214	1/13 (8%) with liver carcinoma and 3/13 (23%) with adenoma in the 214-mg/kg-d group	NDr[d]	NDr	NDr[d]	DeAngelo et al. (1989) [conference proceeding] (abstract only)	PR

Table 2. Summary of Potentially Relevant Data for DNOP (CASRN 117-84-0)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference	Notes[b]
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	20/0, ICR rat, inhalation, 2 hr/d, 3 d/wk, 4−16 wk	NR	No effects reported	NDr	NDr	NDr	Lawrence et al. (1975)	PR
Developmental	ND							
Reproductive	ND							
Carcinogenic	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects and a human equivalent concentration (HEC in mg/m^3) for inhalation noncancer effects. Values are converted to a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure.
Doses were converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm) and then converted from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Notes: IRIS = utilized by IRIS, date of last update; PS = principal study; PR = peer reviewed; NPR = not peer reviewed.
[c]Study author-adjusted doses (converted from % in food to mg/kg-d); all other adjusted doses were calculated for this PPRTV document.
[d]NOAELs/LOAELs are not identified because only abstract of study was available.
[e]This value is a NOEL (no-observed-effect level) rather than a NOAEL.

NA = not applicable; ND = no data; NDr = not determinable; NR = not reported; NR/Dr = not reported in study but determined from data.

HUMAN STUDIES

Oral Exposures

The effects of oral exposure to DNOP in humans have not been evaluated in any subchronic, chronic, developmental, reproductive, or carcinogenic studies.

Inhalation Exposures

The effects of inhalation exposure to DNOP in humans have been evaluated in one case-control reproductive study of endometriosis (Reddy et al., 2006) and one neurological cohort study involving persons proximal to a petroleum processing facility (Kilburn and Warshaw, 1995). These studies are summarized below. No additional subchronic, chronic, developmental, or carcinogenic human studies were identified.

Reproductive Studies

Reddy et al. (2006)

In a case-control study, Reddy et al. (2006) investigated the association between phthalate exposure and endometriosis in Indian women. The study group consisted of 49 infertile women who were diagnosed with endometriosis using laparoscopy and were recruited from a hospital and research center serving the region of Andhra Pradesh, India. There were two control groups used in this study. Control Group 1 consisted of 38 women who were attending the same hospital for other gynecological conditions (but were confirmed negative for endometriosis by laparoscopy). All women in Control Group 1 were infertile and 17% reported dyspareunia (painful intercourse), 26% complained of mild dysmenorrhea (pain during menstruation), and 6% complained of severe dysmenorrhea. Control Group 2 consisted of 21 women who had visited the same hospital for laparoscopic tubal sterilization. These women were fertile and had no evidence of endometriosis or any other gynecological conditions. The authors reported that all of the women in the case and control groups had no history of occupational exposure to reproductive toxicants, lived in urban areas, were nonsmokers, and did not consume alcohol.

The authors collected and analyzed blood samples using gas chromatography to determine the concentrations of di-*n*-butyl phthalate (DNBP), di-*n*-octyl phthalate (DNOP), butyl benzyl phthalate (BBP), and diethyl hexyl phthalate (DEHP). When the authors compared the fertility histories of the three study groups, they found that the groups had comparable ages of menarche, durations of infertility (exposed and Control Group 1), ages, and body mass index (BMI). Only pain during intercourse differed among these three groups, with women with endometriosis more commonly reporting this problem (34% versus 17% in Control Group 1).

The results showed that there were significant differences in the blood concentrations of phthalate esters in women with endometriosis compared with women without the condition. The mean blood concentration of DNBP was 0.44 µg/mL in cases diagnosed with endometriosis compared with 0.08 µg/mL in Control Group 1 and 0.15 µg/mL in Control Group 2; mean concentrations of BBP were 0.66 µg/mL in cases diagnosed with endometriosis compared with 0.12 µg/mL in Control Group 1 and 0.11 µg/mL in Control Group 2; mean concentrations of DNOP were 3.32 µg/mL in cases diagnosed with endometriosis compared with 0.00 µg/mL in Control Group 1 and 0.00 µg/mL in Control Group 2; and mean concentrations of DEHP were 2.44 µg/mL in cases diagnosed with endometriosis compared with 0.50 µg/mL in Control Group 1 and 0.45 µg/mL in Control Group 2. The correlation between phthalate concentration and severity of endometriosis was statistically significant and strong for all of the phthalates examined ($r = +0.57$ and $p < 0.0001$ for DNOP).

The authors concluded that this study suggests an association between phthalate ester exposure (exposure route unknown) and endometriosis in Indian women. The authors also noted that DNOP was found at the highest concentration of all of the phthalates examined, followed by DEHP. The results of this study support a study performed by Cobellis et al. (2003) that reported that women with endometriosis had higher serum concentrations of DEHP compared with those without this condition. However, Cobellis et al. (2003) did not measure levels of DNOP. Although this study is limited by its inability to separate the effects of the individual phthalate esters, it is a well-conducted epidemiologic study that lends support to the literature that indicates that DNOP causes reproductive effects in animals.

Neurological Studies

Kilburn and Warshaw (1995)

In a cohort study conducted in Louisiana, Kilburn and Warshaw (1995) investigated neurobehavioral endpoints in residents living near a motor oil and chemical reprocessing plant that was in operation from 1966−1983. The study included those individuals living beyond the plant's dispersion and drainage areas. A large number of chemicals were identified at the combustion site, including methylene chloride, chloroform, trichloroethylene, polychlorinated biphenyls (PCBs), toluene, styrene, chlorobenzene, arsenic, and DNOP. No measurements taken during the time of the plant's operation were available. The study group consisted of 77 women and 54 men between the ages of 15 and 65 years old who were living near the plant and were identified during the course of a class action law suit against the plant (thus recall bias may complicate the interpretation of self-reported symptoms). This group had resided near the site for an average of 9.3 years during the plant's operation. A randomly-selected reference group of 37 women and 29 men was identified from a nearby town and matched for sex and age. Self-administered surveys were designed to assess the demographics, occupational histories, toxic exposures, and neurological and medical histories of the treatment and reference groups. Exposure was not measured directly, but the maximum concentrations of DNOP in the sludge and soil were measured to be 770 ppb and 960 ppb, respectively. Duration of residence and distance from the plant were used as surrogates for exposure measurements. Trained health professionals, blind to the subjects' status, administered a neurophysiological (blink, balance, reaction time, and color discrimination) and neuropsychological (recall; intelligence; visual attention and integrative capacity; constructional, interpretative, and integrative capacity; decision making; peripheral sensation; and discrimination) test batteries. The results of the tests were adjusted for a 1.4-year average difference in educational attainment between the exposed and reference groups.

The results showed that there were significant differences in simple and choice reaction times, body balance, and cognitive and perceptual motor functions between the cohort and reference groups. The effects remained significant after adjusting for age and education. Blink reflex latency and eye closure speed were reported to be normal in both groups. Differences in recall and memory were not significant. Self-reported symptom frequencies and scores for depression, anger, confusion, tension, and fatigue were elevated in the exposed group, which the study authors reported as indicators of depression. Confounding from other diagnosed disorders or occupational disorders were reported as minimal. None of the surrogates for exposure were found to be correlated with the effects identified within the exposed population; however, the range of surrogate values within the group was limited.

The authors concluded that this study suggests an association between exposure to the combustion products and the neurological effects that were observed in the local residents. A large number of chemicals were found at this site, some of which are known toxins. While DNOP was identified on the site, no conclusion regarding the toxicity of DNOP can be made from the available study data.

Carcinogenicity

The carcinogenic effects of inhalation exposure to DNOP have not been evaluated in humans.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure to DNOP in animals have been evaluated in two subchronic (Smith et al., 2000 and Poon et al., 1997), two chronic (Carter et al., 1989; DeAngelo et al., 1989), and three reproductive (Heindel et al., 1989; NTP, 1985; Kwack et al., 2009) studies. No developmental studies were identified. Two carcinogenicity studies were identified (Carter et al., 1989; DeAngelo et al., 1989), but only the abstracts are available for these studies. A number of short-term toxicity studies are available and are presented in the "Other Data" section and summarized in Table 3B.

Subchronic Studies

Smith et al. (2000)

In the rat component of a peer-reviewed subchronic-duration study, Smith et al. (2000) administered doses of 0-, 1000-, or 10,000-ppm DNOP (>99% pure) in the diet to groups of 5 male Fisher 344 (F344) rats for 2 or 4 weeks. Adjusted daily doses are calculated as 0, 100, or 1000 mg/kg-day, respectively, using standard body weight and food consumption rates (0.18 kg and 0.018 kg/day, respectively) because experimental data were not available (U.S. EPA, 1988). Rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Researchers followed the NIH *Guide for the Care and Use of Laboratory Animals* (U.S. DHEW, 1978). Animals were housed in polycarbonate cages, and the room was kept on a 12-hour light/dark cycle. Diet consisted of NIH-07 pelletized feed and deionized water ad libitum. The authors did not report whether this study was Good Laboratory Practice (GLP) compliant.

Smith et al. (2000) sacrificed, weighed, and necropsied the animals following treatment. Blood was collected, and the livers were removed. The authors recorded the relative liver weights and measured liver samples for gap junctional intercellular communication (GJIC), replicative DNA synthesis, and peroxisomal beta-oxidation (PBOX) activity. The authors used two-way analysis of variance (ANOVA) followed by a Dunnett's test to evalute the statistical differences ($p \leq 0.05$) between the groups.

Smith et al. (2000) presented the results in graphs, which were digitized, and the information is presented in Table B.1. The authors noted significant increases in the relative liver weights (113% of controls) and PBOX activities in animals treated with 1000 mg/kg-day compared with controls at 2 weeks. However, at 4 weeks, there were no significant differences between the groups. The authors also noted elevated periportal DNA synthesis in rats administered 1000 mg/kg-day at 2 and 4 weeks (421% and 1370% of controls, respectively). The authors concluded that, because the chronic data are limited for DNOP, it is difficult to

understand the exact significance of the studied endpoints. Based on the effects observed in the liver (increased relative liver weight and elevated periportal DNA synthesis), a NOAEL of 100 mg/kg-day and a LOAEL of 1000 mg/kg-day are identified.

Poon et al. (1997)

Poon et al. (1997) is selected as the principal study for the derivation of subchronic and chronic p-RfD values. Poon et al. (1997) investigated the oral toxicity of DNOP (99% pure; in diet and 4% corn oil) in a 13-week, peer-reviewed study in Sprague-Dawley rats. Young male rats (105−130 g) and female (93−111 g) were obtained from Charles River Laboratories. It is unknown if the study was conducted in compliance with GLP. The study authors administered 0-, 5-, 50-, 500-, or 5000-ppm DNOP daily via diet to groups of 10 animals per sex per dose. The study authors calculated average daily doses of 0, 0.4, 3.5, 36.8, and 350.1 mg/kg-day for males and 0, 0.4, 4.1, 40.8, and 402.9 mg/kg-day for females. The study authors measured animal body weights and food consumption weekly throughout the course of the study. Clinical observations were made daily. At sacrifice, hematology (hematocrit, hemoglobin, red blood cell, platelet, and total and differential white blood cell counts) and serum biochemistry (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities, and albumin, calcium, cholesterol, glucose, inorganic phosphate, potassium, sodium, bilirubin, uric acid, creatinine, blood urea nitrogen, and total protein levels) were measured. The testes, epididymides, adrenal, aorta, bone marrow, brain, esophagus, eyes, heart, intestinal tract, kidneys, liver, mammary glands, mandibular and mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spleen, stomach, trachea and lungs, thyroid and parathyroid, tongue, urinary bladder, and uterus were fixed, sectioned, and stained. It is unclear if histopathologic evaluation was conducted in a blinded fashion. The authors performed a one-way ANOVA and Duncan's multiple range tests.

Poon et al. (1997) reported no signs of clinical toxicity or changes in food consumption in any of the animals exposed to DNOP. Similarly, no significant changes in organ weights were reported in either male or female animals (see Table B.2). Hematology and serum biochemistry measurements were not significantly altered, with the exception of increased calcium levels in males exposed to 350.1 mg/kg-day (117% of controls) and increased inorganic phosphate levels in females exposed to 4.1 mg/kg-day (see Table B.3). Hepatic EROD levels were 3-fold higher (308% of controls) in males and 2-fold higher (212% of controls) in females exposed to the high-dose levels (see Table B.4). No increase in peroxisome proliferation was observed (observational, no quantitative data presented). The levels of DNOP in the livers of the animals of all treatment groups were very low or below the detection limit. However, concentrations of DNOP were 3- to 6-fold higher in the adipose tissue compared with the liver of the high-dose animals (see Table B.5).

Poon et al. (1997) observed "mild microscopic changes" in the livers and thyroids of both sexes of treated animals (see Tables B.6 and B.7). All animals in the high-dose group (10/10 in both sexes) showed a moderate increase in zonation of the liver. Many of the animals in this group also showed mild-to-moderate increases in the perivenous cytoplasmic vacuolization (9/10 males; 5/10 females) in addition to increased perivenous cytoplasmic volume. Mild nuclear prominence was observed in the liver interstitium of a number of rats in the high-dose group (7/10 males; 10/10 females). The study authors also reported mild-to-moderate, dose-dependent anisokaryosis, nuclear hyperchromicity, and vesiculation in treated male rats (observational, no quantitative data presented). Follicles in the thyroid were found to be reduced

in size, and small decreases in colloid density were reported in animals in the high-dose group. None of the other examined endpoints were significantly altered, including morphological effects on male reproductive organs.

Poon et al. (1997) concluded that DNOP is not a peroxisome proliferator at the levels tested in this subchronic study. Despite not observing an effect on liver weight, the study authors concluded that the observed increase in EROD activity indicates that DNOP is a 3-methylcholanthrene-type enzyme inducer. The study authors identified a NOAEL of 36.8 mg/kg-day based on "all gross, histopathological, and biochemical changes" (the study authors reported a NOAEL of 36.6 mg/kg-day; however, this is believed to be a typo, and the correct NOAEL is 36.8 mg/kg-day). Mild-to-moderate cytoplasmic vacuolation accompanied by other hepatic histological changes, thyroid histopathology, and increased EROD activity support the identification of a LOAEL of 350.1 mg/kg-day and a corresponding NOAEL of 36.8 mg/kg-day.

Smith et al. (2000)

In the mouse component of the previously summarized peer-reviewed subchronic study, Smith et al. (2000) administered 0-, 500-, or 10,000-ppm DNOP to groups of 5 male $B6C3F_1$ mice via diet for 2 or 4 weeks. The adjusted daily doses are calculated as 0, 90, and 1804 mg/kg-day, respectively, based on standard body weight and food consumption rates (0.0316 kg and 0.0057 kg/day, respectively) because no experimental data were available (U.S. EPA, 1988). The mice were kept under the same conditions as the rats in this study (see Smith et al., 2000). The authors did not report whether this study was GLP compliant.

Smith et al. (2000) conducted a similar analysis as was performed previously in rats. Again, the results were presented graphically, which were digitized, and the information is presented in Table B.8. The authors found elevated levels of PBOX at both 2 and 4 weeks in mice administered 1804 mg/kg-day (control values not reported). PBOX levels were elevated at 4 weeks in mice administered 90 mg/kg-day (see Table B.8). No other significant changes were noted. The authors concluded that, because chronic data are limited for DNOP, it is difficult to understand the exact significance of the studied endpoints, and may not be a negative health effect. Based on the observed changes in PBOX levels, a LOAEL of 90 mg/kg-day is identified. The LOAEL is the lowest dose administered, preventing the identification of a NOAEL.

