UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
BETHESDA, MD 20814

Memorandum

Date: March 08, 2010

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THROUGH: Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences
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FROM : Kent R. Carlson, Ph.D., Toxicologist, Directorate for Health Sciences

SUBJECT : Toxicity Review of **Di-*n*-Octyl Phthalate (DnOP)**

The following memo provides the U.S. Consumer Product Safety Commission's (CPSC's) Health Sciences' staff assessment of the potential toxicity associated with **DnOP**.

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition. First, it must be toxic under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial illness or injury during or as a result of reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards under the FHSA (CPSC, 1992; summarized at 16 CFR §1500.135)

The FHSA addresses both acute and chronic hazards. While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic health hazards, the manufacturer is required to label a product appropriately according to the requirements of the FHSA. The first step in the risk assessment process is hazard identification, that is, a review of the available toxicity data for the chemical under consideration and a determination of whether the chemical is considered "toxic" under the FHSA. Chronic toxicity data (including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are assessed by the CPSC staff using guidelines issued by the Commission (CPSC, 1992). If it is concluded that a substance is toxic under the FHSA due to chronic toxicity, then a

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quantitative assessment of exposure and risk is performed to evaluate whether the chemical may be considered a "hazardous substance" under the FHSA. This memo represents the first step in the risk assessment process; that is, the hazard identification step.

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Table of Contents

Tables

Figures

Appendices

Abbreviations

ADI	Acceptable daily intake
ASI	Average severity index
AUC	Area under the concentration time curve
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention (U.S.)
CERHR	Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program (U.S.)
CHAP	Chronic Hazard Advisory Panel
CMA	Chemical Manufacturers Association
CPSC	Consumer Product Safety Commission (U.S.)
DEHP	Di-(2-ethylhexyl) phthalate
DENA	Diethylnitrosamine
DnDP	Di-*n*-decyl phthalate
DnHP	Di-*n*-hexyl phthalate
DnOP	Di-*n*-octyl phthalate
DOP	Di-octyl phthalate (DEHP)
EPA	Environmental Protection Agency (U.S.)
FHSA	Federal Hazardous Substances Act
Gd	Gestational day
GGT	Gamma-glutamyl transpeptidase
GJIC	Gap junction intercellular communication
IUR	Inventory Update Reporting database (U.S. EPA)
Ld	Lactation day
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
LPS	Lipopolysaccharide
MEHP	Mono-(2-ethylhexyl) phthalate
MCHpP	Mono-(7-carboxy-*n*-heptyl) phthalate
MCMP	Mono-carboxymethyl phthalate
MCPP	Mono-(3-carboxypropyl) phthalate
MCPeP	Mono-(5-carboxy-*n*-pentyl) phthalate
MHOP	Mono-hydroxy-*n*-octyl phthalate
MnOP	Mono *n*–octyl phthalate
MOOP	Mono-(7-oxo-*n*-octyl) phthalate
MRT	Mean residence time
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PNd	Postnatal day

PHA	Phytohemagglutinin
PPAR	Peroxisome proliferator-activated receptor
PPARα	Peroxisome proliferator-activated receptor, alpha isoform
PVC	Polyvinyl chloride
SER	Smooth endoplasmic reticulum
TRI	Toxics Release Inventory (U.S. EPA)
VRT	Variance of residence time

Executive Summary

Di-*n*-Octyl phthalate (DnOP) is a commonly used plasticizer found in a variety of consumer products. Acute toxicology testing in animals demonstrates that DnOP has low acute toxicity and is only a mild dermal irritant. Longer multiple dose studies show that DnOP primarily affects the liver, kidneys, thyroid, and possibly immune function. Reproductive, developmental, and carcinogenic effects have not been conclusively determined for DnOP. Acute and subchronic Acceptable Daily Intakes (ADIs) of 1.0 mg/kg-day and 0.368 mg/kg-day, respectively, have been generated based on DnOP-induced adverse effects in the liver.

1. Introduction

This document is a review of current hazard information for **DnOP**. It is intended to be utilized as part of an individual and cumulative phthalate risk assessment. This assessment was prepared from a variety of review articles (NICNAS, 2008; OME, 2005; NTP, 2003; ATSDR, 1997; Government of Canada, 1993) as well as supplemental independent studies retrieved from a literature search up to January 2009.

Nomenclature-related confounding issues have been discovered for DnOP. Di(2-ethylhexyl) phthalate (DEHP) is commonly termed **di-Octyl phthalate** (**DOP**) in the published literature and marketing/supplier information reports (i.e., Innua Petrochemicals Ltd, 2008). DEHP, however, is distinct from DnOP in both hazard and exposure potential. For this reason, DEHP/DOP hazards have been detailed in a separate report.

2. Physico-chemical Characteristics

The DnOP structure is comprised of a pair of eight-carbon esters linked to a benzene-dicarboxylic acid ring and is the straight chain analog to DEHP. The ester side chains are in an *ortho* configuration, in contrast to those found in isophthalates (*meta*) or terephthalates (*para*). DnOP is one of a variety of plasticizers (Appendix 3) used in the production of polyvinyl chloride plastics, cellulose ester resins, and polystyrene resins.

Structural descriptors, names and synonyms, registry numbers, and physico-chemical characteristics of DnOP can be seen in Tables 2.1, 2.2, 2.3, and 2.4, respectively.

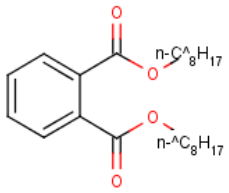
Table 2.1 Structural Descriptors and Molecular Formulas of DnOP (ChemIDplus Lite, 2009)	
lnChl notation	lnChl=1/C24H38O4/c1-3-5-7-9-11-15-19-27-23(25)21-17-13-14-18-22(21)24(26)28-20-16-12-10-8-6-4-2/h13-14,17-18H,3-12,15-16,19-20H2,1-2H3
Smiles notation	C1(c(C(OCCCCCCCCC)=O)cccc1)C(OCCCCCCCCC)=O
Molecular	 $C_{24}H_{38}O_4$; $C_6H_4[COO(CH_2)_7CH_3]_2$; MW = 390.56

Table 2.2 Names and Synonyms of DnOP (ChemIDplus Lite, 2009)	
Synonyms	1,2-Benzenedicarbonic acid, dioctyl ester; 1,2-Benzenedicarboxylic acid, dioctyl ester; 4-09-00-03180 (Beilstein Handbook Reference); AI3-15071 (USDA); BRN 1915994; Benzenedicarboxylic acid di-n-octyl ester; CCRIS 6196; Celluflex DOP; DNOP; Di-n-octyl phthalate; Dicapryl phthalate; Dinopol NOP, Dioctyl 1,2-benzenedicarboxylate; Dioctyl o-benzenedicarboxylate; Dioctyl phthalate; Dioktylester kyseliny ftalove; Dioktylester kyseliny ftalove (Czech); EINECS 204-214-7; HSDB 1345; NSC 15318; Octyl phthalate; Phthalic acid; dioctyl ester; Polycizer 162; Px-138; RCRA waste number U107; Vinicizer 85; n-Octyl phthalate; o-Benzenedicarboxylic acid, dioctyl ester
Systematic Name	1,2-Benzenedicarboxylic acid, 1,2-dioctyl ester; Dioctyl phthalate; Phthalic acid, dioctyl ester
Superlist Name	1,2-Benzenedicarboxylic acid, dioctyl ester; Di-n-octyl phthalate; Dioctyl phthalate; Phthalic acid, dioctyl ester; RCRA waste no. U107