Chronic Studies

Carter et al. (1989)

In an abstract, Carter et al. (1989) describe a carcinogenicity study in which 0 or 1% DNOP was administered via the diet to groups (number unreported) of male F344 rats for 65 weeks. The adjusted daily dose for 1% DNOP is 789.5 mg/kg-day, and the human equivalent dose is 214 mg/kg-day, calculated using 0.38 kg as the standard body weight and 0.03 kg/day as the food consumption rate based on values presented by U.S. EPA (1988). The authors reported "numerous liver nodules" in DNOP-treated rats; however, the full study report could not be obtained. In the abstract, the authors reported 3-fold increases in hepatic *N*-acetyl-β-glucosaminidase, β-galactosidase, α-mannosidase, and aryl sulfatase levels. Additionally, cathepsin D and β-glucuronidase levels were increased. The abstract authors concluded that the upregulation of glycosidases may cause sublethal autolysis, and they proposed that this activity may result in tumor induction. These data support the previously summarized study by

Poon et al. (1997) in the identification of enzyme induction and pathologies in the liver. Because only the abstract of this study was available, further evaluation of the carcinogenicity of DNOP could not be accomplished.

DeAngelo et al. (1989)

In the abstract of another carcinogenicity study, DeAngelo et al. (1989) completed a partial hepatectomy and administered a single dose of 30-mg/kg diethylnitrosamine (DEN; a complete carcinogen) to groups of 12−18 male F344 rats and then dosed them with DNOP (0.5% or 1.0%) or DEHP (0.1%, 0.5%, or 2.0%) via the feed for 60−65 weeks. The authors dosed comparable groups that were not initiated with DEN. A control group received the diet and DEN alone, and a positive control group was administered 0.05% phenobarbital (PB; 11 mg/kg-day) in the drinking water. The adjusted daily doses of 395 and 789.5 mg/kg-day and the human equivalent doses of 107 and 214 mg/kg-day DNOP are calculated based on the standard body weight of 0.38 kg and food consumption rate of 0.03 kg/day (U.S. EPA, 1988) because experimental data were not available. It is unclear if this study was GLP-compliant because only an abstract from the Proceedings of the American Association for Cancer Research could be obtained.

After sacrifice, the animals were examined for liver tumors (no other tumor types were examined). Rats that were given only DEN had a 6% incidence of carcinomas (1/18 animals), while the positive control group that received PB had a 94% incidence of carcinomas (16/17 animals). The authors reported that DEHP did not increase the incidence of carcinomas at any dose in the DEN-initiated rats, whereas DNOP did increase the carcinoma incidence to 54% (7/13) at 107-mg/kg-day DNOP and 61% (11/18) at 214-mg/kg-day DNOP. In the animals that were not initiated with DEN, only the group dosed at 214-mg/kg-day DNOP developed liver neoplasms (1/13 [8%] with carcinomas; 3/13 [23%] with adenomas). The authors concluded that DNOP can promote liver carcinomas in rats initiated with DEN through a mechanism other than peroxisome proliferation. Furthermore, DNOP may be carcinogenic without an initiator chemical. This summary was published in a conference proceeding; upon further research, it appears that this study was never published in full. Because only the abstract of this study was available, further evaluation of the carcinogenicity of DNOP could not be accomplished.

Reproductive Studies

Heindel et al. (1989)

In a peer-reviewed, continuous breeding, reproductive toxicity study, Heindel et al. (1989) administered diets containing 0, 1.25, 2.5, or 5.0% DNOP (Midwest Research Institute; >99% pure and stable in feed at room temperature for up to 2 weeks) to male and female CD-1 mice (20 pairs per group) for 7 days prior to and during a 98-day cohabitation period. The authors calculated average daily doses of 0, 1820, 3620, and 7460 mg/kg-day, respectively (calculated by Morrissey et al., 1989). A group consisting of 40 pairs of control animals received feed only. The study was conducted using the Reproductive Assessment by Continuous Breeding Protocol (RACB). This protocol describes four possible tasks: (1) a 14-day dose-setting study with 5 doses and a control group (8 animals per sex per group); (2) a continuous breeding phase with a control group of 40 breeding pairs and 3 dose groups with 20 pairs per group; (3) a 1-week crossover mating trial after the Task 2 litter is weaned using 3 groups of 20 pairs (conducted if Task 2 is positive for reproductive effects); and (4) offspring assessment (conducted if Task 2 is negative for reproductive effects).

Heindel et al. (1989) obtained mice from Charles River Breeding Laboratories, Inc. (Kingston, NY) that were 11 weeks old when the breeding phase began. All animals were quarantined for at least 2 weeks. Animals were initially housed in groups that were segregated by sex during the quarantine and premating periods and then were subsequently housed as breeding pairs or individually. The cages were solid-bottom polypropylene or polycarbonate with stainless steel wire lids. Rodent chow (NIH-07) and deionized/filtered water were provided ad libitum. The room was kept at 23 ± 2ºC (humidity unreported) and maintained on a 14-hour light/10-hour dark cycle. Although this study does not include a GLP certificate, according to Morrisey et al. (1989), all RACB protocol studies are GLP compliant. This study is an acceptable reproductive study based on the reported methodology. However, analysis of the developmental endpoints is limited in the RACB protocol because the offspring are not examined for skeletal or soft tissue anomalies; thus, this study is not considered an acceptable developmental study.

After 98 days of cohabitation in Task 2, Heindel et al. (1989) housed animals individually, and dosing was continued. Any litters born after the continuous breeding phase were weaned and then provided with the dosed feed. These animals were then used for Task 4. The endpoints that were evaluated included clinical signs, parental body weight, fertility (number of adults producing a litter/number of breeding pairs), litters per pair, live pups per litter, proportion of pups born alive, sex of the live pups, pup body weights within 18 hours of birth, and food and water consumption. At the end of the task, the authors necropsied the F0 animals and measured their body weights; organ weights; epididymal sperm motility, morphology, and number; and estrous cyclicity (monitored for 7 days using vaginal lavage). Organs selected for microscopic evaluation were fixed in 10% neutral buffered formalin (Bouin's fixative for the testes), embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Task 3 was not completed for DNOP because the results of Task 2 were not positive for reproductive effects.

Task 4 however, was completed because Task 2 did not indicate reproductive toxicity. The control and high-dose F1 animals were reared, weaned, and dosed until sexually mature (74 ± 10 days) using the same concentrations of DNOP in the diet as their parents. These animals were then cohabitated for 7 days and dosed until necropsy at 95 days. The adjusted daily doses were calculated as 0 and 8640 mg/kg-day (calculated by Morrissey et al., 1989). The endpoints that were examined following mating and necropsy of the F1 mice were the same as those examined in the F0 mice, which included an examination of the F2 offspring for the following: live pups per litter, proportion of pups born alive, sex of the live pups, and pup body weights within 18 hours of birth.

In order to test for dose-related trends (Tasks 2 and 4), Heindel et al. (1989) employed the Cochran-Armitage test. The authors used the Fisher's exact test to make pairwise comparisons between the control and DNOP-dosed groups. The number of litters and live pup indices were calculated for each fertile pair, and group means were calculated for each dose level. The live pup index was calculated as the number of pups born alive divided by the total number of pups produced by each pair. The sex ratio was calculated by dividing the number of male pups born alive by the total number of live pups produced by each pair. The authors used the Kruskal-Wallis test to determine the overall differences between the treatment group means and the Jonckheere's test to determine the ordered differences. The authors used the Wilcoxon-Mann-Whitney *U*-test to make pairwise comparisons of the treatment group means. In order to adjust for any effect that the number of pups per litter had on the average pup weight,

the authors performed analysis of covariance (ANCOVA) using the average litter size as the covariate (live and dead pups). The authors then used the *F*-test and *t*-test to evaluate for overall equality and pairwise equality, respectively, for least-squares estimates of dose-group means. Analyses were performed for males, females, and both sexes in order to account for any sex differences within the parameters that were examined. Organ weights were adjusted for total body weight using ANCOVA. The Kruskal-Wallis and Wilcoxon-Mann-Whitney *U*-tests were used to analyze unadjusted body and organ weights. Finally, the authors used Jonckheere's test to detect any dose-related trends (Tasks 2 and 4).

Heindel et al. (1989) reported that they did not find any treatment-related alterations in physical appearance, body-weight gain, or food consumption in the Task 2 animals. In addition, data indicated that there were no significant treatment-related effects on fertility or reproductive performance in the parental animals. Because there were no reproductive effects observed in Task 2, the F1 animals were cohabitated and mated. The mating indices (percentage of plug-positive animals divided by the number of cohabitated animals) were 80% for the control mice and 95% for the treated F1 mice, and the fertility indices were 75% and 90%, respectively. The number of pups born alive, pup sex, and pup weights were not affected in the F2 generation. However, treatment with 8640-mg/kg-day DNOP significantly increased (by >10% over control) the liver weight in both sexes of the F1 animals, kidney weight in F1 females, and the seminal vesicle weight in F1 males (see Tables B.9 and B.10). Other reproductive parameters, terminal body weights, and reproductive organ weights of the F1 animals of both sexes were not significantly affected by the highest dose of DNOP.

Heindel et al. (1989) concluded that DNOP is not a reproductive toxicant at concentrations of up to 7460 mg/kg-day in CD-1 mice. The authors compared DNOP with other phthalates of varying alkyl chain lengths and concluded that phthalates with side chains containing 3−7 carbons (DNOP has a side chain of 8 carbons) are the phthalates that are toxic to the reproductive system. Based on the lack of any kind of effects in the F0 generation, a no-observed-effect level (NOEL) of 7460 mg/kg-day was determined; a LOAEL could not be identified. Although DNOP did not cause reproductive effects in either generation, a LOAEL of 8640 mg/kg-day is identified based on decreased liver, kidney, and seminal vesicle weights observed at this dose level in the F1 generation. The identification of a NOAEL for the F1 generation is precluded because the only dose identified is the LOAEL.

NTP (1985)

NTP (1985) published a GLP-compliant, multigenerational reproductive toxicity study that exposed male and female CD-1 mice (Charles River Laboratories) to DNOP (Midwest Research Institute, ≥98% pure) in feed. This study, like Heindel et al. (1989), was completed under the RACB. The F0 generation of mice (20 per sex per group) was administered 1.25, 2.50, or 5.0% DNOP in the diet. A control group consisting of 40 males and 40 females received feed only. Adjusted daily doses of 1820, 3620, and 7460 mg/kg-day, respectively, for the F0 males are calculated using average food consumption and body-weight data that were reported by the study authors. Adjusted daily doses of 1699, 3411, and 7120 mg/kg-day, respectively, are calculated for the F0 females using time-weighted average food consumption and body-weight values that were reported by the study authors. Food and water were provided ad libitum. F0 animals were exposed to DNOP for 1 week prior to mating, 14 weeks of cohabitation, and 3 weeks after cohabitation (18 weeks in total). The authors measured the body weight, number of litters produced, number of live and dead pups per litter, mean male and female pup body

weights per litter, percentage of infertile pairs, and number of abnormal pups along with a brief description of any abnormalities. Litters born during the 14 weeks of cohabitation were examined and immediately sacrificed. Pups born during the 3 weeks after cohabitation were allowed to remain with their mothers. The authors did not report the sacrifice or necropsy of the F0 generation after the delivery of the pups. NTP (1985) is an acceptable reproductive study based on the reported methodology. However, the developmental endpoints are limited in the RACB protocol because the offspring are not examined for skeletal or soft tissue anomalies; therefore, this is not considered an acceptable developmental study.

F1 pups from pairs in the high-dose group and the control group were allowed to mature for approximately 70 days after weaning. Pups from the high-dose group received DNOP treatment through lactation until weaning at 3 weeks and then received 5% DNOP in the feed for approximately 13 weeks (16 weeks of total exposure time). The adjusted daily doses were calculated using average body weight and food consumption data that were reported in the study for Weeks 27−31 (from the time of mating until necropsy). The authors reported body weights at weaning (Week 19) but did not record body weights again until mating (Week 27); therefore, an adjusted daily dose based on the time-weighted average body weight would be skewed towards a greatly reduced body weight. The adjusted doses based on averages from the final 5 weeks are much more conservative estimates. These values are 8101 mg/kg-day for males and 9438 mg/kg-day for females. After weaning, 20 males and 20 females from each group were cohabitated for up to 7 days. Mating continued for the full 7 days or until a copulatory plug was found. The authors then examined the same reproductive parameters described above for litters born to the F1 generation. F1 pups were weighed at 3 weeks, on the first day of cohabitation, and once a week thereafter. F1 food consumption was monitored during the week of cohabitation and once per week thereafter. F1 pups were necropsied, and absolute and body weight-adjusted liver, kidney, right epididymis, right cauda, right testis, seminal vesicles, and prostate gland weights were recorded.

The authors used the Kruskal-Wallis test for trend analysis and the Jonckheere's test for ANOVA. Comparisons of proportions were made using the Mann-Whitney *U*-test, chi-squared test, and the Cochran-Armitage test. Data from pairs in which one or both partners died were excluded from statistical analyses.

NTP (1985) reported the death of 3 mice (2 females and 1 male) in the control group and 2 mice (1 female and 1 male) of the F0 generation in the 3620-mg/kg-day dose group (3411 mg/kg-day for females). The authors did not report any effects on body weight (see Tables B.11 and B.12) or food consumption (see Tables B.13 and B.14) in F0 males or females. Treatment had no significant effects on the number of litters delivered per mated pair, number of live pups per litter, sex ratio, or average or adjusted live pup weights of pups born to the F0 generation.

For the F1 generation, successful mating and fertility ratios of the treated mice were comparable to the controls. There were no significant differences in parental body weight (see Tables B.15 and B.16), food consumption, number of litters delivered per mated pair, number of live pups per litter, sex ratio, or average or adjusted live pup weights (F2 generation). Significantly increased absolute and relative liver weights (123% and 128%, respectively) and significantly decreased absolute and relative weights of the seminal vesicles (87% and 89%, respectively) were reported in male mice treated with 8101 mg/kg-day (see Table B.17). No

other significant organ-weight differences were reported in treated males. Necropsy revealed significantly increased absolute and relative kidney weights (111% and 110%, respectively) and absolute and relative liver weights (124% and 122%, respectively) in female mice treated with 9438 mg/kg-day (see Table B.18).

The authors concluded that DNOP did not affect fertility or reproduction in adult or second generation CD-1 mice. Because there were no effects of any kind reported in the F0 generation, a NOEL of 7120 mg/kg-day is identified; no LOAEL could be identified. Based on the increased liver weights in F1 male and female mice, a LOAEL of 8101 mg/kg-day was identified. Because F1 mice were only treated at one dose level, the establishment of a NOAEL for the F1 generation is precluded.

Kwack et al. (2009)

In a third peer-reviewed, reproductive study, Kwack et al. (2009) examined the systemic and sperm toxicity of several phthalate esters including DNOP (purity unreported) when administered to SD rats by gavage. The doses were 250 mg/kg-day for the monoesters and 500 mg/kg-day for the phthalate diesters for 4 weeks to male rats (number of animals not reported) from 6 to 10 weeks of age.

DNOP did not cause systemic changes including increases in food consumption, body weight, or organ weight (thymus, heart, liver, spleen, kidney, adrenal, testis, and epididymis examined). No changes in red blood cell counts or hematocrit, hemoglobin, platelets, or mean corpuscular hemoglobin were observed. The only serum chemistry parameter significantly altered was alkaline phosphatase (ALP), which was increased 3.5-fold. DNOP significantly lowered sperm counts and sperm motility in epididymal sperm. DNOP was intermediate among the phthalates for potency in this regard.

DNOP caused a significant decrease (38% of control) in sperm counts and in sperm motility (31% of control). Other sperm motility indicators including linearity, straightness, beat cross frequency, amplitude of head displacement, curvilinear velocity, straight-line velocity, and average path velocity were unchanged.

Because only one dose was used, a NOAEL cannot be established; however, a LOAEL of 500 mg/kg-day is established for sperm counts and sperm motility.

Inhalation Exposure

The effects of inhalation exposure to DNOP have been evaluated in one chronic animal study (Lawrence et al., 1975). Studies evaluating DNOP inhalation exposure have not been evaluated in subchronic, developmental, reproductive, or carcinogenicity studies.

Short-Term Studies

The short-term effects of inhalation exposure to DNOP have not been evaluated in animals.

Subchronic Studies

The subchronic effects of inhalation exposure to DNOP have not been evaluated in animals.

Chronic Studies

Lawrence et al. (1975)

Lawrence et al. (1975) investigated the inhalation toxicity of DNOP (purity unreported) in a 16-week study on male ICR rats. The study authors saturated the air with DNOP vapors and exposed groups of 20 animals to the vapors for 2 hours per day, 3 days per week, for 4−16 weeks. The actual exposure period could be considered less than chronic; however, the duration of the study was chronic. The exposure cannot be adjusted to continuous exposure because the authors did not measure the concentration used in the study. Following 4, 8, 12, and 16 weeks, 5 mice were sacrificed. It is unknown if the study was conducted in compliance with GLP. The lungs and other unspecified tissues were removed and preserved for histopathology. The study authors reported no effects associated with exposure to DNOP, although data supporting this conclusion were not presented in the study report.

The lack of any exposure measurements and descriptions of the methods and results limits the usefulness of this study. For these reasons, neither a NOAEL nor a LOAEL can be identified. This study does not support the derivation of a subchronic RfC value due to the lack of reported methods and data.

Developmental Studies

The developmental effects of inhalation exposure to DNOP have not been evaluated in animals.

Reproductive Studies

The reproductive effects of inhalation exposure to DNOP have not been evaluated in animals.

Carcinogenecity Studies

The carcinogenic effects of inhalation exposure to DNOP have not been evaluated in animals.

Other Data (Short-Term Tests, Other Examinations)

Several studies are identified and presented in Table 3A that report the genotoxic activity of DNOP in prokaryotic organisms (Goodyear Tire and Rubber Company, 1982a,b; Zeiger et al., 1982, 1985; Sato et al., 1994; Shibamoto and Wei, 1986; Seed, 1982). Other types of genotoxicity studies are not identified. Table 3B presents summaries of DNOP tumor promoter carcinogenicity studies (Carter et al., 1992; DeAngelo et al., 1986), short-term studies (NTP, 1985; Oishi and Hiraga, 1980, 1982; Hinton et al., 1986; Mann et al, 1985; Lake et al., 1984, 1986; Jones et al., 1993; Oishi, 1990; Foster et al., 1980), metabolism/toxicokinetic studies (Albro and Moore, 1974; Calafat et al., 2006; Silva et al., 2005), mode-of-action/mechanistic studies (Mann et al., 1985; Gray et al., 1983; Hinton et al., 1986; Zacharewski et al., 1998), an in vitro neurotoxicity study (Teranishi and Kasuya, 1980), in vitro reproductive studies (Fredricsson et al., 1993; Gray and Beamand, 1984), and an in vitro cytotoxicity study (Jones et al., 1975).

Table 3A. Summary of DNOP Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation[c]		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	Liquid suspension assay with *Escherichia coli* strains W3100 and P3478	10−2000 µg DNOP/plate	–	–	Not mutagenic to *Escherichia coli*	Goodyear Tire and Rubber Company (1982a)
	Ames assay with *Salmonella typhimurium* strains TA98, TA1535, TA1537, and TA100 with and without S9 activation	10−3200 µg DNOP/plate	–	–	Not mutagenic to *Salmonella typhimurium*	Goodyear Tire and Rubber Company (1982b)
	Preincubation modification of the Ames assay with *S. typhimurium* strains TA98, TA100, TA1535, TA1537; incubated at 37ºC for 2 d	100−10,000 µg DNOP/plate (5 doses)	–	–	Not mutagenic to *Salmonella typhimurium*	Zeiger et al. (1982); Zeiger et al. (1985)
	Preincubation procedure with *Salmonella typhimurium* strain TA98	0.25−500 µmol DNOP/plate	–	–	Not mutagenic to *Salmonella typhimurium*	Sato et al. (1994)
	Modified Ames assay with *Salmonella typhimurium* strains TA98 and TA100 with and without S9 activation	NR	–	–	Not mutagenic to *Salmonella typhimurium;* concentrations of 20−300 µg/plate reported for synthetic rubber extract, but unclear if same concentration range used for DNOP	Shibamoto and Wei (1986)
	Salmonella typhimurium strain TA100; mutation to 8-azaguanine resistance and histidine prototrophy assessed	NR	–	–	Concentrations ranged up to 10 mM for other chemicals tested; no DNOP concentration reported	Seed (1982)
SOS repair induction	SOS chromotest with *Escherichia coli* PQ37	0.025−50 µmol DNOP/plate	–	–	Mutagenicity of Trp-1 slightly suppressed by DNOP	Sato et al. (1994)

Table 3A. Summary of DNOP Genotoxicity Studies						
			Results[b]			
Endpoint	Test System	Dose Concentration[a]	Without Activation	With Activation[c]	Comments	References
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal missegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	ND					
Chromosomal aberrations	ND					
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					
Genotoxicity studies in mammals—in vivo						
Chromosomal aberrations	ND					
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					

Table 3A. Summary of DNOP Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation[c]		
Mouse biochemical or visible specific locus test	ND					
Dominant lethal	ND					
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results; highest dose tested for negative results.
[b]+ = positive; ± = equivocal or weakly positive; − = negative; T = cytotoxicity; NA = not applicable; ND = no data; NDr = not determined; NR = not reported; NR/Dr = not reported by the study author, but determined from data.
[c]S9 from rat liver induced with phenobarbital, aroclor, or 5,6-benzoflavone.

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity studies other than oral/inhalation	Male F344 rat; 2/3 partial hepatectomy and single injection of 30 mg/kg of the initiator DEN; 10 d later, 0.5% or 1.0% DNOP administered in diet for 26 wk; immunohistochemistry and histochemistry for neoplasms (gamma-glutamyl transpeptidase [GGT] and glutathione *S*-transferase [GST-P])	Increased GGT levels compared with controls treated with DEN; GGT and GST-P expression increased in liver compared with controls treated with DEN, although this could not be definitively localized to foci or nodules; absolute liver weight not affected by DNOP although slight nonsignificant increase in relative liver weight	Acts as promoter under study conditions	Carter et al. (1992)
	Male Sprague-Dawley rat (5/group); 2/3 partial hepatectomy and single injection of 30-mg/kg DEN (initiator); treated with 0 or 1% DNOP in diet for 7 d/wk for 10 wk; hepatotoxicity evaluated	Significant increase ($p < 0.05$) in GGT+ foci and GGT activity at 1% DNOP compared with controls and animals administered bis(2-ethylhexyl) phthalate (DEHP), MEHP, or 2-ethylhexanol (2-EH); carnitine acetyltransferase (CAT) activity increased in animals treated with DNOP	No concurrent liver enlargement in animals treated with DNOP; DNOP may be a promoter of carcinogenic activity	DeAngelo et al. (1986)
Short-term studies	Male Wister rat (young); administered diets containing 2% mono-*n*-octyl phthalate (MNOP) for 1 wk; serum levels evaluated	Significantly increased levels of serum nonesterified fatty acids; decreased levels of triglycerides and total cholesterol; increased percentage of oleic acids in serum triglycerides; free cholesterol and serum lipoperoxide not significantly altered in treated rats; enlarged liver	Possible hepatotoxicity along with serum lipid effects	Oishi and Hiraga (1982)

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Short-term studies	Male Wistar rat (6 rats in control, 4 rats in exposure group); administered via diet; 0 or 2000 mg/kg-d dose for 3, 10, or 21 d	Accumulation of fat in the centrilobular zone of the liver and fatty necrosis observed at 10 and 21 d; relative liver weight significantly increased at 10 and 21 d; peroxisome proliferation and hepatomegaly at 21 d; smooth endoplasmic proliferation and loss of rough endoplasmic reticulum (unclear at which sacrifice time this effect was observed); serum thyroxine levels decreased at 21 d with ultrastructural changes in thyroid	Indications of liver and thyroid toxicity with severity increasing with duration of exposure	Hinton et al. (1986); Mann et al. (1985)
	Male (unspecified number) Sprague-Dawley rat; 1000 mg/kg-d DNOP by gastric intubation for 14 d; relative liver weight evaluated and hepatic microsomal activities measured	Significantly higher relative liver weight (4.2 ± 0.1 g/100 g-bw) compared with control (3.6 ± 0.1 g/100 g-bw); 7-ethoxycoumarin *O*-deethylase (ECOD) activity significantly lower than controls	Acute hepatotoxic effects at 1000 mg/kg-d; less correlation between in vivo and in vitro effects for DNOP than for other phthalate esters	Lake et al. (1984, 1986)
	Male Wistar rat (unspecified number); 2% DNOP administered via diet for 1 wk; body weight and food consumption, relative organ weights, serum levels, and zinc concentrations in tissue assessed	Relative liver weight significantly higher than control; serum levels of testosterone and dihydrotestosterone not significantly affected; zinc concentrations significantly decreased in the testes	2% DNOP does not cause significant testicular atrophy, but has hepatic effects	Oishi and Hiraga (1980)
	Wistar rat (3/group; 2-g/kg DNOP administered via gavage for 2 d; testicular tissue examined for changes in Leydig cells	No changes observed to the seminiferous tubular structure or Leydig cell morphology	No testicular toxicity	Jones et al. (1993)
	Male Wistar rat; in vitro; mitochondrial fraction of testes incubated with DNOP for 2 min; mitochondrial oxygen consumption measured	Significant decrease in mitochondrial oxygen consumption at 1.3 µmol/mL	May induce testicular atrophy under the experimental conditions	Oishi (1990)

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Short-term studies	Male Wistar rat (5/group); single dose of 2 mg/kg DNOP administered via gavage and animals sacrificed 6 hr later; mitochondrial fraction of testes prepared and mitochondrial oxygen consumption measured	Significant decrease in the respiration control ratio and oxygen consumption	May induce testicular atrophy under the experimental conditions. DNOP causes decreased mitochondrial function with potential fertility effects in male rats	Oishi (1990)
	Male Sprague-Dawley rat (12/group); 2800 mg/kg-d administered via gavage for 4 d; testes removed, weighed, fixed, sectioned, and stained	No significant effects observed	No testicular toxicity	Foster et al. (1980)
	Male and female (8/sex) CD-1 mouse; 0.0, 0.50, 1.25, 2.50, 5.0, or 10.0% DNOP administered via diet for 2 wk; animals observed for clinical toxicity	No effect on daily food consumption or body weight; significant number of males (6/8) and females (4/8) in the 10.0% dose group displayed rough coats	DNOP may cause clinical toxicity in CD-1 mice at 10.0% DNOP.	NTP (1985)
Metabolism/ toxicokinetic	Male CD rat; 0.2-mL DNOP administered via gavage at 24-hr intervals; urine collected for 48 hr following initial dose	Urine contained 31.0% of the phthalate moiety; alkyl side chain permitted a series of α- and β-oxidations of the carboxyl-terminated metabolites; phthalic acid is a minor metabolite	Indicates oral absorption; high occurrence of oxidative metabolites	Albro and Moore (1974)
	Female Sprague-Dawley rat; single gavage dose of 300-mg/kg DNOP; 24-hr urine samples collected and analyzed for metabolites; concurrent human samples taken from a random population without documented exposure to phthalates	Urinary levels of mono-(3-carboxypropyl) phthalate (MCPP) highest (225 μg/mg creatinine) and higher than mono-*n*-octyl phthalate (MNOP) (0.4 μg/mg creatinine); MCPP also detected in 86% of human samples with a mean of 1.4 ng/mL; however, MCPP can also result from other phthalate esters	MCPP more abundant in urine than MNOP, indicating primary metabolism to MNOP followed by oxidative metabolism to compounds such as MCPP	Calafat et al. (2006)

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Female Sprague-Dawley rat (2/group); single oral dose of 300-mg/kg DNOP; urinary metabolite levels measured	MNOP, MCPP, and parent compound detected, with MCPP considered the major metabolite; biphasic excretion pattern; levels decreased significantly after the first day; MCPP, mono-(7-carboxy-*n*-heptyl) phthalate (MCHpP), mono-hydroxy-*n*-octyl phthalate (MHOP), and mono-oxo-*n*-octyl phthalate (MOOP) still detectable after 4 d	Authors concluded that metabolism of DNOP in rats results in a high percentage of oxidative metabolic products in urine; MCPP and select MNOP oxidation products present in urine at higher levels than MNOP	Silva et al. (2005)
Mode–of-action/ mechanistic	Male Wistar rat; 4/time period (6 in control); 2% DNOP administered via diet; animals sacrificed after 3, 10, or 21 d; peroxisomal enzyme and hepatic enzyme activities assessed	Increased activity of cyanide-insensitive palmitoyl CoA oxidation observed in animals sacrificed at Day 10 and Day 21; percentage of catalase activity as part of the liver homogenate significantly increased at Days 10 and 21; 5'-nucleotidase, glucose-6-phosphatase, and succinate dehydrogenase activities significantly decreased at 21 d. and smooth endoplasmic reticulum proliferation and the loss of rough endoplasmic reticulum beginning at 3 d.	DNOP causes hepatotoxic effects in this protocol	Mann et al. (1985); Hinton et al. (1986)
	Primary hepatocytes from male Sprague-Dawley rat; 0.2-mM DNOP or MNOP administered for 48−72 hr	DNOP: carnitine acetyltransferase activity 202% of control; MNOP: carnitine acetyltransferase activity 660% of control; carnitine palmitoyltransferase activity 234% of control; no effect on peroxisome levels	DNOP: slowly hydrolyzed; few effects compared with 2-ethylhexyl ester; MNOP: most potent of straight-chained monoesters; increased enzyme levels due to effects other than peroxisome proliferation	Gray et al. (1983)