Table 2.3 Registry Numbers for DnOP (ChemIDplus Lite, 2009; ATSDR, 1997)	
CAS Registry Number	117-84-0
Other Registry Number	8031-29-6
System Generated Number	000117840
NIOSH RTECS	TI 1925000
EPA hazardous wastes	U 107
Hazardous Substances Database (HSDB)	1345

Table 2.4 Physico-chemical Properties of DnOP	
Color	Colorless (NICNAS, 2008)
Odor	Odorless (NICNAS, 2008)
Physical State	Organic liquid (NICNAS, 2008)
Water Solubility	**0.0005mg/L** (Staples *et al.,* 1997; Ellington, 1999; NTP, 2003); 0.02mg/L water @ 25C (ChemIDplus Lite, 2009); 0.49-0.51 µg/L @ 25C (no-stir vs. stir; Ellington, 1999); 0.40 µg/L @ 20C (Letinski *et al.,* 1999, cited in Ellington, 1999); 0.003 g/L @ 25 C (NICNAS, 2008); 0.00046-3.0 mg/L (Staples *et al.*, 1997)
Vapor Pressure	**1.00E-07 mm Hg @ 25 C** (Staples *et al.,* 1997; NTP, 2003; ChemIDplus Lite, 2009); $1.44x10^{-4}$ mm Hg @ 25 C (ATSDR, 1997); $2.2*10^{-7}$-$1.9*10^{-4}$ (Staples *et al.,* 1997)
Melting Point	**-25 C** (Staples *et al.,* 1997; NTP, 2003; ATSDR, 1997; NICNAS, 2008; ChemIDplus Lite, 2009)
Boiling Point	**390 C**; 101.3 kPa (NTP, 2003; NICNAS, 2008)
Flash Point	**219 C** (NICNAS, 2008; ATSDR, 1997)
Specific Gravity (g/mL)	**0.978** @ 25C (Staples *et al.,* 1997; NTP, 2003)
Log P (octanol-water; K_{ow})	**8.06** (Staples *et al.,* 1997; Ellington, 1999); 5.22 (ATSDR, 1997); 8.1 (ChemIDplus Lite, 2009); 8.16-8.18 (no stir vs. stir; Ellington, 1999); 5.22 (NICNAS, 2008); 5.22-8.54 (Staples *et al.,* 1997)
K_{oc} (L/kg; suspended solids)	**$2.0*10^{6}$** (Staples *et al.,* 1997)
Henry's Law Constant	**$1.03*10^{-4}$ atm-m^3/mole** (Staples *et al.,* 1997); 2.57E-06 atm-m^3/mole @ 25C (ChemIDplus Lite, 2009); 0.55, 6.68E-03 atm-m^3/mole (NICNAS, 2008), $5.5x10^{-6}$ to $6.68x10^{-5}$ H atm/m^3/mole (ATSDR, 1997)
Atmospheric OH Rate Constant	**2.06E-11 cm^3/molecule-sec @ 25 C** (ChemIDplus Lite, 2009)
Density	**978 kg/m^3 (g/ml) @ 25 C** (NICNAS, 2008)
Storage Stability	DnOP concentration was 82-104% of target concentrations immediately after mixing with feed and degradation was negligible after one month (at room temperature) when mixed in with test diets (Poon *et al.,* 1997)

3. Manufacture, Supply, and Use

In general, DnOP is manufactured commercially by esterifying phthalic anhydride with n-octanol, or by reacting n-octylbromide with phthalic anhydride. This reaction is facilitated by heating and can take place either at atmospheric pressure or under a vacuum.

The 2006 EPA non-confidential Inventory Update Reporting (IUR) database lists only two importers of DnOP in the United States, ICC Chemical Corporation (NY, NY) and Vinmar Overseas LTD (Houston, TX). These companies have a combined production/importation volume of between 10 and 50 million pounds of liquid DnOP (with a purity of > 90%). This range is similar to commercial production of DnOP reported for the US in 1994 (10 million pounds; Kavlock *et al.,* 2002 cited in Silva *et al.,* 2005). Additional companies, Georgia Gulf Chemicals and Vinyls, LLC, Condea Vista Company, and LG Chemical America, Inc. have also been reported in the IUR with no additional details when searched using dates prior to 2006. IUR searches did not detail international suppliers of DnOP-containing mixtures (Chemos GmbH -

Germany, BP Chemicals LTD – United Kingdom, and Annecis Tatham – United Kingdom) noted in general online web searches.

Production or release of DnOP is not currently reported in the Toxics Release Inventory (TRI, 2009) in contrast to other phthalates such as di-(2-ethylhexyl) phthalate (EPA, 2006). Earlier TRI results, however, state that at least 55-99 facilities manufactured or processed DnOP at one point in time (EPA, 1992a; ATSDR, 1997). Delisting from the TRI was initiated by a petition from Vista Chemical Corporation (EPA, 1992b) in cooperation with Teknor Apex (EPA, 1992c) and Aristech (EPA, 1992d), DnOP manufacturers. Delisting was supported by an EPA hazard characterization that concluded "DnOP (1) cannot reasonably be anticipated to cause teratogenic effects, immunotoxicity, or neurotoxicity and (2) cannot reasonably be anticipated to cause liver toxicity, kidney toxicity, or reproductive or developmental toxicity, except, at relatively high dose levels… In addition, EPA cannot characterize the hazard potential and therefore cannot establish that DnOP can reasonably be anticipated to cause cancer" (EPA, 1993a).

DnOP is primarily used as a plasticizer in plastic production. It imparts flexibility and other mechanical properties to various types of plastics. DnOP's principal use occurs in conjunction with two other C6-C10 phthalate esters, di-n-hexyl phthalate (DnHP; CAS No. 84-75-3) and di-n-decyl phthalate (DnDP; CAS no. 84-77-5). DnOP has been reported to be at 20% volume/volume in these mixtures with the remaining phthalate mixture at 1% DnHP and 79% DnDP (Jahnke *et al.,* 2005 cited in NICNAS, 2007). C6-C10 mixtures (and by default, DnOP) are reportedly used in a wide variety of consumer products (Table 3.1). They also have industrial uses in the creation of bottle cap liners and conveyor belts, as dyes, catalysts, or initiator carriers in plastics production, and as a chemical intermediate in the manufacture of plastisols (ATSDR, 1997). DnOP has also served as a fluid for use in electrical capacitors (ATSDR, 1997).