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode–of-action/ mechanistic	In vitro rat hepatocytes; incubated with 0.05-, 0.1-, or 0.25-mM DNOP; DNA, protein, and enzyme (including cytochrome P450 and cytochrome b_5) estimation conducted; fat metabolism investigated in a separate assay	Signs of systemic toxicity such as blebbing and vacuolation at 0.25 mM; increased lipid accumulation in all treated groups; fat metabolism assay showed that isolated hepatocytes in rats fasted in the early or late afternoon had increased incorporation of 1-^{14}C-palmitate into triglyceride and cholesterol esters and an increase in fatty acid oxidation	Results suggest connection between the observed early hepatic changes and subsequent liver tumor formation in rats	Hinton et al. (1986)
	17β-estradiol (E2)-dependent recombinant *Saccharomyces cervevisae* strain PL3 incubated with 10 µm DNOP at 30ºC and photographed every 24 hr	Did not support estrogen receptor-mediated growth of PL3	No estrogen receptor-mediated growth	Zacharewski et al. (1998)
	Uterine tissues from 22-d-old Sprague-Dawley rat collected, weighed, homogenized on ice, and centrifuged to separate the cytosol; cytosol incubated with 1 nM [^{3}H]E2 and 1−1000 µM DNOP for 30 min at 30°C and then cooled to 4°C; specific binding of [^{3}H]E2 measured	Did not compete with [^{3}H]E2 for binding with estrogen receptor at any concentration tested	DNOP does not bind with the estrogen receptor	Zacharewski et al. (1998)
	Mature, ovariectomised female Sprague-Dawley rat (10/group); orally dosed with 20, 200, or 2000 mg/kg DNOP in sesame oil for 4 d; vaginal lavages performed once per day to assess vaginal cornification	Statistically significant, but not dose-dependent, decreases in body weight; no significant differences in uterine wet weights; no significant induction of vaginal cornification at any concentration tested	DNOP does not display estrogen receptor-mediated estrogenic activity in vivo	Zacharewski et al. (1998)

Table 3B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode–of-action/ mechanistic	Transiently transfected MCF-7 human breast cancer estrogen receptor-positive cells and stably transfected HeLa cells; concentrations of 0.1, 1, or 10 μM phthalates; incubated for 24 hr and assayed for luciferase activity	No significant induction of luciferase activity at any concentration	DNOP does not bind with the estrogen receptor	Zacharewski et al. (1998)
Immunotoxicity	Adjuvant effects of di-*n*-butyl-, di-*n*-octyl-, di-iso-nonyl- and di-iso-decyl phthalate were studied	Adjuvant effect was accepted to be present if a statistical increase in antibody production occurred in a test group as compared to an ovalbumin control group together with the fulfillment of dose-response relationships	Phthalates with 8 or 9 carbon atoms in the alkyl side chains were the stronger adjuvants, whereas phthalates with shorter or longer alkyl side chains possessed less adjuvant activity.	Larson et al (2003)
Neurotoxicity	In vitro test; fibroblasts from newborn rat cerebellum in primary culture; concentrations of 1.3×10^{-4}, 7.5×10^{-4}, or 12.5×10^{-4} M	Granulation at 1.3×10^{-4} M and slightly depressed outgrowth at 7.5 and 12.5×10^{-4} M; results not significant	DNOP the least toxic to fibroblasts out of the five esters tested (DNOP, dimethyl phthalate [DMP], diethyl phthalate [DEP], di-*n*-butyl phthalate [DNBP], and di-*n*-heptyl phthalate [DNHP])	Teranishi and Kasuya (1980)
Reproductive toxicity	In vitro test on human spermatozoa transferred to a defined medium by the swim-up procedure or separation by the Percoll gradient; concentrations of 0−640 μM DNOP	Significant, dose-response decrease in motility; DNOP did not affect linearity, mean amplitude of lateral displacement of the sperm head, or velocity	DNOP the least toxic to sperm compared with other phthalate esters tested (dibutyl phthalate [DBP], diethyl phthalate [DEP], DMP, and DEHP)	Fredricsson et al. (1993)
	Cultures of adhered rat Sertoli cells with germ cells (spermatocytes and spermatogonia); treated with 10^{-6}–10^{-4} M DNOP for 24−48 hr	Dose-dependent increase in germ cell detachment after treatment with DNOP; marked detachment at 10^{-4} M and disruption of Sertoli-cell monolayer	Positive for toxicity in male reproductive cells	Gray and Beamand (1984)
Other	Human WI-38 diploid cells; exposed to unreported DNOP concentration for 22 hr	170 μM caused 50% inhibition of growth	DNOP has mid-level ID50 compared with other phthalate esters	Jones et al. (1975)

ND = no data.

Tests Evaluating Carcinogenicity, Genotoxicity, and/or Mutagenicity

All studies evaluating DNOP for mutagenic activities were negative (see Table 3A). DNOP has been tested using reverse mutation and SOS repair induction assays in *Salmonella typhimurium* and *Escherichia coli*. The results of these studies did not indicate that DNOP has mutagenicity ratios that are significantly different from the negative controls, with or without metabolic activation (Goodyear Tire and Rubber Company, 1982a,b; Zeiger et al., 1982, 1985; Sato et al., 1994; Shibamoto and Wei, 1986; Seed, 1982). In vivo and in vitro genotoxicity studies performed on mammalian eukaryotic organisms, mammalian cells, mammals, or subcellular models were not identified.

Other Toxicity Studies (Exposures Other Than Oral or Inhalation)

Carcinogenicity

Two studies investigated the ability of DNOP to act as a liver tumor promoter using surgical procedures and the administration of DEN as an initiating agent.

Carter et al. (1992)

Carter et al. (1992) performed a 2/3 partial hepatectomy on male F344 rats (unspecified number of animals; control group included) and dosed them with 30-mg/kg DEN to initiate carcinogenesis. Ten days later, the authors administered 0, 0.5%, or 1.0% DNOP via the diet for 26 weeks. The authors evaluated neoplastic activity by measuring gamma-glutamyl transpeptidase (GGT) and glutathione S-transferase (GST-P) enzyme levels in the liver. Immunohistochemical methods detected a significant increase in the percentage of the liver expressing GGT after the administration of both concentrations of DNOP. The volume percentage of liver expressing GST-P was significantly greater in animals treated with both DEN and 1.0% DNOP than those treated with DEN alone. No changes in absolute liver weight were reported; however, the authors did note a slight increase in the relative liver weight after DNOP treatment. The authors concluded that DNOP acted as a tumor promoter in conjunction with DEN as an initiator.

DeAngelo et al. (1986)

DeAngelo et al. (1986) performed a 2/3 partial hepatectomy on male Sprague Dawley rats (five animals per goup; control group included) and dosed them with 30-mg/kg DEN to initiate carcinogenesis prior to the administration of DNOP. Animals were treated with 0 or 1.0% of DNOP via the diet for 7 days per week for 10 weeks. After sacrifice by carbon dioxide asphyxiation, the authors measured the body and liver wet weights, performed gross examinations of the livers, measured enzyme levels, and stained and counted the number of GGT+ foci. The authors found a significant increase in GGT+ foci and GGT activity in animals administered 1.0% DNOP compared with the controls and in animals that were administered DEHP, MEHP, or 2-EH. In addition, carnitine acetyltransferase (CAT) activity was increased in animals treated with DNOP compared with controls, although no concurrent liver enlargement was observed in these animals. These results suggest that DNOP is a promoter of carcinogenic activity. However, neither of these carcinogenicity studies can be considered for reference derivation due to the lack of methodology and data presented in the abstracts.

Short-Term Studies

Oishi and Hiraga (1982)

In a short-term study, Oishi and Hiraga (1982) administered diets containing 2% mono-*n*-octyl phthalate (MNOP), a metabolite of DNOP, to young male Wistar rats (number

not specified) and evaluated serum parameters after 1 week of administration. The authors reported significant increases in nonesterified fatty acids in the serum, decreases in triglycerides and total cholesterol levels, an increased percentage of oleic acids in serum triglyceride levels, and enlarged livers. These results indicate that MNOP significantly alters serum lipid components and may be hepatotoxic.

Hinton et al. (1986)

Hinton et al. (1986) administered concentrations of 2000-mg/kg-day DNOP to male Wistar rats (6 rats in control group; 4 per exposure group) via the diet for 3, 10, or 21 days. Rats were then sacrificed, blood was drawn, and organs were removed for analysis. Administration of DNOP led to the accumulation of fat in the centrilobular zone of the liver and fatty necrosis in those animals dosed for 10 and 21 days. Liver weight was also increased at 10 and 21 days, and peroxisome proliferation and hepatomegaly was noted at 21 days. The authors posited that these hepatic changes may be indicative of subsequent tumor formation. Effects on the thyroid were also reported. Serum thyroxine (T4) reportedly decreased on Days 3, 10, and 21 (47, 59, and 76% of the control, respectively), while serum triiodothyronine (T3) increased to 133% of the control on Day 21. The study authors also reported ultrastructural changes in the thyroid that included an increase in the number and size of the lysosomes, enlarged Golgi apparatus, and damaged mitochondria. The results suggest a connection between the observed early hepatic changes and subsequent liver tumor formation in rats

Lake et al. (1984, 1986)

In another study, Lake et al. (1984, 1986) administered 1000-mg/kg-day DNOP by gavage to an unspecified number of male Sprague-Dawley rats for 14 days. The authors measured the relative liver weight and activity of the hepatic microsomes. Relative liver weight was significantly increased (117%) in treated animals compared with controls. The authors also reported increased palmitoyl-CoA oxidation (125%), enoyl-CoA hydratase heat labile activity (165%), carnitine acetyltransferase activity (305%), lauric acid hydroxylation (125%), and ethylmorphine *N*-demethylase activity (125%) as well as reduced D-amino acid oxidase activity (55%), EROD activity (55%), and ECOD activity (70%). While the liver appeared to be the target organ for DNOP toxicity, peroxisome proliferation was not observed.

Oishi and Hiraga (1980)

To investigate testicular effects of DNOP in male rats, Oishi and Hiraga (1980) administered 2.0% DNOP via the diet for 1 week and then measured food consumption, relative organ weights, serum levels of hormones, and zinc concentrations in the testicular tissue (zinc deficiency can cause atrophy and retarded growth). The relative liver weight was significantly increased in the treated animals compared with the controls. Zinc concentrations in the testes were significantly decreased (91%) in the treated animals compared with the controls; however, serum levels of testosterone and dihydrotestosterone were not significantly affected by treatment with DNOP. The authors concluded that, while the dietary administration of 2.0% DNOP did not result in testicular atrophy, it did produce hepatic toxicity.

Jones et al. (1993)

Jones et al. (1993) administered 2-g/kg DNOP via gavage to 3 male Wistar rats for 2 days and examined the testicular tissue for changes to Leydig cells. No changes were observed in the structure of the seminiferous tubules or Leydig cell morphology of the treated rats; thus, the authors concluded that DNOP is not a testicular toxin.

Oishi (1990)

In an in vivo experiment, Oishi (1990) administered a single dose of 2-g/kg DNOP via gavage to male Wistar rats (5 per group). Six hours later, the rats were sacrificed, and the mitochondrial function of the testes was examined. As in the in vitro experiment, there was a significant decrease in the mitochondrial oxygen consumption as well as a significant decrease in the respiratory control ratio in the treated rats. In another experiment, Oishi (1990) incubated the mitochondrial fraction from the testes of male Wistar rats with DNOP for 2 minutes and measured mitochondrial oxygen consumption. DNOP did not affect oxygen consumption up to a concentration of 0.65 μmol/mL, but there were significant decreases in oxygen consumption at 1.3 μmol/mL. The effects seen in this study suggest that DNOP causes decreased mitochondrial function with potential fertility effects in male rats.

Foster et al. (1980)

In another in vivo test of testicular toxicity, Foster et al. (1980) administered 2800-mg/kg-day DNOP via gavage to male Sprague-Dawley rats (12 per group) for 4 days. After sacrifice, the testes were removed, weighed, fixed, sectioned, stained, and examined. The treated animals did not display any clinical effects (body weight or food consumption changes). Testicular weights were unaffected, and there were no histological indications of toxicity in the testes. The testicular zinc content was not significantly affected by treatment. The results of this study and the Oishi and Hiragi (1980) in vivo study do not support the results of the in vitro assays (Oishi, 1990; Jones et al., 1993) that suggest DNOP may be toxic to the testes of male rats.

NTP (1985)

NTP (1985) administered 0.0, 0.50, 1.25, 2.50, 5.0, or 10.0% DNOP to male and female CD-1 mice (8 per sex) via the diet for 2 weeks. All animals were observed for clinical toxicity. The authors reported that a significant number of males (6/8) and females (4/8) in the 10.0%-dose group had rough coats; no other effects were noted.

Metabolism/Toxicokinetic Studies

Several studies have examined the metabolism and toxicokinetics of DNOP.

Albro and Moore (1974)

Albro and Moore (1974) administered 0.2-mL DNOP neat via gavage to male CD rats at 24-hour intervals. Urine testing indicated that 31.0% of the phthalate moiety remained, that there were many oxidative metabolites present, and that phthalic acid was a relatively minor metabolite. The study authors concluded that DNOP is initially metabolized to carboxyl-terminated metabolites and then α- and β-oxidations of the alkyl side chains produce a high incidence of oxidative metabolites.

Calafat et al. (2006)

Calafat et al. (2006) found similar results. The authors administered a single dose of 300-mg/kg DNOP via gavage to female Sprague-Dawley rats and analyzed 24-hour urine samples for metabolites. The study authors also analyzed human urine samples collected from a random population with no documented exposure to phthalates and measured phthalate metabolites. Mono-(3-carboxypropyl) phthalate (MCPP) was the most abundant metabolite detected in the experimental rats (average of 255 μg/mg creatinine). In contrast, MNOP was detected at an average of 0.4 μg/mg creatinine. MCPP was detected in 86% of the human urine

samples (mean: 1.4 ng MCPP/mL). While MCPP is a primary metabolite of DNOP, it is also a minor metabolite of other phthalates (e.g., DBP). However, other phthalates produce much smaller quantities of MCPP than DNOP (e.g., 0.6 µg/mg creatinine MCPP from DNOP metabolism and 11.6 µg/mg creatinine MCPP from DBP). The observed production of MCPP, as compared with the monoester, suggests that DNOP is primarily metabolized through an oxidative pathway.

Silva et al. (2005)

In another metabolic study, Silva et al. (2005) administered single oral doses of 300-mg/kg DNOP to 2 female Sprague-Dawley rats and measured urinary metabolite levels. As in the Calafat et al. (2006) study, MCPP was considered the major metabolite. MNOP and the parent compound were detected at much smaller concentrations. The authors observed a biphasic excretion pattern with metabolite levels decreasing significantly after the first day. Other DNOP oxidative metabolites identified included mono-carboxymethyl phthalate (MCMP), mono-(7-carboxy-*n*-heptyl) phthalate (MCHpP), mono-(5-carboxy-*n*-pentyl) phthalate (MCPeP), and isomers of mono-hydroxy-*n*-octyl phthalate (MHOP), and mono-oxo-*n*-octyl phthalate (MOOP), all of which remained detectable in the urine 4 days after administration.

Mode-of-Action/Mechanistic Studies

Mann et al. (1985)

Mann et al. (1985) examined the mechanisms of liver toxicity that were observed in rats in a previously described study (Hinton et al., 1986) in which the authors administered 2.0% DNOP via the diet for 3, 10, or 21 days. Mann et al. (1985) noted peroxisome proliferation and hepatomegaly at 21 days and smooth endoplasmic reticulum proliferation and the loss of rough endoplasmic reticulum beginning at 3 days, which remained apparent through 21 days. The percentage of catalase activity as part of the liver homogenate was significantly increased at 10 and 21 days, and 5'-nucleotidase, glucose-6-phosphatase, and succinate dehydrogenase were significantly decreased at 21 days. There was also a slight, significant increase in cyanide-insensitive palmitoyl Coenzyme A oxidation at both 10 and 21 days.

Gray et al. (1983)

Using an in vitro assay, Gray et al. (1983) exposed primary male Sprague-Dawley rat hepatocytes to 0.2-mM DNOP or MNOP for 48−72 hours. DNOP produced a 202% increase (significant when compared with controls) in carnitine acetyltransferase activity. The administration of MNOP resulted in a 660% increase in carnitine acetyltransferase activity and a 234% increase in carnitine palmitoyltransferase activity. Peroxisome numbers were unaffected by 48-hour treatment with 0.2-mM MNOP. The authors concluded that MNOP was the most potent straight-chained monoester examined and that it is much more potent than the parent compound, DNOP.

Hinton et al. (1986)

Using a similar in vitro assay, Hinton et al. (1986) incubated rat hepatocytes with 0.05-, 0.1-, or 0.25-mM DNOP and measured protein and enzyme levels and fat metabolism. In agreement with Gray et al. (1983), the authors did not observe any increase in cyanide-insensitive palmitoyl Coenzyme A oxidation. Systemic toxicity was seen at 0.25 mM, which included signs such as blebbing and vacuolation, but no increase in cell death. All treated groups showed increased lipid accumulation. In a separate fat metabolism assay, the authors noted that hepatocytes isolated from fasted rats or those fed ad libitum in the afternoon showed

increased incorporation of 1-^{14}C-pamitate into triglyceride and cholesterol esters and an increase in fatty acid oxidation. Rats fed ad libitum in the morning showed minor changes.

Zacharewski et al. (1998)

Zacharewski et al. (1998) utilized an estrogen receptor-mediated yeast assay in which 17β-estradiol (E2)-dependent recombinant *Saccharomyces cervevisae* strain PL3 was incubated with 10 μm DNOP on a selective medium at 30ºC and photographed every 24 hours. The results indicated that DNOP does not exhibit ER-mediated growth of PL3, suggesting it is not estrogenic in vitro.

Due to evidence of the estrogenic activities of other phthalate esters, a series of experiments on the estrogenicity of DNOP were conducted by Zacharewski et al. (1998). In an in vitro competitive ligand-binding assay, uterine tissues were collected from 22-day-old Sprague-Dawley rats, weighed, homogenized, and centrifuged in order to separate the cytosol. The cytosol was then incubated with 1 nM [^{3}H]E2 and 1−1000 μM DNOP for 30 minutes at 30°C and then cooled to 4°C. The results suggest that DNOP is not estrogenic because DNOP did not compete with [^{3}H]E2 for binding to the estrogen receptor at any of the concentrations tested.

Using another assay, Zacharewski et al. (1998) administered to mature, ovariectomized female Sprague-Dawley rats (10 per group) oral doses of 20, 200, or 2000 mg/kg DNOP in sesame oil over 4 days. The authors performed vaginal lavages once per day to access vaginal cornification. Signs of clinical toxicity were also investigated. Statistically significant, but not dose-dependent, decreases in body weight were observed at all three doses. Uterine wet weight was not affected by treatment with DNOP, and vaginal cornification was not found to be significantly increased in the treated animals. These results indicate that DNOP does not have estrogen receptor-mediated estrogenic activity in vivo.

Using a gene expression in vitro assay, Zacharewski et al. (1998) utilized MCF-7 human breast cancer estrogen receptor-positive cells and HeLa cells transfected with a chimeric receptor/reporter system to assess binding of DNOP to the human estrogen receptor. These cells were exposed to concentrations of 0.1, 1, or 10 μM DNOP for 24 hours and then assayed for luciferase activity as an indicator of estrogen receptor binding. DNOP did not cause significant induction of luciferase activity at any concentration, providing further support that DNOP is not estrogenic in vitro.

Immunotoxicity

There are several studies by Larsen and colleagues that address immunological effects of phthalates. In the Larsen et al. (2003) study, the adjuvant effects of di-*n*-butyl-, di-*n*-octyl-, di-iso-nonyl- and di-iso-decyl phthalate are studied in a screening model. Ovalbumin, used as the model antigen, was injected subcutaneously in the neck region of BALB/cJ mice with the selected phthalate in concentrations from 2–2000 μg/ml. Additionally, the mice were boosted once or twice with ovalbumin alone. Immunization with ovalbumin alone, the ovalbumin control group, served as the baseline for antibody production, whereas aluminium hydroxide served as the positive control. The levels of ovalbumin-specific IgE, IgG1, and IgG2a antibodies in sera were determined. Adjuvant effect was accepted to be present if a statistical increase in antibody production occurred in a test group as compared to an ovalbumin control group together with the fulfillment of dose-response relationships. Adjuvant effect varied strongly between the

phthalates investigated. Phthalates with 8 or 9 carbon atoms in the alkyl side chains were the stronger adjuvants, whereas phthalates with shorter or longer alkyl side chains possessed less adjuvant activity. Adjuvant effects were apparent either from the IgE or the IgG1 response or both, whereas no effect was seen on the IgG2a response.

Neurotoxicity

Teranishi and Kasuya (1980)

Using an in vitro assay, Teranishi and Kasuya (1980) incubated primary fibroblasts from newborn rat cerebellum with concentrations of 1.3×10^{-4}, 7.5×10^{-4}, or 12.5×10^{-4} M DNOP. Although the results were not significant, the authors noted granulation in the fibroblasts exposed to 1.3×10^{-4} M DNOP and slightly depressed outgrowth of cells exposed to $7.5 \times 10^{-4}$and 12.5×10^{-4} M DNOP. DNOP was the least toxic phthalate ester to fibroblasts of the esters studied (DNOP, DMP, DEP, DNBP, and DNHP).

Reproductive Toxicity

Two studies investigated the in vitro reproductive toxicity of DNOP in human males.

Fredricsson et al. (1993)

Fredricsson et al. (1993) transferred human spermatozoa to a defined medium by either the swim-up procedure or separation by Percoll gradient. The spermatozoa were then exposed to concentrations of 0−640 µM DNOP and examined for effects to their motility and morphology. The results indicated that there was a significant, dose-response decrease in motility. However, DNOP treatment did not affect linearity, mean amplitude of lateral displacement of the sperm head, or velocity at any concentration. Although this indicates that DNOP may slightly affect male reproduction, the authors considered it to be the least toxic chemical to sperm when compared with DBP, DEP, DMP, and DEHP.

Gray and Beamand (1984)

Gray and Beamand (1984) treated cultures of rat germ cells (spermatocytes and spermatogonia) that were adhered to Sertoli cells in concentrations of 10^{-6}−10^{-4} M DNOP for 24−48 hours. The authors reported a dose-dependent increase in germ cell detachment after treatment with DNOP and marked detachment and disruption of the Sertoli-cell monolayer at 10^{-4} M DNOP. Although these two in vitro studies suggest that DNOP treatment may cause male reproductive effects, there is very little in vivo data to support this hypothesis (Heindel et al., 1989; NTP, 1985).

Other Studies

Jones et al. (1975)

In order to examine the cytotoxicity of DNOP that could potentially leach out of certain medical devices, Jones et al. (1975) exposed human WI-diploid cells to an unreported concentration of DNOP for 22 hours. A concentration of 170-µM DNOP caused a 50% inhibition in the growth of cells. Compared with other phthalate esters, DNOP was in the middle of the range of calculated ID50 values (i.e., dose causing 50% inhibition in growth).

DERIVATION OF PROVISIONAL VALUES

Tables 4 and 5 present a summary of the noncancer reference and cancer values, respectively. IRIS data are indicated in the table, if available.

Table 4. Summary of Noncancer Reference Values for DNOP (CASRN 117-84-0)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Mild-to-moderate cytoplasmic vacuolation in the liver	1×10^{-1}	NOAEL	36.8	300	Poon et al. (1997)
Chronic p-RfD (mg/kg-d)	Rat/M	Mild-to-moderate cytoplasmic vacuolation in the liver	1×10^{-2}	NOAEL	36.8	3000	Poon et al. (1997)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = not determined

Table 5. Summary of Cancer Values for DNOP (CASRN 117-84-0)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	NDr			
p-IUR	NDr			

NDr = not determined

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

Liver effects (changes in histopathology, enzyme activity, and weight) were observed in subchronic (Smith et al., 2000; Poon et al., 1997), chronic (Carter et al., 1989; DeAngelo et al., 1989), and reproductive studies (F1 generation—Heindel et al., 1989; NTP, 1985). Sperm effects were also noted (Kwack et al., 2009) but occurred at higher doses than those eliciting effects on the liver. **The Poon et al. (1997) study is selected as the principal study for the derivation of the subchronic p-RfD value.** It is a published, peer-reviewed study that provides sufficient information in the materials and methods section. Details of the Poon et al. (1997) study are provided in the "Review of Potentially Relevant Data" section of this document. This study employed the lowest doses of any studies found in the database, and provides the most sensitive indication of toxicity. The critical effect selected from this study is mild-to-moderate cytoplasmic vacuolation observed in the liver of rats. This effect was supported by mild–to-moderate accentuation of zonation, endothelial prominence, anisokaryosis, and nuclear

hyperchromicity. Cytoplasmic vacuolation had a high incidence (9/10 males; 5/10 females) at the highest dose (350.1 mg/kg-day in males and 402.9 mg/kg-day in females), corresponding to a NOAEL of 36.8 mg/kg-day in males and 40.8 mg/kg-day in females. These histopathological changes were the most sensitive endpoint identified in this study or any other study found in the database. Enzymatic induction also occurred; hepatic EROD was increased 3-fold in high-dose males and 2-fold in high-dose females. Benchmark dose (BMD) analysis of the data from Poon et al. (1997) do not show adequate model fits in the low-dose region of the curve (i.e., none of the models showed a goodness of fit greater than 0.1).

Other oral in vivo studies support the identification of the liver as the target organ of toxicity for DNOP. Several studies have reported changes in liver weight or liver enzymes (NTP, 1985; Heindel et al., 1989; Smith, 2000; Carter et al., 1989). Absolute and relative liver weights were significantly increased in male mice (23% and 28%, respectively) dosed with 8101-mg/kg-day DNOP in feed and in female mice (24% and 22%, respectively) dosed with 9438-mg/kg-day DNOP in feed (NTP, 1985). Decreased liver weights were reported in Heindel et al. (1989) at 8640-mg/kg-day DNOP in the diet. Enzymatic changes in the liver were supported by Smith et al. (2000) who found elevated levels of PBOX in mice administered 1804-mg/kg-day DNOP in the feed at both 2 and 4 weeks and in mice administered 90-mg/kg-day DNOP in the feed at 4 weeks. Carter et al. (1989) reported a 3-fold increase in hepatic *N*-acetyl-β-glucosaminidase, β-galactosidase, α-mannosidase, and aryl sulfatase levels and an increase in cathepsin D and β-glucuronidase levels in mice dosed with 789.5-mg/kg-day DNOP. Two chronic studies also reported increases in liver nodules or liver tumors following DNOP administration, although the full text of these studies could not be obtained (Carter et al., 1989; DeAngelo et al., 1989). As a result, these chronic studies were not suitable for use in the derivation of a chronic RfD.

The histopathological and enzymatic liver effects described by Poon et al. (1997) were not accompanied by any significant changes in liver weight. Thoolen et al. (2010) discussed this issue and indicated that hepatocellular hypertrophy is characterized by the enlargement of the hepatocyte cytoplasm and other alterations in cytosolic protein and/or organelle numbers that can be considered an "adaptive response" to chemical stress. However, excessive hypertrophy can lead to hepatocellular degeneration and necrosis. The high incidence of numerous histopathological changes and the consistency across the Poon et al. (1997) study and other studies suggest that DNOP ultimately overcomes homeostatic mechanisms in the liver. Therefore, given the weight of evidence of liver effects described in the principal and supporting studies as well as the high incidence of the critical effect (mild-to-moderate cytoplasmic liver vacuolation in 9/10 male and 5/10 female rats that is accompanied by the accentuation of zonation, endothelial prominence, anisokaryosis, and nuclear hyperchromicity), this effect is considered the most sensitive for the derivation of a subchronic p-RfD. The $NOAEL_{ADJ}$ of 36.8 mg/kg-day in male rats is chosen as the POD to derive the subchronic p-RfD.

$$\textbf{Subchronic p-RfD} = \textbf{NOAEL}_{\textbf{ADJ}} \div \textbf{UF}_{\textbf{C}}$$
$$= 36.8 \text{ mg/kg-day} \div 300$$
$$= \mathbf{1 \times 10^{-1}\ mg/kg\text{-}day}$$

Table 6 summarizes the uncertainty factors for the subchronic p-RfD for DNOP.

Table 6. Uncertainty Factors for Subchronic p-RfD for DNOP		
UF	**Value**	**Justification**
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans.
UF_D	3	A UF_D of 3 is applied because the database includes two acceptable two-generation reproduction studies in mice (Heindel et al., 1989; NTP, 1985), but there are no acceptable developmental studies.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was utilized.
$UF_C \leq 3000$	300	

The confidence of the subchronic p-RfD for DNOP is medium, as explained in Table 7 below.

Table 7. Confidence Descriptors for Subchronic p-RfD for DNOP		
Confidence Categories	**Designation[a]**	**Discussion**
Confidence in study	M	Confidence in the key study is medium. Poon et al. (1997) is peer reviewed, but it is unknown if the study was conducted in compliance with GLP. The critical effect of cytoplasmic vacuolation of the liver is supported by other liver effects observed in this study as well as a number of other studies that reported significant liver alterations.
Confidence in database	M	The database includes subchronic toxicity studies in two species (rat and mouse), two chronic toxicity studies in rats, and two two-generation reproductive studies in mice but no developmental toxicity studies.
Confidence in subchronic p-RfD[b]	M	The overall confidence in the subchronic p-RfD is medium.

[a]L = low; M = medium; H = high.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

Because the data from the two chronic-duration studies on oral DNOP exposure (Carter et al., 1989; DeAngelo et al., 1989) was only available in abstract form and a thorough evaluation of the chronic toxicity of DNOP could not be performed, **the Poon et al. (1997) study was also selected as the critical study for derivation of the chronic p-RfD value.** The same critical effect (mild-to-moderate cytoplasmic vacuolation in the liver that is supported by mild-to-moderate accentuation of zonation, endothelial prominence, anisokaryosis, and nuclear hyperchromicity) as that used to derive the subchronic p-RfD value was used to derive the chronic p-RfD, and the $NOAEL_{ADJ}$ of 36.8 mg/kg-day in male rats is chosen as the POD. The

subchronic p-RfD derivation section of this document further describes the justification for the selection of this study as the principal study and provides a summary of the critical effects.

Chronic p-RfD = $\mathbf{NOAEL_{ADJ} \div UF_C}$
= 36.8 mg/kg-day ÷ 3000
= $\mathbf{1 \times 10^{-2}}$ **mg/kg-day**

Table 8 summarizes the uncertainty factors for the chronic p-RfD for DNOP.

Table 8. Uncertainty Factors for the Chronic p-RfD for DNOP

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to interspecies extrapolations to account for potential toxicokinetic and toxicodynamic differences between rats and humans.
UF_D	3	A UF_D of 3 is applied because the database includes two acceptable two-generation reproduction studies in mice (Heindel et al., 1989; NTP, 1985), but there are no acceptable developmental studies.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	10	A UF_S of 10 is applied for using data from a subchronic-duration study to assess potential effects from chronic-duration exposure because data for evaluating response from chronic-duration exposure are unavailable or insufficient.
$UF_C \leq 3000$	3000	

The confidence of the chronic p-RfD value is medium, as explained in Table 9 below.