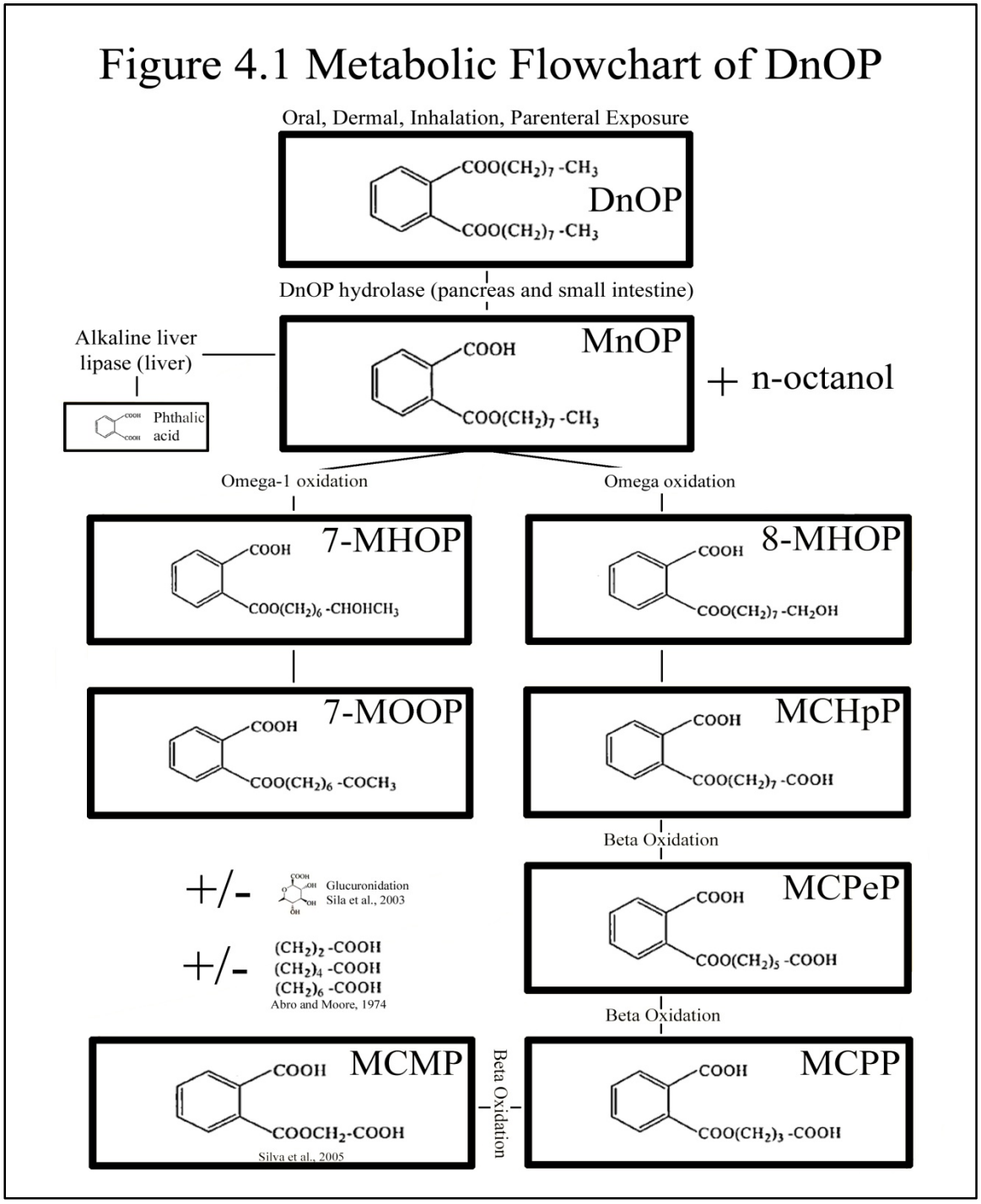
Table 3.1 Products Reported to Contain DnOP*									
Consumer Products Created with C6-C10 phthalate mixture (and DnOP)**									Other
Arts-Crafts	**Auto**	**Home Maintenance**	**Home Office**	**Inside Home**	**Landscape/ Yard**	**Personal Care**	**Pesticides**	**Pet care**	
Seam cements (NICNAS, 2008)	Traffic cones (NICNAS, 2008)	Flooring tiles (NICNAS, 2008	Notebook covers (NICNAS, 2008)	Vinyl furniture upholsteries (NICNAS, 2008)	Canvas tarps (NICNAS, 2008)	Cosmetics (NICNAS, 2008)	Active ingredients (NICNAS, 2008)	Flea Collars (NICNAS, 2008)	Children's Toys (NICNAS, 2008)
Adhesives (NICNAS, 2008)	Automobile hoses (NICNAS, 2008)	Carpet tiles (NICNAS, 2008)		Shower curtains (NICNAS, 2008)	Gloves (NICNAS, 2008)				Shoes (NICNAS, 2008)
Polymer modeling clays (Stopford *et al.,* 2003)	Automobile upholsteries (ATSDR, 1997)	Swimming pool liners (NICNAS, 2008)		Carpetback coatings (Silva *et al.,* 2005)	Garden hoses (NICNAS, 2008)				Play and exercise balls (NICNAS, 2008)
		Weather strippings (NICNAS, 2008)		Wall coverings (ATSDR, 1997)					Child care articles (Stringer *et al.,* 1997, 1999)
		Insulations (NICNAS, 2008)		Window shades (ATSDR, 1997)					Adult entertainment toys (Nilsson *et al.,* 2006)
		Polyurethane surface coatings (NICNAS, 2008)		Tablecloths (ATSDR, 1997)					Packaging films (Silva *et al.,* 2005)
		Floor finishes (NICNAS, 2008)							Rainwear (ATSDR, 1997)
		Floor adhesives (NICNAS, 2008)							PVC foams (swords, masks, floor puzzles; Borling *et al.,* 2006)
		Nitrocellulose lacquer coatings (NICNAS, 2008)							
		Wiring and cables (Silva *et al.,* 2005)							

* Note: CPSC shares regulatory jurisdiction with other Federal agencies for some of the products referenced in this table

** Amended categories from the Consumer Product Information Database created by the U.S. Health and Human Services Commission, 2009

4. Toxicokinetics

CPSC staff was unable to find studies involving the absorption, distribution, metabolism, and excretion of DnOP from oral, dermal, or inhalation exposures in humans. This finding has previously been noted in other reviews as a data gap (ATSDR, 1997; NICNAS, 2008). A general diagrammatic representation of DnOP metabolism in rats is provided in Figure 4.1.



Absorption

Oral Exposure

Numerous animal studies indirectly illustrate that DnOP, or a DnOP metabolite, is absorbed following oral exposure. DnOP-induced pathological effects are reported for a variety of organ systems following gavage (Lake *et al.,* 1984, Jones *et al.,* 1993) and feed-based (Mann *et al.,* 1985, Heindel *et al.,* 1989) dosing strategies.

It is currently thought that absorption from the gastrointestinal tract following oral ingestion is rapid and on the order of hours (NICNAS, 2008). It is not known if metabolic conversion to mono-*n*-octyl phthalate (MnOP) via a "DnOP hydrolase" is required before being absorbed (ATSDR, 1997; Lake *et al.,* 1977) or if a DnOP absorption threshold exists in different species (as with DEHP; Albro, 1986).

Esterases that metabolize DnOP to MnOP are present in many tissues (Rowland, 1974 and Rowland *et al.,* 1977 as cited by Kluwe, 1982) and may be the reason that metabolism is similar *in vitro* in human small intestine, rat intestine, ferret intestine, and baboon intestine (Lake *et al.,* 1977; ATSDR, 1997). Similarities may be due to other non-esterase-mediated effects as well, because esterase-mediated metabolism in the intestine is highly species, site, compound, and presentation dependent (Inoue *et al.,* 1979; Van Gelder *et al.,* 2000).

Absorption of DnOP is still largely undescribed in terms of relevant species, metabolites, location of absorption, and carrier media-induced effects. It is currently assumed that species differences in absorption are minimal, but this has not been demonstrated for the parent DnOP, its primary metabolite MnOP, or metabolites that may be subject to enterohepatic circulation. In terms of regulatory importance, a quantitative estimate of absorption (i.e., percent absorption) is ultimately necessary to reduce default considerations currently used in risk assessment (100% absorption). Percent absorption has been estimated in part for other phthalates (Kluwe, 1982; ECB, 2008).

Distribution

Oral Exposure

DnOP is distributed in the blood and tissue compartment primarily as the metabolite, MnOP. Pharmacokinetic parameters for MnOP have been reported for rats gavage dosed with 2000 mg/kg DnOP. Both blood *(peak = 3h; t½ = 3.3h; Area under the concentration time curve (AUC) = 1066 µg-h/mL; Mean residence time (MRT) = 5.4 h; and Variance of residence time (VRT) = 19.5 h^2)* and testes *(peak = 6 hours; t½ = 5.0; AUC = 358 µg-h/mL; MRT = 6.2 h; and VRT = 21.7 h^2)* data suggest that systemic absorption and distribution is rapid (Oishi, 1990; NICNAS, 2008; OME, 2005; NTP, 2003; ATSDR, 1997). Distribution of DnOP to other tissues has also been reported by Poon *et al.,* (1997).

In this study, DnOP residues were found in liver (4-5 ppm ~ detection limit) and adipose tissue (15-25 ppm) 13 weeks following dietary exposure to 5000 mg/kg of DnOP. Reliability of this study has been questioned, however, because of failures to use mass spectrometry to identify DnOP, a lack of analytical blanks, and inconsistency in the data with regard to dose and the biology of hydrolysis and absorption (NTP, 2003).

Knowledge of the distribution of DnOP, MnOP, and other metabolites is still rudimentary. Organ clearance rates and distribution kinetics have not been determined for any DnOP metabolite, animal species, or sensitive subpopulation.

Metabolism

Oral Exposure

Metabolism of diester phthalates such as DnOP occurs first by phase I biotransformation to a monoester (Silva *et al.,* 2003). DnOP is metabolized to MnOP and n-octanol via hydrolysis of a single ester link, or to a minor amount of phthalic acid via hydrolysis of both ester linkages (ATSDR, 1997; OME, 2005). N-octanol is further oxidized to a fatty acid and metabolized by the fatty acid oxidation pathway (NTP, 2003).

Metabolic hydrolysis is thought to be mediated by esterases in the gastrointestinal tissues (Rowland *et al.,* 1977; NICNAS, 2008; NTP, 2003). Esterases that can metabolize DnOP to MnOP are reported for many other tissues *in vivo* as well (Rowland, 1974 and Rowland *et al.,* 1977 as cited by Kluwe, 1982). Metabolic hydrolysis for DnOP is consistent among many species and has been determined *in vitro* using human small intestine and rat, ferret, and baboon intestine (Lake *et al.*, 1977; ATSDR, 1997). Rapid hepatic conversion from DnOP to MnOP and phthalic acid has been confirmed in studies using rat liver microsomes (Silva *et al.,* 2005; NICNAS, 2008).

In contrast to metabolism by tissues, hydrolysis of DnOP by gut content enzymes is not significant for DnOP. In rats, only 10% of an administered DnOP dose (1 mg/ml) is hydrolyzed following 16 hours of incubation with intestinal contents. In general, hydrolytic activity in this study was suggested to be enzymatic in nature, since it was not affected by bacterial sterilization of the gut or filtrative removal of all cellular material (Rowland, 1974 and Rowland *et al.,* 1977 as cited by Kluwe, 1982).

In rats, MnOP is further metabolized by oxidative processes into mono-(3-carboxypropyl) phthalate (MCPP) and other lesser metabolites such as (mono-carboxymethyl phthalate (MCMP), mono-(5-carboxy-n-pentyl) phthalate (MCPeP), mono-(7-carboxy-n-heptyl) phthalate (MCHpP), isomers of mono-hydroxy-n-octyl phthalate (MHOP; e.g., mono-(7-hydroxy-n-octyl) phthalate), mono-oxo-n-octyl phthalate (MOOP; e.g., mono-(7-oxo-n-octyl) phthalate) and phthalic acid (PA). The relative urinary concentrations 24 hours after DnOP (300 mg/kg) administration are reported as: MCPP = 163.6 µg/ml, MCHpP = 71.6 µg/ml, MHOP = 23.6 µg/ml, MOOP = 21.2 µg/ml, MCPeP = 11.3 µg/ml, PA = 2.68 µg/ml, MCMP = 0.83 µg/ml, and MnOP = 0.278 µg/ml (Silva *et al.,* 2005). The major urinary metabolite, MCPP, results from an initial omega-oxidation and two beta-oxidations of the n-octyl side chain (ATSDR, 1997; see Figure 4.1). The production of the oxidative metabolite MHOP has been verified *in vitro* in rat liver microsomes (Silva *et al.,* 2005).

MnOP or other oxidized metabolites are subsequently catalyzed with glucuronic acid to form glucuronide conjugates (phase II biotransformation). Glucuronidation is important in making DnOP metabolites more polar and water soluble prior to excretion. The extent to which DnOP metabolites are conjugated has not been determined empirically. This is due in part to

urinary metabolite detection methods which enzymatically strip the glucuronide conjugate from the metabolite with β-glucuronidase prior to analysis (Silva *et al.,* 2005). Rats can glucuronidate similar phthalates (i.e., DEHP), but don't excrete glucuronide conjugated metabolites (Albro, 1986). The distribution of glucuronide-bound and free metabolites is important to understand, since the relative toxicity of DnOP is determined to a large extent by the free metabolites and enterohepatic recirculation of deconjugated metabolites (Silva *et al.,* 2003).