Table 9. Confidence Descriptors for the Chronic p-RfD for DNOP

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	Confidence in the key study is medium. Poon et al. (1997) is peer reviewed, but it is unknown if the study was conducted in compliance with GLP. The critical effect of cytoplasmic vacuolation of the liver is supported by other liver effects observed in this study as well as a number of other studies that reported significant liver alterations.
Confidence in database	M	The database includes subchronic toxicity studies in two species (rat and mouse), two chronic toxicity studies in rats, and two two-generation reproductive studies in mice but no developmental toxicity studies.
Confidence in subchronic p-RfD[b]	M	The overall confidence in the chronic p-RfD value is medium.

[a]L = low; M = medium; H = high.
[b]The overall confidence cannot be greater than lowest entry in table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No subchronic or chronic p-RfC values can be derived because no adequate, well-described studies are available. There is only one study available on inhalation exposure to DNOP in animals (Lawrence et al., 1975). The study authors exposed animals 3 days a week for 16 weeks. However, the authors did not provide any exposure measurements or descriptions of the methods and results. Therefore, this study cannot be used for the derivation of an RfC value.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 10 identifies the cancer weight-of-evidence descriptor for DNOP. The abstracts of two chronic bioassays with evidence of DNOP-induced liver tumors (or preneoplastic lesions) are available (DeAngelo et al., 1989; Carter et al., 1989), although the complete studies could not be obtained. In particular, DeAngelo et al. (1989) indicated that, without DEN initiation or surgical alteration, the administration of 1% DNOP (214 mg/kg-day) in the diet increases the incidence of hepatic tumors in male F344 rats. However, no complete carcinogenicity bioassays were located; thus, the available information is not adequate to assess the carcinogenic potential of DNOP.

Table 10. Cancer WOE Descriptor for DNOP

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	Not selected	NA	No human cancer studies are available.
"Likely to Be Carcinogenic to Humans"	Not selected	NA	No strong animal cancer data are available.
"Suggestive Evidence of Carcinogenic Potential"	Not selected	NA	No statistically significant increases in cancer incidence were found in the scientific literature for any animal, at any site, or in any gender.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**Adequate information is not available to assess carcinogenic potential. Available data in abstract form did not contain sufficient information to make a determination.**
"Not Likely to Be Carcinogenic to Humans"	Not selected	NA	No strong evidence of noncarcinogenicity in humans or animals is available.

NA = not applicable.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

The lack of data on the carcinogenicity of DNOP precludes the derivation of quantitative estimates for either oral (p-OSF) or inhalation (p-IUR) exposure. Although two abstracts of chronic bioassays examined the carcinogenic potential of DNOP, there are no full reports available that adequately describe the methodology or present complete data sets; therefore, no carcinogenicity values can be derived.

APPENDIX A. PROVISIONAL SCREENING VALUES

No screening values were calculated.

APPENDIX B. DATA TABLES

Table B.1. DNA Synthesis, PBOX Activities, and Relative Liver Weights of Fisher Rats Exposed to DNOP for 2 or 4 Weeks[a]

Parameter[c,d]		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]		
		0	1000 (100)	10,000 (1000)
2 wk	DNA synthesis (hepatic labeling index, %)	2.08 ± 0.79	2.00 ± 0.43 (96)	8.75 ± 6.16 (421)*
	PBOX activity (-fold increase)	NR	1.13 ± 0.07	3.12 ± 0.28*
	Relative liver weight (% of body weight)	4.29 ± 0.20	4.43 ± 0.20 (103)	4.86 ± 0.30 (113)*
4 wk	DNA synthesis (hepatic labeling index, %)	1.20 ± 0.50	0.96 ± 0.64 (80)	16.45 ± 1.93 (1370)*
	PBOX activity (-fold increase)	NR	1.32 ± 0.07	1.25 ± 0.14
	Relative liver weight (% of body weight)	4.49 ± 0.21	4.42 ± 0.48 (98)	4.79 ± 0.28 (107)

[a]Source: Smith et al. (2000).
[b]Doses converted from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[c]Values expressed as mean ± SD (% of control determined by independent calculations).
[d]All data digitized using GetData Graph Digitizer.

*Significantly different from control ($p \leq 0.05$); data analyzed using two-way ANOVA followed by Dunnett's test.
NR = not reported.

Table B.2. Organ Weights in Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter[c]		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
Male		**0**	**5 (0.4)**	**50 (3.5)**	**500 (36.8)**	**5000 (350.1)**
Sample size		10	10	10	10	10
Final body weight (g)		518 ± 36	540 ± 43 (104)	543 ± 43 (105)	530 ± 61 (102)	534 ± 38 (103)
Liver	Weight (g)	17.1 ± 1.7	18.5 ± 2.1 (108)	18.4 ± 2.1 (108)	18.6 ± 3.4 (109)	18.6 ± 1.5 (109)
	% Body weight	3.31 ± 0.26	3.42 ± 0.38 (103)	3.39 ± 0.27 (102)	3.49 ± 0.25 (105)	3.49 ± 0.15 (105)
Kidney	Weight (g)	1.6 ± 0.1	1.66 ± 0.17 (104)	1.62 ± 0.13 (101)	1.7 ± 0.27 (106)	1.69 ± 0.15 (106)
	% Body weight	0.31 ± 0.02	0.31 ± 0.03 (100)	0.3 ± 0.02 (97)	0.32 ± 0.03 (103)	0.32 ± 0.03 (103)
Testis	Weight (g)	3.46 ± 0.23	3.25 ± 0.42 (94)	3.3 ± 0.19 (95)	3.3 ± 0.26 (95)	3.5 ± 0.23 (101)
	% Body weight	0.67 ± 0.05	0.6 ± 0.08 (90)	0.61 ± 0.05 (91)	0.62 ± 0.07 (93)	0.65 ± 0.05 (97)
Female		**0**	**5 (0.4)**	**50 (4.1)**	**500 (40.8)**	**5000 (402.9)**
Sample size		10	10	10	10	10
Final body weight (g)		296 ± 31	307 ± 33 (104)	302 ± 21 (102)	320 ± 23 (108)	292 ± 32 (99)
Liver	Weight (g)	9.83 ± 1.04	9.66 ± 1.31 (98)	9.83 ± 0.88 (100)	10.25 ± 1.16 (104)	10.3 ± 1.36 (105)
	% Body weight	3.32 ± 0.23	3.15 ± 0.26 (95)	3.26 ± 0.21 (98)	3.2 ± 0.17 (96)	3.52 ± 0.17 (106)
Kidney	Weight (g)	1.02 ± 0.06	1.06 ± 0.18 (104)	0.96 ± 0.17 (94)	1.07 ± 0.1 (105)	1.06 ± 0.07 (104)
	% Body weight	0.35 ± 0.03	0.35 ± 0.05 (100)	0.32 ± 0.07 (91)	0.33 ± 0.02 (94)	0.37 ± 0.03 (106)

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values for weight expressed as mean ± SD (% of control determined by independent calculations).

Table B.3. Hematology and Serum Biochemistry of Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
Male		**0**	**5 (0.4)**	**50 (3.5)**	**500 (36.8)**	**5000 (350.1)**
Sample size		10	10	10	10	10
Hematology[c]	White blood cells (10^3)	5.14 ± 1.23	5.4 ± 2.03 (105)	5.54 ± 2.06 (108)	4.31 ± 1.54 (84)	4.29 ± 1.22 (83)
	Mean corpuscular hemoglobin (pg)	17.64 ± 0.8	17.83 ± 0.82 (101)	17.49 ± 0.6 (99)	17.53 ± 0.64 (99)	17.37 ± 0.69 (98)
	Mean corpuscular volume (µm/m^3)	52.41 ± 2.19	52.74 ± 2.56 (101)	51.8 ± 1.93 (99)	51.9 ± 1.4 (99)	51.27 ± 1.8 (98)
Serum biochemistry[c]	Platelet count (10^3)	911 ± 63	922 ± 81 (101)	895 ± 110 (98)	891 ± 71 (98)	852 ± 72 (94)
	Albumin (g/dL)	3.53 ± 0.22	3.68 ± 0.32 (104)	3.49 ± 0.27 (99)	3.47 ± 0.14 (98)	3.46 ± 0.16 (98)
	Calcium (mg/dL)	8.09 ± 1.56	9.17 ± 0.98 (113)	8.68 ± 1.18 (107)	8.87 ± 0.92 (110)	9.46 ± 1.06 (117)*
	Inorganic phosphate (mg/dL)	6.39 ± 0.569	6.62 ± 1.99 (104)	7.05 ± 0.43 (110)	7.02 ± 0.77 (110)	7.20 ± 0.49 (113)
Female		**0**	**5 (0.4)**	**50 (4.1)**	**500 (40.8)**	**5000 (402.9)**
Sample size		10	10	10	10	10
Hematology[c]	White blood cells[c] (10^3)	3.58 ± 1.51	4.83 ± 1.47 (135)	4.42 ± 1 (123)	4.5 ± 1 (126)	3.91 ± 1.3 (109)
	Mean corpuscular hemoglobin (pg)	18.66 ± 0.6	18.47 ± 0.48 (99)	18.56 ± 0.33 (99)	18.3 ± 0.39 (98)	18.44 ± 0.6 (99)
	Mean corpuscular volume (µm/m^3)	54.43 ± 1.3	54.54 ± 1.52 (100)	54.92 ± 1.9 (101)	53.7 ± 1.1 (99)	53.7 ± 1.4 (99)
Serum biochemistry[c]	Platelet count (10^3)	836 ± 99	923 ± 104 (110)	876 ± 96 (105)	836 ± 85 (100)	817 ± 64 (98)
	Albumin (g/dL)	3.92 ± 0.41	3.83 ± 0.29 (98)	3.91 ± 0.21 (100)	3.92 ± 0.21 (100)	4.08 ± 0.24 (104)
	Calcium (mg/dL)	9.44 ± 0.92	10.05 ± 1.89 (106)	9.65 ± 1.59 (102)	9.79 ± 1.62 (104)	9.33 ± 1.94 (99)
	Inorganic phosphate (mg/dL)	7.22 ± 1.54	7.99 ± 0.68 (111)	8.52 ± 1.14 (118)*	7.64 ± 0.89 (106)	7.96 ± 1.08 (110)

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values are expressed as the mean ± SD (% of control determined by independent calculations).

*Significant ($p < 0.05$) using one-way ANOVA, *t*-test, or Duncan's Multiple Range test.

Table B.4. Ethoxyresorufin-*O*-deethylase Activity in Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter[c,d]	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
Male	**0**	**5 (0.4)**	**50 (3.5)**	**500 (36.8)**	**5000 (350.1)**
Ethoxyresorufin-*O*-deethylase activity	0.12 ± 0.01	0.13 ± 0.02 (106)	0.14 ± 0.03 (111)	0.15 ± 0.03 (125)	0.38 ± 0.20 (308)*
Parameter	**Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]**				
Female	**0**	**5 (0.4)**	**50 (4.1)**	**500 (40.8)**	**5000 (402.9)**
Ethoxyresorufin-*O*-deethylase activity	0.16 ± 0.03	0.17 ± 0.03 (103)	0.17 ± 0.04 (103)	0.20 ± 0.04 (122)	0.35 ± 0.06 (212)*

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values expressed as the mean ± SD, nmol/min/mg protein (% of control determined by independent calculations).
[d]Data were extracted from the study graph(s) using GetData Graph Digitizer, Graph Digitizer Software version 2.24.

*Significant ($p < 0.05$) using one-way ANOVA, *t*-test, or Duncan's Multiple Range test.

Table B.5. Liver and Adipose Tissue Residues in Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter[c]	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
Male	**0**	**5 (0.4)**	**50 (3.5)**	**500 (36.8)**	**5000 (350.1)**
Liver	<3	<3	<3	<3	5 ± 4
Adipose tissue	<3	<3	4 ± 2	7 ± 7	15 ± 4
Female	**0**	**5 (0.4)**	**50 (4.1)**	**500 (40.8)**	**5000 (402.9)**
Liver	<3	<3	4 ± 2	5 ± 3	4 ± 2
Adipose tissue	<3	7 ± 5	<3	<3	25 ± 7

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values expressed as the mean ± SD, ppm wet weight.

Table B.6. Histopathology of Male Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
		0	**5 (0.4)**	**50 (3.5)**	**500 (36.8)**	**5000 (350.1)**
Peroxisomes (% cell area)		4.53	1.50	2.59	1.75	5.50
Liver[c]	Accentuation of zonation	1/10 (10) [0.1]	2/10 (20) [0.2]	1/10 (10) [0.1]	1/10 (10) [0.1]	10/10 (100) [3.1]
	Anisokaryosis	1/10 (10) [0.1]	0/10	4/10 (40) [0.3]	5/10 (50) [0.4]	9/10 (90) [1.9]
	Nuclear hyperchromicity	0/10	0/10	2/10 (20) [0.3]	3/10 (30) [0.4]	5/10 (50) [1.0]
	Perivenous cytoplasmic vacuolation	0/10	0/10	0/10	0/10	9/10 (90) [2.7]
	Endothelial prominence	0/10	0/10	0/10	0/10	7/10 (70) [1.1]
Thyroid[c]	Reduced follicle size	4/10 (40) [0.4]	5/10 (50) [0.6]	6/10 (60) [1.1]	6/10 (60) [1.0]	5/10 (50) [0.8]
	Decreased colloid density	0/10	0/10	3/10 (30) [0.3]	5/10 (50) [0.2]	6/10 (60) [0.4]
Testis[c]	Seminiferous tubule atrophy	7/10 (70) [0.8]	5/10 (50) [0.6]	6/10 (60) [0.5]	6/10 (60) [0.4]	5/10 (50) [0.4]
Epididymis[c]	Bilateral reduction in sperm density	0/10	0/10	0/10	0/10	0/10

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values expressed as the number of lesions observed/number examined (% incidence) [severity score]; percentage was calculated. Values in brackets denote the average severity score where 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. For tissue changes that were focal, locally extensive, and multiple, a score of less than the integer is assigned. These scores are as follows: minimally focal = 0.25; minimal, locally extensive = 0.5; minimal, multifocal = 0.75; mild, focal = 1.25; mild, locally extensive = 1.50; mild, multifocal = 1.75; etc.

Table B.7. Histopathology of Female Sprague-Dawley Rats After Oral Exposure to DNOP for 13 Weeks[a]

Parameter		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]				
		0	**5 (0.4)**	**50 (4.1)**	**500 (40.8)**	**5000 (402.9)**
Peroxisomes (% cell area)		3.69	ND	ND	ND	4.15
Liver[c]	Accentuation of zonation	5/10 (50) [0.4]	6/10 (60) [0.4]	9/10 (90) [0.7]	10/10 (100) [0.8]	10/10 (100) [1.6]
	Anisokaryosis	9/10 (90) [1.5]	10/10 (100) [2.0]	10/10 (100) [2.3]	10/10 (100) [2.5]	10/10 (100) [3.0]
	Nuclear hyperchromicity	3/10 (30) [0.6]	10/10 (100) [2.1]	9/10 (90) [1.6]	10/10 (100) [1.9]	10/10 (100) [2.0]
	Perivenous cytoplasmic vacuolation	0/10	0/10	0/10	0/10	5/10 (50) [1.2]
	Endothelial prominence	0/10	0/10	5/10 (50) [0.5]	9/10 (90) [0.9]	10/10 (100) [1.5]
Thyroid[c]	Reduced follicle size	4/10 (40) [0.4]	6/10 (60) [0.7]	6/10 (60) [0.6]	5/10 (50) [1.0]	8/10 (80) [1.6]
	Decreased colloid density	2/10 (20) [0.1]	0/10	1/10 (10) [0.1]	5/10 (50) [0.3]	4/10 (40) [0.2]

[a]Source: Poon et al. (1997).
[b]Doses were converted to adjusted daily doses by the study authors.
[c]Values expressed as the number of lesions observed/number examined (% incidence) [severity score]; percentage was calculated. Values in brackets denote the average severity score where 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. For tissue changes that were focal, locally extensive, and multiple a score of less than the integer is assigned. These scores are as follows: minimally focal = 0.25; minimal, locally extensive = 0.5; minimal, multifocal = 0.75; mild, focal = 1.25; mild, locally extensive = 1.50; mild, multifocal = 1.75; etc.