The ability to glucuronidate and deglucuronidate can differ significantly within a species, age class, or disease state. Dwivedi *et al.,* (1987) demonstrated that rat strains can differ as much as 2-fold in serum and liver β–glucuronidase activity. Miyagi and Collier (2007) revealed that development of human neonatal hepatic "total" UDP-glucuronosyltransferase (UGT) enzyme activity doesn't mature until 20 months of age and also that β–glucuronidase activity (responsible for hydrolysis/enterohepatic recirculation) is highest in the neonatal liver and decreases to adult levels by 4 months. No significant differences in UGT activities have been reported for different genders or ethnicities.

The shift from metabolic cleavage and recirculation to conjugation and clearance in the neonate is an important consideration and directly impacts the choice of animal models for further metabolic and toxicological research. The rat model may be a good choice for early human life stages, since rats don't excrete glucuronide-conjugates of higher molecular weight phthalates (i.e., DEHP). Other species that do excrete glucuronide conjugates (i.e., mice) might better represent later human developmental stages.

Potential DnOP metabolites have been outlined to some extent in the reviewed papers. DnOP metabolite distribution (organ compartments), conjugation states (bound or free), metabolic rates (i.e., such as first order kinetics), enterohepatic recirculation, and metabolic saturation states have not been described, however, for different species and human sensitive and general subpopulations.

Excretion

Oral Exposure

The parent compound, DnOP, is not found in the urine, feces, or other excretory compartments because it is hydrolytically transformed to MnOP in the gastrointestinal tract (NICNAS, 2008; Albro and Moore, 1974; OME, 2005; NTP, 2003; ATSDR, 1997). MnOP is excreted in the urine. After gavage dosing 559 mg/kg-day DnOP to rats, 31% of the administered dose is recovered in the urine as a mixture of MnOP and phthalic acid (Albro and Moore, 1974). MCPP is also a major urinary metabolite of DnOP (in terms of concentration) and has been estimated to be over 560-fold higher than MnOP concentrations in 24 hour urine samples of Sprague–Dawley rats dosed with DnOP (Silva *et al.,* 2005; Calafat *et al.,* 2006; NICNAS, 2008). At least five other oxidative metabolites and phthalic acid have also been identified as minor products in the urine (Silva *et al.,* 2005; NICNAS, 2008).

Urinary excretion of DnOP metabolites follows a biphasic pattern and concentrations of these metabolites decrease dramatically after the first 24 hours. Even so, low concentrations of MCPP, MCHpP, MHOP, and MOOP are still detectable after 4 days. Some metabolites such as MCPP are also found in the urine of rats dosed with di-isooctyl phthalate, di-isononyl phthalate, di-isodecyl phthalate, di-(2-ethylhexyl) phthalate, and di-*n*-butyl phthalate, but at much lower concentrations when compared to DnOP (Calafat *et al.,* 2006).

DnOP metabolites that accumulate in the urine have been described. As with metabolism, the metabolite distribution (excreta compartments – urine, feces, breath), conjugation states (bound or free), and excretion or clearance rates (first order kinetics) have not been robustly described for DnOP.

Overall Toxicokinetic Conclusion

Oral Exposure

Overall, orally ingested DnOP is thought to be quickly metabolized to MnOP in the gut, absorbed, oxidatively metabolized to other metabolites in the serum or liver, and then circulated in the blood as free or conjugated monoester or monoester metabolites prior to excretion primarily via urine (Figure 4.1).

Dermal Exposure

No studies have reported quantitative information regarding the absorption, distribution, metabolism, or excretion of DnOP or its metabolites following dermal exposures (ATSDR, 1997). It is expected, however, that because of its lipophilic nature, DnOP will be absorbed to some extent via this route of exposure (Kluwe, 1982). Systemic processes involved in the distribution, metabolism, and excretion of DnOP following dermal exposures are expected to be the same as that from oral exposures.

Inhalation Exposure

As with dermal exposures, CPSC staff have found no studies reporting qualitative or quantitative information regarding the absorption, distribution, metabolism, or excretion of DnOP or its metabolites following inhalation exposures. It is expected, however, that because of its lipophilic nature, DnOP will be absorbed to some extent via the inhalation route of exposure (Kluwe, 1982). Systemic processes involved in the distribution, metabolism, and excretion of DnOP following inhalation exposures are expected to be the same as that from oral exposures.

Parenteral Exposure

CPSC staff have found no studies reporting qualitative or quantitative information regarding the absorption, distribution, metabolism, or excretion of DnOP or its metabolites following parenteral (intraperitoneal or intravenous injections) exposures. It is expected, however, that following injection, parent DnOP will be metabolized to some extent in the bloodstream or interstitial fluid by ubiquitous esterases (Rowland, 1974 and Rowland *et al.,* 1977 as cited by Kluwe, 1982). Because of its lipophilicity (Staples *et al.,* 1997), a portion of DnOP is also expected to directly partition into cellular material following injection. Following metabolism, systemic processes involved in the distribution, metabolism, and excretion of DnOP are expected to be the same as that from oral exposures.

5. Hazard Information

In evaluating toxicity data, CPSC staff applies the definition for toxicity in the regulations (16 CFR 1500.3 (c)(2)(ii)) and chronic hazards guidelines (CPSC, 1992) promulgated under the FHSA (15 U.S.C. 1261-1278). A substance or mixture is classified as "known to be toxic" only if there is sufficient evidence in humans, and is regarded as "probably toxic" if there is either limited evidence in humans, or sufficient evidence in animals (Table 5.1).

If a substance is "known to be toxic" or "probably toxic", it is considered "toxic" under the FHSA. If a substance is "possibly toxic", it is not be considered "toxic" under the FHSA.

Table 5.1 Classification of Chronic Hazards under the FHSA

Evidence	Human Studies	Animal Studies
Sufficient evidence	**Known**	**Probable**
Limited evidence	**Probable**	Possible
Inadequate evidence	Possible	---

Acceptable daily intakes (ADI's) values are calculated when a given chemical has sufficient toxicity information available and is considered "toxic" due to chronic effects. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers. In some cases, insufficient data are available to calculate an ADI.

Overall, animal data are sufficient to support the conclusion that DnOP is probably not acutely toxic via the oral route of exposure under the FSHA. Acute oral toxicities (LD_{50}s) for DnOP in at least four reported studies were ≥ 6513 mg/kg. This is in excess of the oral LD_{50} range (50 - 5000 mg/kg) necessary to be termed acutely toxic under the FHSA.

Sufficient animal data and limited human data also support the conclusion that DnOP is probably not corrosive or an ocular or dermal irritant under the FHSA.

In terms of sensitization, contrasting results in animal (negative) and human (negative and positive) data support the conclusion that there is "limited or inadequate human and animal evidence" for the designation of DnOP as a "sensitizer" under the FHSA.

DnOP-induced adverse effects were noted in animal test subject's thyroid, immune system, kidney, and liver in two, three, three, and eight published studies, respectively. Sufficient animal data exist, therefore, to support the conclusion that DnOP has [sub]chronic toxicity under the FHSA.

Inadequate or limited animal data exist when considering DnOP-induced effects as: 1) a carcinogen, 2) a neurotoxicant, 3) a reproductive or developmental toxicant, 4) a dermal or inhalation toxicant, 5) a respiratory irritant, or 6) a genotoxicant. Adverse effects were not

observed or key evidence was missing in the majority of studies presented, limiting the overall conclusions to “possibly toxic” under the FHSA.

In the following discussions, hazard information has been divided into sections thought to be of interest for regulatory matters (i.e., for labeling and other mitigation measures) as well as for biological and pathological consistency. More specifically, hazards have been divided into whether the exposure is singular or repeated. Hazards associated with repeated exposures have been further divided into groupings based on the affected organ system (i.e., hepatic, neurological, hematologic, etc.) and discussed in terms of the exposure duration (acute, 14 days or less; intermediate, subchronic, 15 - 364 days; and long-term, chronic, greater than 365 days). Specific study information can be reviewed in tabular (Appendix 1) or narrative (Appendix 2) form.

Single-Dose Toxicity

Acute Oral Toxicity

Studies determining the acute toxicity of DnOP in humans were not found. For other animals, DnOP has lower acute oral toxicity in mice (LD_{50} = 6513 mg/kg, GTPZAB, 1973; >12,800 to 13,000 mg/kg-bw, Dogra *et al.,* 1989; Eastman Kodak Company, 1978 cited in NICNAS, 2008) than in rats (LD_{50} = 53,700 mg/kg, Dogra *et al.,* 1987; 47,000 mg/kg, Balynina and Berezovkaia, 1976).

Acute oral LD_{50}s are much lower for mice than that obtained via intraperitoneal injection (65,700 mg/kg in Goldemberg and Safrin, 1977 and Lawrence *et al.,* 1975). In contrast, acute oral LD_{50}s for rats are similar to that obtained via intraperitoneal injection (>48,900 mg/kg in Singh *et al.,* 1972). The di-C6-C10 mixture (containing 20% DnOP) also has low acute oral toxicity when tested in rats (LD_{50} > 2000; 39,900 - 61,000; and > 30,720 mg/kg-bw; Huels, 1965 and 1988 cited in ECB, 2000a and NICNAS, 2007).

Methodological details involving the conduct of many of the acute oral toxicity studies were not provided in the originating studies. Details omitted include the number and strain of animals, DnOP doses, timing of mortality, and clinical signs. Methodological deficiencies were not thought to be significant enough to overshadow the fact that all of the acute mouse and rat oral LD_{50}s cited for DnOP were consistently higher than the oral LD_{50} range (50 - 5000 mg/kg) required by the FHSA to conclude that a chemical is acutely toxic. The weight of evidence including probable animal data are sufficient, therefore, to support the conclusion that **DnOP does not fit the designation of "acutely toxic" under the FHSA (16 CFR §1500.3(c)(2)(i)(A))**.

Acute Dermal Toxicity

CPSC staff found only one study that has reported information regarding the acute dermal toxicity of DnOP. In this study, guinea pigs had an acute dermal LD_{50} of 75 mL/kg-bw (Bisesi, 1994; CMA, 1999 cited in NICNAS, 2008). No other study factors were reported in the Bisesi or NICNAS reports. In addition, a di-C6-C10 mixture (containing 20% DnOP) had low acute dermal toxicity when tested in rabbits (LD_{50} > 20,000 mg/kg-bw; Huels cited in ECB, 2000a).

As with the acute oral studies, methodological details involving the conduct of the acute dermal toxicity studies (i.e., dose level in mg/kg, incubation period and state) have not been provided. These facts, combined with a lack of additional studies, support the conclusion that **there is "inadequate evidence" for the designation of DnOP as "acutely toxic" under the FHSA (16 CFR §1500.3(c)(2)(i)(C))**.

Acute Inhalation Toxicity

CPSC staff did not find any information regarding the acute inhalation toxicity of DnOP. The di-C6-C10 mixture (containing 20% DnOP), however, had low acute inhalation toxicity when tested in rats (no death after 8 hours in a saturated atmosphere; Huels cited in ECB, 2000a).

The lack of acute inhalation toxicity data for DnOP can be considered a data gap and supports the conclusion that **there is "inadequate evidence" for the designation of DnOP as "acutely toxic" under the FHSA (16 CFR §1500.3(c)(2)(i)(B))**.

Primary Skin Irritation

DnOP is a mild skin irritant when applied to depilated guinea pig skin or rabbit skin (Eastman Kodak Company, 1978; Marhold, 1986; RTECS, 2004; Government of Canada, 1993; NICNAS, 2008). The di-C6-C10 mixture is also, at most, a mild skin irritant in rabbits when tested by the OECD TG 404 method (Scientific Associates, 1975; Huels, 1989; and ECB, 2000a; NICNAS, 2007). Designation of DnOP as a mild skin irritant is supported by research of Kanerva *et al.,* (1997) in which DOP (2% weight/weight; construed in this case to be DnOP because DEHP was also directly tested in this study) occlusive irritation patch testing in 173 humans resulted in an irritation incidence of 1.2% (i.e., essentially negative).

Methodological details involving the conduct of the skin irritation studies have not been provided. Details omitted include the number and strain of animals, DnOP doses, occlusion or not, timing of effects, and clinical signs. Even so, the weight of evidence including limited human data and probable animal data are sufficient to support the conclusion that **DnOP does not fit the definition of "corrosive" as outlined in the FHSA (16 CFR §1500.3(c)(3)) or designation as a "primary irritant" under the FHSA (16 CFR §1500.3(c)(4))**.

Primary Eye Irritation

Administration of DnOP caused mild conjunctival irritation in guinea pigs (Eastman Kodak Company, 1978 cited in NICNAS, 2008) or mild eye irritation in rabbits (with 500 mg dose for 24 hours; RTECS, 2004; Marhold, 1986 cited in NICNAS, 2008). In another study, instillation of 20 mg DnOP into the eyes of rabbits induced severe ocular consequences (Anonymous, 1946 cited in NICNAS, 2008). Dosing with the di-C6-C10 mixture also induced a mild eye irritation in rabbits (Scientific Associates, 1975b; Huels, 1989; ECB, 2000a; and NICNAS, 2007).

Methodological details involving the conduct of the primary eye irritation studies have not been provided. Details omitted include the number and strain of animals, DnOP doses, timing of effects, and clinical signs. The weight of evidence including probable animal data are sufficient to support the conclusion that **DnOP does not fit the designation of an "eye irritant" under the FHSA (16 CFR §1500.3(c)(4))**.

Respiratory Irritation

Evidence from one case report suggests that DnOP is a mild respiratory irritant (NICNAS, 2008). In this case, respiratory irritation was reported in workers exposed to a mixture of phthalates (including DnOP).