ND = not determinable.

Table B.8. DNA Synthesis, PBOX Activities, and Relative Liver Weights of Mice Exposed to DNOP for 2 or 4 Weeks[a]

Parameter[c,d]		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)[b]		
		0	**500 (90)**	**10,000 (1804)**
2 wk	DNA synthesis (hepatic labeling index, %)	2.80 ± 1.16	2.95 ± 0.85 (105)	3.64 ± 0.31 (130)
	PBOX activity (-fold increase)	NR	0.90 ± 0.15	1.12 ± 0.15*
	Relative liver weight (% of body weight)	5.52 ± 0.51	5.21 ± 0.31 (94)	5.91 ± 0.54 (107)
4 wk	DNA synthesis (hepatic labeling index, %)	2.16 ± 0.69	2.24 ± 0.62 (104)	2.85 ± 1.54 (132)
	PBOX activity (-fold increase)	NR	1.73 ± 0.08*	2.03 ± 0.23*
	Relative liver weight (% of body weight)	5.82 ± 0.42	5.40 ± 0.23 (93)	5.71 ± 0.42 (98)

[a]Source: Smith et al. (2000).
[b]Doses converted from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[c]Values expressed as the mean ± SD (% of control determined by independent calculations).
[d]All data digitized using GetData Graph Digitizer.

*Significantly different from control ($p \leq 0.05$); determined using two-way ANOVA followed by Dunnett's test.
NR = not reported.

Table B.9. Mean Body Weights, Organ Weights, and Sperm Parameters in Male CD-1 Mice After Dietary Exposure to DNOP Using a Continuous Breeding Protocol (F1 Generation)[a]

Parameter		Exposure Group, % (Adjusted Daily Dose, mg/kg-d)[b]	
		0	**5 (8640)**
No. of animals		20	20
Weight[c]	Body (g)	36.69 ± 0.86	35.5 ± 0.86 (97)
	Liver (g)	1.96 ± 0.06	2.42 ± 0.08 (123)*
	Kidneys (g)	0.70 ± 0.02	0.70 ± 0.02 (100)
	Right epididymis (mg)	49 ± 1	50 ± 1.6 (102)
	Right cauda epididymis (mg)	19.1 ± 0.7	18.3 ± 0.4 (96)
	Right testis (mg)	131 ± 5	131 ± 6 (100)
	Seminal vesicles (mg)	429 ± 2	374 ± 9 (87)*
	Prostate (mg)	27 ± 2	28 ± 2 (104)
Sperm parameters[c]	Concentration (10^6 sperm/g)	1118 ± 64	1239 ± 78 (111)
	% Motile	94 ± 1	94 ± 1 (100)
	% Abnormal sperm	5.0 ± 0.6	3.5 ± 0.4 (70)

[a]Source: Heindel et al. (1989).
[b]Converted by authors based on average feed consumption.
[c]All parameters expressed as the mean ± SE (% of control determined by independent calculations).

*Significantly different from control ($p < 0.05$).

Table B.10. Mean Body Weights, Organ Weights, and Estrous Cycle Length in Female CD-1 Mice After Dietary Exposure to DNOP Using a Continuous Breeding Protocol (F1 Generation)[a]

Parameter		Exposure Group, % (Adjusted Daily Dose, mg/kg-d)[b]	
		0	**5 (8640)**
No. of animals		20	20
Weight[c]	Body (g)	30.03 ± 0.82	30.61 ± 0.54 (102)
	Liver (g)	1.88 ± 0.06	2.34 ± 0.05 (124)*
	Kidneys (g)	0.479 ± 0.014	0.533 ± 0.011 (111)*
Estrous cycle length (days)		4.63 ± 0.1	4.83 ± 0.2 (104)

[a]Source: Heindel et al. (1989).
[b]Converted by authors based on average feed consumption.
[c]All parameters expressed as the mean ± SE (% of control determined by independent calculations).

*Significantly different from control ($p < 0.01$).

Table B.11. Body Weights of F0 Male CD-1 Mice After Oral Exposure to DNOP for 18 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]			
		0	**1.25 (1820)**	**2.5 (3620)**	**5.0 (7460)**
Wk 1	Sample size	40	20	20	20
	Body weight (g)	35.3 ± 0.30	34.9 ± 0.52 (99)	34.6 ± 0.38 (98)	34.4 ± 0.45 (97)
Wk 2	Sample size	40	20	20	20
	Body weight (g)	36.4 ± 0.30	35.9 ± 0.54 (99)	35.7 ± 0.45 (98)	35.4 ± 0.49 (97)
Wk 3	Sample size	40	20	20	20
	Body weight (g)	35.4 ± 0.30	35.2 ± 0.51 (99)	35.0 ± 0.46 (99)	34.6 ± 0.46 (98)
Wk 6	Sample size	40	20	20	20
	Body weight (g)	37.4 ± 0.47	36.7 ± 0.73 (98)	36.3 ± 0.51 (97)	36.6 ± 0.65 (98)
Wk 10	Sample size	40	20	20	20
	Body weight (g)	39.9 ± 0.65	38.9 ± 0.79 (97)	38.0 ± 0.61 (95)	39.0 ± 0.88 (98)
Wk 14[d]	Sample size	39[e]	20	19[e]	20
	Body weight (g)	40.3 ± 0.72	39.8 ± 0.92 (99)	38.8 ± 0.75 (96)	39.5 ± 1.07 (98)

[a]Source: NTP (1985).
[b]Study authors estimated the administered dose in mg/kg-day based on food consumption and body-weight data gathered over the duration of the study.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]All animals were sacrificed during Week 17 of the study.
[e]One male died during Week 12 of the study.

Table B.12. Body Weights of F0 Female CD-1 Mice After Oral Exposure to DNOP for 18 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]			
		0	1.25 (1699)	2.5 (3411)	5.0 (7120)
Wk 1	Sample size	40	20	20	20
	Body weight (g)	27.8 ± 0.24	27.7 ± 0.50 (100)	27.0 ± 0.47 (97)	27.4 ± 0.44 (99)
Wk 2	Sample size	40	20	20	20
	Body weight (g)	28.9 ± 0.28	28.6 ± 0.53 (99)	29.0 ± 0.62 (100)	29.4 ± 0.45 (102)
Wk 3	Sample size	40	20	20	20
	Body weight (g)	31.5 ± 0.27	32.0 ± 0.50 (102)	30.9 ± 0.45 (98)	31.6 ± 0.47 (100)
Wk 6	Sample size	40	20	20	20
	Body weight (g)	36.3 ± 0.43	37.7 ± 0.84 (104)	36.6 ± 0.66 (101)	36.3 ± 0.60 (100)
Wk 10	Sample size	38[d]	20	20	20
	Body weight (g)	49.3 ± 1.07	49.2 ± 1.75 (100)	47.0 ± 1.84 (95)	49.2 ± 1.65 (100)
Wk 14[e]	Sample size	38	20	19[f]	20
	Body weight (g)	42.2 ± 0.89	44.0 ± 1.35 (104)	43.2 ± 1.9 (102)	44.8 ± 1.77 (106)

[a]Source: NTP (1985).
[b]Doses were converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Time-Weighted Average Food Consumption per Day × (1 ÷ Time-Weighted Average Body Weight) × (Days Dosed ÷ Total Days); Time-Weighted Average Food Consumption per Day and Time-Weighted Average Body Weight were calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Two females died after Week 6 of the study.
[e]All animals were sacrificed during Week 17 of the study except for pregnant or nursing animals.
[f]One female died during Week 13 of the study.

Table B.13. Food Consumption by F0 Male CD-1 Mice After Oral Exposure to DNOP for 18 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]			
		0	**1.25 (1820)**	**2.5 (3620)**	**5.0 (7460)**
Wk 1	Sample size	40	20	20	20
	Food consumption (g/d)	5.42 ± 0.06	5.32 ± 0.13 (98)	5.42 ± 0.12 (100)	4.85 ± 0.08 (89)
Wk 2	Sample size	40	20	20	20
	Food consumption (g/d)	5.59 ± 0.09	5.73 ± 0.23 (103)	5.75 ± 0.15 (103)	5.98 ± 0.17 (107)
Wk 6	Sample size	40	20	20	20
	Food consumption (g/d)	5.39 ± 0.09	5.33 ± 0.10 (99)	5.21 ± 0.12 (97)	5.87 ± 0.17 (109)
Wk 10	Sample size	40	20	20	20
	Food consumption (g/d)	5.07 ± 0.09	5.24 ± 0.12 (103)	5.04 ± 0.11 (99)	5.38 ± 0.12 (106)
Wk 14[d]	Sample size	39[e]	20	19[e]	20
	Food consumption (g/d)	5.30 ± 0.10	5.25 ± 0.15 (99)	4.91 ± 0.11 (93)	5.20 ± 0.15 (98)

[a]Source: NTP (1985).
[b]Study authors estimated the dose in mg/kg-day using food consumption and body-weight data gathered over the duration of the study.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]All animals were sacrificed during Week 17 of the study.
[e]One male died during Week 12 of the study.

Table B.14. Food Consumption by F0 Female CD-1 Mice After Oral Exposure to DNOP for 18 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]			
		0	1.25 (1699)	2.5 (3411)	5.0 (7120)
Wk 1	Sample size	40	20	20	20
	Food consumption (g/d)	5.52 ± 0.08	5.65 ± 0.19 (102)	5.96 ± 0.14 (108)	6.09 ± 0.16 (110)
Wk 2	Sample size	40	20	20	20
	Food consumption (g/d)	5.59 ± 0.09	5.73 ± 0.23 (103)	5.75 ± 0.15 (103)	5.98 ± 0.17 (107)
Wk 6	Sample size	40	20	20	20
	Food consumption (g/d)	5.39 ± 0.09	5.33 ± 0.10 (99)	5.21 ± 0.12 (97)	5.87 ± 0.17 (109)
Wk 10	Sample size	38[d]	20	20	20
	Food consumption (g/d)	5.10 ± 0.09	5.24 ± 0.12 (103)	5.04 ± 0.11 (99)	5.38 ± 0.12 (105)
Wk 14[e]	Sample size	38	20	19[f]	20
	Food consumption (g/d)	5.34 ± 0.10	5.25 ± 0.15 (98)	4.88 ± 0.11 (91)	5.20 ± 0.15 (97)

[a]Source: NTP (1985).
[b]Doses were converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Time-Weighted Average Food Consumption per Day × (1 ÷ Time-Weighted Average Body Weight) × (Days Dosed ÷ Total Days); Time-Weighted Average Food Consumption per Day and Time-Weighted Average Body Weight are calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Two females died after Week 6 of the study.
[e]All animals were sacrificed during Week 17 of the study except for pregnant or nursing animals.
[f]One female died during Week 13 of the study.

Table B.15. Body Weights of F1 Male CD-1 Mice After Oral Exposure to DNOP for 16 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]	
		0	5.0 (8101)
Sample size		20	20
Body weight (g)	Wk 19−20[d]	12.5 ± 0.70	11.4 ± 0.60 (91)
	Wk 27	34.3 ± 0.74	33.1 ± 0.67 (97)
	Wk 28	32.9 ± 0.69	31.9 ± 0.70 (97)
	Wk 29	33.9 ± 0.69	32.8 ± 0.74 (97)
	Wk 30	34.6 ± 0.70	33.7 ± 0.77 (97)
	Wk 31	35.5 ± 0.78	34.6 ± 0.84 (97)

[a]Source: NTP (1985).
[b]Doses are converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Average Food Consumption per Day × (1 ÷ Time-Average Body Weight) × (Days Dosed ÷ Total Days); Average Food Consumption per Day and Average Body Weights were calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Represents weight at weaning; pups were weaned during Weeks 19−20 of the study.

Table B.16. Body Weights of F1 Female CD-1 Mice After Oral Exposure to DNOP for 14 Weeks in a Reproductive Study[a]

Parameter[c]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]	
		0	5.0 (9438)
Sample size		20	20
Body weight (g)	Wk 19−20[d]	11.1 ± 0.44	11.0 ± 0.38 (99)
	Wk 27	27.0 ± 0.76	26.1 ± 0.56 (97)
	Wk 28	28.5 ± 0.77	28.4 ± 0.55 (100)
	Wk 29	33.6 ± 1.15	34.8 ± 0.88 (104)
	Wk 30	39.6 ± 2.60	41.9 ± 2.65 (106)
	Wk 31	29.7 ± 0.71	29.9 ± 0.59 (101)

[a]Source: NTP (1985).
[b]Doses are converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Average Food Consumption per Day × (1 ÷ Time-Average Body Weight) × (Days Dosed ÷ Total Days); Average Food Consumption per Day and Average Body Weight are calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Represents weight at weaning; pups were weaned during Weeks 19−20 of the study.

Table B.17. Terminal Body Weights and Absolute and Relative Organ Weights of F1 Male CD-1 Mice After Oral Exposure to DNOP for 14 Weeks in a Reproductive Study[a]

Parameter[c,d]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]	
		0	5.0 (8101)
Sample size		20	20
Terminal body weight (g)		36.690 ± 0.859	35.540 ± 0.857 (97)
Liver	Absolute weight (g)	1.962 ± 0.058	2.422 ± 0.082 (123)***
	Adjusted weight (g)	1.923 ± 0.042	2.461 ± 0.042 (128)****
Kidney	Absolute weight (g)	0.702 ± 0.021	0.697 ± 0.018 (99)
	Adjusted weight (g)	0.695 ± 0.017	0.705 ± 0.017 (101)
Right epididymis	Absolute weight (mg)	49.225 ± 1.471	50.365 ± 1.623 (102)
	Adjusted weight (mg)	48.609 ± 1.271	50.981 ± 1.271 (105)
Right cauda	Absolute weight (mg)	19.050 ± 0.655	18.305 ± 0.438 (96)
	Adjusted weight (mg)	18.864 ± 0.493	18.491 ± 0.493 (98)
Right testis	Absolute weight (g)	0.131 ± 0.005	0.131 ± 0.006 (100)
	Adjusted weight (g)	0.128 ± 0.005	0.134 ± 0.005 (105)
Seminal vesicles	Absolute weight (g)	0.429 ± 0.017	0.374 ± 0.009 (87)*
	Adjusted weight (g)	0.425 ± 0.013	0.377 ± 0.013 (89)**
Prostate gland	Absolute weight (mg)	27.035 ± 1.782	27.795 ± 1.839 (103)
	Adjusted weight (mg)	27.014 ± 1.846	27.816 ± 1.846 (103)

[a]Source: NTP (1985).
[b]Doses are converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Average Food Consumption per Day × (1 ÷ Time-Average Body Weight) × (Days Dosed ÷ Total Days); Average Food Consumption per Day and Average Body Weight are calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Equation used for adjusting organ weights to body weight was not provided.