The case report involves exposure to multiple phthalates. Respiratory irritation, therefore, cannot be directly correlated to DnOP exposure. The lack of DnOP-induced respiratory irritation data and undescribed study methodologies are data gaps that support the conclusion that **there is "inadequate evidence" for the designation of DnOP as "a respiratory irritant"**.

Sensitization

DnOP-induced sensitization has been evaluated in animal studies, human patch exposure tests, and occupational studies. In animals, a sensitization response was not evoked in depilated guinea pigs induced and then challenged with DnOP (Eastman Kodak Company, 1978 cited in NICNAS, 2008). In addition, sensitization responses were not reported for guinea pigs in a test conducted with the di-C8-C10 alkyl ester (containing DnOP) under Good Laboratory Practices (GLP) and OECD test guidelines (ECB, 2000b; NICNAS, 2008). In humans, 2% DnOP did not evoke a sensitization response in an occlusive allergic patch study using 173 subjects (Kanerva *et al.,* 1997).

A lack of sensitization responses contrast with those reported in other occupational studies. In one study, a group of 30 plastic shoe factory workers with skin lesions and a group of 30 plastic shoe factory workers without skin lesions were patch tested for sensitivity to a "standard battery of substances" plus four widely used plasticizers. Six workers in the groups with skin lesions were positive for sensitization reactions to phthalates and five were positive for reactions to coal tar. Although not clearly explained, reactions were also observed to di-octyl phthalate (ECB, 2000a; NICNAS, 2008). In the second report, a single worker developed asthmatic symptoms following exposure to aerosolized DnOP in an inhalation chamber (ECB, 2000a; NICNAS, 2008). Asthmatic symptoms were alleviated by the administration of sodium cromolgycate, a mast cell stabilizer.

As with other acute studies, methodological details aren't available for supporting the sensitization potential of DnOP. In the Eastman Kodak study, "no further details were reported" for the summary (ATSDR, 1997), and in the di-C8-C10 summary (ECB, 2000b), information on the relative proportion of DnOP in the mixture was not addressed, even though OECD 406 guidelines were followed for the actual study. Neither study summary reported positive control results. In the Kanerva (1997) patch study, the shoe factory worker study, and the asthma study, the induction doses, challenge doses, or estimates of previous occupational exposures were not reported. Since these were hypothetically post-exposure, a standard induction and then challenge protocol was also not utilized, limiting potential positive results to people with high enough occupational exposures to DnOP to have simulated an "induction". Durations in at least one of these studies (2 days induction/challenge and 2 - 4 days of observation) were also far shorter than that typically used to generate a *de novo* sensitization response in laboratory animals (1-3 week induction with challenge at 1 - 2 weeks post induction; EPA, 1998d). In addition, cross sensitization by other phthalates or chemicals could not be ruled out in the occupational studies, since exposures to multiple phthalates and other chemicals were involved in the workplace.

Even though animal data illustrate that DnOP is not a sensitizer, human occupational data suggest that sensitization may occur following exposures. These contrasting arguments support the conclusion that **there is "limited or inadequate human and animal evidence" for the designation of DnOP as a "sensitizer" under the FHSA (16 CFR §1500.3(c)(5)(i))**.

Repeat-Dose Toxicity

General Effects (i.e., **food or water consumption, body weight, etc.)**

Both acute and subchronic feeding studies suggest that body weight and food consumption are not adversely affected to any great extent following dosing with DnOP. Decrements in body weight and food consumption were not seen in male Sprague-Dawley rats exposed to 2800 mg/kg-day DnOP via oral gavage once a day for 4 days (Foster *et al.,* 1980) or male and female Sprague-Dawley rats exposed to 350.1-402.9 mg/kg-day DnOP in feed for 13 weeks (Poon *et al.,* 1997). Only marginal decrements in body weight and food consumption were observed in Wistar rat studies using higher doses (2266, 2078, and 1906 mg/kg-day) of DnOP in feed for short periods of time (3, 10, or 21 days; Mann *et al.,* 1985). Statistically significant decrements in body weight and food consumption have been reported, however, following dosing of Wistar rats with 1000 mg/kg-day MnOP (a DnOP metabolite) in feed for 7 days (Oishi and Hiraga, 1980).

Published studies suggest that mice may be relatively less sensitive than rats to the effects of DnOP when considering body weight and food consumption. Treatment of female CD-1 mice dams with 9780 mg/kg-day DnOP via oral gavage once a day during gestation days (Gd) 6 - 13 (Hardin *et al.,* 1987), male CD-1 mice with 15,000 mg/kg-day in feed for 14 days (Heindel *et al.,* 1989), and male and female CD-1 mice with 7500 mg/kg-day in feed for 105 days (Heindel *et al.,* 1989) had no effect on body weight gain or food consumption in either the non-parental, F_0, or F_1 generations.

Decrements in food consumption and body weight are tied closely to dosing strategy. In these studies, adverse effects were seen in other organ systems at doses in which body weights and food consumption were not affected. This suggests that dosing strategies in these studies were adequate and that pathologies were not resultant from changes in body weight or food consumption. Minor inconsistencies such as the reporting of absolute body weights instead of body weight gains did not affect the overall conclusion that non-organ based effects are not the most sensitive hazard endpoint for DnOP.

Hepatotoxicity

An abundance of data suggests that the liver is one of the primary organs affected by DnOP or its metabolites.

Male Sprague-Dawley rats (6 per group) exposed to 1000 mg/kg-day DnOP daily via oral gavage for 14 days developed significantly increased relative liver weights, marginally increased palmitoyl-CoA oxidation, increased enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, and ethylmorphine N-demethylase activity. Significant reductions in activity were observed in D-amino acid oxidase, 7-ethoxyresorufin O-deethylase, and 7-ethoxycoumarin O-deethylase (Table 5.2). Changes were not observed in the whole homogenate protein, cytochrome p450s, or microsomal proteins. Slightly higher doses of DnOP (2000 mg/kg-day) did not result in increased numbers of peroxisomes when viewed ultrastructurally (Lake *et al.,* 1984). Largely the same results were reported in additional testing when male Sprague-Dawley rats were gavage dosed with 1000 mg/kg-day DnOP once a day for 14 days (Lake *et al.,* 1986). Overall, *in vivo* treatment with DnOP increased relative liver weight, marginally increased palmitoyl-CoA oxidation, marginally increased enoyl-CoA hydratase heat labile and ethylmorphine N-demethylase activity, and reduced lauric acid 11-12 hydroxylation and 7-ethoxycoumarin O-deethylase activity.