*Significantly different from control ($p < 0.05$); Wilcoxon rank-sum test was used.
**Significantly different from control ($p < 0.05$); ANCOVA F-test was used.
***Significantly different from control ($p < 0.01$); Wilcoxon rank-sum test was used.
****Significantly different from control ($p < 0.01$); ANCOVA F-test was used.

Table B.18. Terminal Body Weights and Absolute and Relative Organ Weights of F1 Female CD-1 Mice After Oral Exposure to DNOP for 14 Weeks in a Reproductive Study[a]

Parameter[c,d]		Exposure Group, % food (Adjusted Daily Dose, mg/kg-d)[b]	
		0	5.0 (9438)
Sample size		20	20
Terminal body weight (g)		30.030 ± 0.824	30.610 ± 0.544 (102)
Liver	Absolute weight (g)	1.883 ± 0.059	2.339 ± 0.054 (124)*
	Adjusted weight (g)	1.899 ± 0.043	2.323 ± 0.043 (122)**
Kidney	Absolute weight (g)	0.479 ± 0.014	0.533 ± 0.011 (111)*
	Adjusted weight (g)	0.482 ± 0.012	0.531 ± 0.012 (110)**

[a]Source: NTP (1985).
[b]Doses are converted from percentage of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), then from ppm to mg/kg-day using the following equation: $Dose_{ADJ}$ = Dose × Average Food Consumption per Day × (1 ÷ Time-Average Body Weight) × (Days Dosed ÷ Total Days); Average Food Consumption per Day and Average Body Weight are calculated from recorded data.
[c]Values expressed as the mean ± SE (% of control determined by independent calculations).
[d]Equation for adjusting organ weights to body weight was not provided.

*Significantly different from control ($p < 0.01$); Wilcoxon rank-sum test was used.
**Significantly different from control ($p < 0.01$); ANCOVA F-test was used.

APPENDIX C. BMD OUTPUTS

BMDS provided no adequate model fits to the Poon et al. (1997) data sets.

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2010) Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH. As cited in HSDB (Hazardous Substances Data Bank). Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Accessed on December 14, 2010. 625688.

Albro, PW; Moore, B. (1974) Identification of the metabolites of simple phthalate diesters in rat urine. *J Chromatogr* 94:209−218. Available online at http://dx.doi.org/10.1016/S0021-9673(01)92368-4. Accessed on December 14, 2010. 094690.

ATSDR (Agency for Toxic Substances and Disease Registry). (1997) Toxicological profile for di-*n*-octylphthalate. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/toxprofiles/tp95-p.pdf. 667238.

Calafat, AM; Silva, MJ; Reidy, JA; et al. (2006) Mono-(3-carboxypropyl) phthalate: A metabolite of di-*n*-octyl phthalate. *J Toxicol Environ Health A* 69(3−4):215−227. Available online at http://www.ncbi.nlm.nih.gov/pubmed/16263692. Accessed on December 14, 2010. 667240.

CalEPA (California Environmental Protection Agency). (2008) All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as on December 18, 2008. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/air/allrels.html. Accessed on December 14, 2010. 595416.

CalEPA (California Environmental Protection Agency). (2009) OEHHA toxicity criteria database. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/risk/ChemicalDB/index.asp. Accessed on December 14, 2010. 595417.

Carter, JH; Carter, HW; DeAngelo, AB; et al. (1989) Sub-lethal autolysis in livers of rats exposed to phthalates [Abstract]. *J Cell Biol* 109(4):182A. 667241.

Carter, JH; Richmond, RE; Carter, HW; et al. (1992) Quantitative image cytometry of hepatocytes expressing gamma-glutamyl transpeptidase and glutathione S-transferase in diethylnitrosamine-initiated rats treated with phenobarbital and/or phthalate esters. *J Histochem Cytochem* 40(8):1105−1115. 667242.

Cobellis, L; Latini, G; De Felice, C; et al. (2003) High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod* 18(7):1512−1515. 673446.

CPSC (Consumer Product Safety Commission). (2010) Toxicity review for di-*n*-octyl phthalate. CPSC, Bethesda, Maryland. Available online at http://www.cpsc.gov/about/cpsia/toxicityDNOP.pdf. Accessed on February 22, 2011. 688912.

DeAngelo, AB; Garrett, CT; Daniel, FB. (1989) Di-*n*-octyl phthalate but not di(2-ethylhexyl)phthalate promotes diethylnitrosamine initiated hepatocellular carcinoma in the male F344 rat. In: Gakkai, NG (ed.). Eightieth annual meeting of the American Association for Cancer Research: proceedings. Baltimore, MD: American Association for Cancer Research, pp. 204. 667256.

DeAngelo, AB; Garrett, CT; Manolukas, LA; et al. (1986) Di-*n*-octylphthalate (DOP), a relatively ineffective peroxisome inducing straight chain isomer of the environmental contaminant di(2-ethylhexyl)phthalate (DEHP), enhances the development of putative preneoplastic lesions in the rat liver. *Toxicology* 41(3):279−288. 667247.

ECHA (European Chemicals Agency). (2010) Evaluation of the new scientific evidence concerning the restrictions contained in Annex VXII to Regulation (EC) No. 1907/2006 (REACH): Review of the available information for di-*n*-octyl phthalate (DNOP). ECHA, Helsinki, Finland. Available online at http://echa.europa.eu/doc/reach/restrictions/dnop_echa_review_report_2010_6.pdf. Accessed on December 14, 2010.

Foster, PMD; Thomas, LV; Cook, MW; et al. (1980) Study of the testicular effects and changes in zinc excretion produced by some *n*-alkyl phthalates in the rat. *Toxicol Appl Pharmacol* 54(3):392−398. Available online at http://dx.doi.org/10.1016/0041-008X(80)90165-9. Accessed on December 14, 2010. 094701.

Fredricsson, B; Moller, L; Pousette, A; et al. (1993) Human sperm motility is affected by plasticizers and diesel particle extracts. *Basic Clin Pharmacol Toxicol* 72(2):128−133. 001297.

Goodyear Tire and Rubber Company. (1982a) DNA damage by dioctyl phthalate basf, tank 28 in the e. Coli pol a1 - assay. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, DC; Report no. 878210368. 667245.

Goodyear Tire and Rubber Company. (1982b) Mutagenicity evaluation of dopine (Dioctyl phthalate) (Basf, tank28) Salmonella typhimurium/microsome bioassay with cover letter. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, DC; Report no. 878210367. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0206046. 667246.

Gray, TJ; Beamand, JA. (1984) Effect of some phthalate esters and other testicular toxins on primary cultures of testicular cells. *Food Chem Toxicol* 22(2):123−131. Available online at http://dx.doi.org/10.1016/0278-6915(84)90092-9. 673423.

Gray, TJ; Lake, BG; Beamand, JA; et al. (1983) Peroxisomal effects of phthalate esters in primary cultures of rat hepatocytes. *Toxicology* 28(1−2):167−179. 667260.

Heindel, JJ; Gulati, DK; Mounce, RC; et al. (1989) Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. *Toxicol Sci* 12(3):508−518. Available online at http://dx.doi.org/10.1016/0272-0590(89)90024-9. Accessed on December 14, 2010. 063443.

Hinton, RH; Mitchell, FE; Mann, A; et al. (1986) Effects of phthalic acid esters on the liver and thyroid. *Environ Health Perspect* 70:195−210. Available online at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474287/pdf/envhper00441-0183.pdf. 630618.

IARC (International Agency for Research on Cancer). (2010) Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC. Available online at http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php. Accessed on December 14, 2010. 597416.

Jones, AE; Kahn, RH; Groves, JT; et al. (1975) Phthalate ester toxicity in human cell cultures. *Toxicol Appl Pharmacol* 31:283−289. 667267.

Jones, HB; Garside, DA; Liu, R; et al. (1993) The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. *Exp Mol Pathol* 58(3):179−193. Available online at http://dx.doi.org/10.1006/exmp.1993.1016. 667268.

Kilburn, KH; Warshaw, RH. (1995) Neurotoxic effects from residential exposure to chemicals from an oil reprocessing facility and superfund site. *Neurotoxicol Teratol* 17(2):89−102. Available online at http://dx.doi.org/10.1016/0892-0362(94)00057-K. 075000.

Kwack, SJ., Kim, KB., Kim, HS., et al. (2009) Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. *J Toxicol Environ Health* 72(21−22):1446−1454

Lake, BG; Gray, TJ; Gangolli, SD. (1986) Hepatic effects of phthalate esters and related compounds: In vivo and in vitro correlations. *Environ Health Perspect* 67:283−290. 667276.

Lake, BG; Rijcken, WR; Gray, TJ; et al. (1984) Comparative studies of the hepatic effects of di-*n*-octylphthalate and mono-*n*-octylphthalate, di-(2-ethylhexyl)phthalate and clofibrate in the rat. *Acta Pharmacol Toxicol* 54(3):167−176. 667277.

Larsen, ST, Lund, RM; Nielsen, GD; et al. (2003) Adjuvant effect of di-*n*-butyl-, di-*n*-octyl-, di-iso-nonyl- and di-iso-decyl phthalate in a subcutaneous injection model using BALB/c mice. *Bas Clin Pharm Tox* 91(5):264−272.

Lawrence, WH; Malik, M; Turner, JE; et al. (1975) A toxicological investigation of some acute, short-term, and chronic effects of administering di-2-ethylhexyl phthalate (DEHP) and other phthalate esters. *Environ Res* 9:1−11. Available online at http://dx.doi.org/10.1016/0013-9351(75)90043-2. 063450.

Mann, AH; Price, SC; Mitchell, FE; et al. (1985) Comparison of the short-term effects of di(2-ethylhexyl) phthalate, di(*n*-hexyl) phthalate, and di(*n*-octyl) phthalate in rats. *Toxicol Appl Pharmacol* 77:116−132. Available online at http://dx.doi.org/10.1016/0041-008X(85)90273-X. 667282.

Morrissey, RE; Lamb, JC; Morris, RW; et al. (1989) Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. *Fundam Appl Toxicol* 13(4):747−777. Available online at http://dx.doi.org/10.1093/toxsci/13.4.747. 673407.

NIOSH (National Institute for Occupational Safety and Health). (2010) NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare, Atlanta, GA. Available online at http://www.cdc.gov/niosh/npg/npgdcas.html. Accessed on December 14, 2010. 625692.

NTP (National Toxicology Program). (1985) Di-*n*-octyl phthalate: reproduction and fertility assessment in CD-1 mice when administered in feed. NTP, Research Triangle Park, NC. Available online at http://www.ntis.gov/search/product.aspx?ABBR=PB85218147. 667286.

NTP (National Toxicology Program). (2011) 12th Report on carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp.niehs.nih.gov/?objectid=03C9AF75-E1BF-FF40-DBA9EC0928DF8B15. 737606.

Oishi, S. (1990) Effects of phthalic acid esters on testicular mitochondrial functions in the rat. *Arch Toxicol* 64(2):143−147. 667290.

Oishi, S; Hiraga, K. (1980) Testicular atrophy induced by phthalic acid esters: effect on testosterone and zinc concentrations. *Toxicol Appl Pharmacol* 53:35−41. Available online at http://dx.doi.org/10.1016/0041-008X(80)90378-6. 061572.

Oishi, S; Hiraga, K. (1982) Effects of monoesters of *O*-phthalic acid on serum lipid composition of rats. *Toxicol Lett* 14(1−2):79−84. Available online at http://www.ncbi.nlm.nih.gov/pubmed/7157420. 673044.

OSHA (Occupational Safety and Health Administration). (2006) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC; OSHA Standard 1915.1000. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286. Accessed on December 14, 2010. 625691.

Poon, R; Lecavalier, P; Mueller, R; et al. (1997) Subchronic oral toxicity of di-*n*-octyl phthalate and di(2-ethylhexyl) phthalate in the rat. *Food Chem Toxicol* 35(2):225−239. Available online at http://dx.doi.org/10.1016/S0278-6915(96)00064-6. 667295.

Reddy, BS; Rozati, R; Reddy, BV. (2006) Association of phthalate esters with endometriosis in Indian women. *BJOG* 113(5):515−520. 667422.

Sato, T; Nagase, H; Sato, K; et al. (1994) Enhancement of the mutagenicity of amino acid pyrolysates by phthalate esters. *Environ Mol Mutagen* 24(4):325−331. 667297.

Seed, JL. (1982) Mutagenic activity of phthalate esters in bacterial liquid suspension assays. *Environ Health Perspect* 45:111−114. 667298.

Shibamoto, T; Wei, CI. (1986) Mutagenicity of materials extracted from synthetic rubber. *Agric Biol Chem* 50:513−514. 673406.

Silva, MJ; Kato, K; Gray, EL; et al. (2005) Urinary metabolites of di-*n*-octyl phthalate in rats. *Toxicology* 210(2−3):123−133. 111416.

Smith, JH; Isenberg, JS; Pugh, G; et al. (2000) Comparative in vivo hepatic effects of di-isononyl phthalate (DINP) and related C7-C11 dialkyl phthalates on gap junctional intercellular communication (GJIC), peroxisomal beta-oxidation (PBOX), and DNA synthesis in rat and mouse liver. Toxicol Sci 54(2):312−321. Available online at http://dx.doi.org/10.1093/toxsci/54.2.312. 667301.

Teranishi, H; Kasuya, M. (1980) The effects of phthalate esters on fibroblasts in primary culture. *Toxicol Lett* 6:11−15. 667302.

Thoolen, B; Maronpot, RR; Harada, T; et al. (2010) Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol* 38(Suppl 7):5S−81S. 688914.

U.S. DHEW (Department of Health, Education and Welfare). (1978) Guide for the care and use of laboratory animals. Washington, DC: DHEW; publication No 78-23.

U.S. EPA (Environmental Protection Agency). (1987) Health and environmental effects profile for phthalic acid esters (PAEs). Office of Research and Development, Cincinnati, OH; PB89-120158.

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Cincinnati, OH; EPA/600/6-87/008. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855#Download. Accessed on January 5, 2011. 064560.

U.S. EPA (Environmental Protection Agency). (1994) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC; EPA/600/R-94/904. Available online at http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=60001G8L.txt. 596444.

U.S. EPA (Environmental Protection Agency). (2010) Health Effects Assessment Summary Tables (HEAST). Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC; EPA/540/R-97/036. Available online at http://epa-heast.ornl.gov/. Accessed on December 14, 2010. 595422.

U.S. EPA (Environmental Protection Agency). (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765−17817. Available online at http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF. 086237.

U.S. EPA (Environmental Protection Agency). (2009) 2009 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC; EPA/822/R-06/013. Available online at http://www.epa.gov/waterscience/drinking/standards/dwstandards2009.pdf. Accessed on December 14, 2010. 644141.

U.S. EPA (Environmental Protection Agency). (2010) Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris/. Accessed on December 14, 2010. 003752.

WHO (World Health Organization). (2010) Online catalogs for the Environmental Health Criteria Series. Available online at http://www.who.int/ipcs/publications/ehc/en/. Accessed on December 14, 2010. 595424.

Zacharewski, TR; Meek, MD; Clemons, JH; et al. (1998) Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. *Toxicol Sci* 46(2):282−293. Available online at http://dx.doi.org/10.1093/toxsci/46.2.282. 667308.

Zeiger, E; Haworth, S; Mortelmans, K; et al. (1985) Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in Salmonella. *Environ Mol Mutagen* 7(2):213−232. 059552.

Zeiger, E; Haworth, S; Speck, W; et al. (1982) Phthalate ester testing in the National Toxicology Program's environmental mutagenesis test development program. *Environ Health Perspect* 45:99−101. 094545.