In these publications, enzymatic measurements are associated with peroxisomal fatty acid β-oxidation and microsomal induction processes. These processes are chosen for investigation because metabolites of phthalates have been shown to induce the liver to inappropriately synthesize triglycerides and increase the synthesis of peroxisomal and microsomal fatty acid oxidases (Hinton *et al.,* 1986). Peroxisomal beta-oxidation is the mechanism by which long- and very long-chain fatty acids in the Acyl-CoA format are irreversibly metabolized in the peroxisome to generate Acetyl-CoA molecules. In peroxisomes, this activity is coupled to the generation of hydrogen peroxide (Reddy, 2001). Overall activity of the peroxisomal fatty acid β-oxidation cycle is determined by measuring CN-insensitive palmitoyl-CoA (fatty acid) oxidation (Lake, 1993). The function of β-oxidation is also assessed by determining the activity of carnitine acetyltransferase, an enzyme that transports activated acyl-groups into peroxisomes, and enoyl-CoA hydratase, a peroxisomal enzyme that facilitates the hydration of fatty acids. Microsomal induction and activity are determined by observing the hydrolysis of lauric acid, a substrate used for measuring the activity of CYP4A isoenzymes; 7-ethoxyresorufin O-deethylase, an enzyme for measuring CYP1A1; ethylmorphine N-demethylase, an enzyme for measuring CYP3A; 7-ethoxycoumarin O-deethylase, a cytochrome P450-dependent microsomal enzyme (Tamasi *et al.,* 2004); and D-amino oxidase, a matrix-bound enzyme important in amino acid catabolism (Mannaerts and Van Veldhoven, 1993).

Table 5.2 Liver Toxicity Data in Sprague-Dawley Rats Gavage Dosed with DnOP (Lake *et al.*, 1984)		
Liver Parameter	**Control (corn oil)**	**1000 mg/kg-day DnOP (percent of control)**
Relative liver weight (g liver/100 g body weight ± Standard Error Mean [SEM])	3.6 ± 0.1	4.2 ± 0.1 (115; P < 0.01)
Enoyl-CoA Hydrolase – heat labile activity (µmol/min/mg homogenate protein)	1.6 ± 0.1	2.6 ± 0.3 (165)
Carnitine acetyltransferase (nmol/min/mg homogenate protein)	4.1 ± 0.4	12.6 ± 2.4 (305)
Lauric acid hydrolase (nmol/hr/mg microsomal protein)	170 ± 8	214 ± 31 (125)
Palmitoyl-CoA oxidation (nmol/min/mg homogenate protein)	5.6 ± 0.3	6.9 ± 0.5 (125)
Ethylmorphine N-demethylase (nmol/hr/mg microsomal protein)	660 ± 71	811 ± 25 (125)
D-amino oxidase (nmol/hr/mg microsomal protein)	121 ± 5	68 ± 3 (55; P < 0.01)
7 ethoxyresorufin O-deethylase (nmol/hr/mg microsomal protein)	8.4 ± 1.2	4.5 ± 0.6 (55; P < 0.01)
7 ethoxycoumarin O-deethylase (nmol/hr/mg microsomal protein)	140 ± 6	97 ± 11 (70; P < 0.05)

Adverse ultrastructural observations were further described by Poon *et al.,* (1997). In Sprague-Dawley rats exposed to DnOP in feed for 13 weeks, DnOP induced a moderately accentuated zonation (M), anisokaryosis (M), nuclear hyperchromicity (M), perivenous cytoplasmic vacuolation (M & F), and endothelial prominence (M) in high dose group livers (350.1 - 402.9 mg/kg-day for M - F, respectively; *see footnote in Table 5.3*). A moderate accentuation of zonation, and endothelial prominence and moderate nuclear hyperchromicity were also seen in female rats exposed to lower doses of DnOP (40.8 and 0.4 mg/kg-day, respectively, Table 5.3). Significant increases in ethoxyresorufin-O-deethylase activity (M, 350.1; F, 402.9 mg/kg-day) were also observed, contrasting those seen in Lake *et al.,* (1984). Differences in enzymatic activity were possibly due to the longer exposure period in the study by Poon *et al.,* (1997). In Poon's study, ultrastructural changes were not associated with changes in absolute or relative liver weight, aminopyrine-N-demethylase activity, aniline hydroxylase activity, or the number or percent area of peroxisomes at any of the test dose levels (Table 5.3; Poon *et al.,* 1997).

Table 5.3 DnOP-induced Liver Effects in Male and Female Sprague-Dawley Rats (Poon *et al.*, 1997)					
Adverse Effect	**Dose (mg/kg-day) in Feed**				
	0 (corn oil)	**0.4**	**3.5 - 4.1**	**36.8 - 40.8**	**350.1 - 402.9**
Absolute liver weight in male rats, g ± stddev (% body weight)	17.1 ± 1.7 (3.31)	18.5 ± 2.1 (3.42)	18.4 ± 2.1 (3.39)	18.6 ± 3.4 (3.49)	18.6 ± 1.5 (3.49)
Absolute liver weight in female rats, g ± stddev (% body weight)	9.83 ± 1.04 (3.32)	9.66 ± 1.31 (3.15)	9.83 ± 0.88 (3.26)	10.25 ± 1.16 (3.20)	10.30 ± 1.36 (3.52)
Male Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	0.120	0.126	0.131	0.150	0.375 ($P < 0.05$)
Female Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	0.163	0.165	0.165	0.198	0.345 ($P < 0.05$)
Male liver accentuation of zonation (Average Severity Index; ASI)[1]	1 (0.1)	2 (0.2)	1 (0.1)	1 (0.1)	10 (3.1)
Male liver anisokaryosis (ASI)[2]	1 (0.1)	0	4 (0.3)	5 (0.4)	9 (1.9)
Male liver nuclear hyperchromaticity (ASI)[3]	0	0	2 (0.3)	4 (0.4)	5 (1.0)
Male liver perivenous cytoplasmic vacuolation (ASI)[4]	0	0	0	0	9 (2.7)
Male liver endothelial prominence (ASI)[5]	0	0	0	0	7 (1.1)
Female liver accentuation of zonation (ASI)[1]	5 (0.4)	6 (0.4)	9 (0.7)	10 (0.8)	10 (1.6)
Female liver anisokaryosis (ASI)[2]	9 (1.5)	10 (2.0)	10 (2.3)	10 (2.5)	10 (3.0)
Female liver nuclear hyperchromaticity (ASI)[3]	3 (0.6)	10 (2.1)	9 (1.6)	10 (1.9)	10 (2.0)
Female liver perivenous cytoplasmic vacuolation (ASI)[4]	0	0	0	0	5 (1.2)
Female liver endothelial prominence (ASI)[5]	0	0	5 (0.5)	9 (0.9)	10 (1.5)

ASI – Average Severity Index

[1]*Accentuated zonation – enhanced differentiation of hepatic acinar zones 1-3*

[2]*Anisokaryosis – significant variation in the sizes of hepatic cell nuclei*

[3]*Nuclear hyperchromicity – increased nuclear density*

[4]*Perivenous cytoplasmic vacoulation – vacoulation in the cytoplasm of cells in hepatic zone 1*

[5]*Endothelial prominence – perivenous, periarterial, and/or sinusoidal enlargement of endothelial cells*

Hepatic ultrastructural changes induced by DnOP may have altered the ability of the rats to excrete DnOP metabolites, since major elements for glucuronidation (glucuronosyltransferase) and deglucuronidation (β–glucuronidase) are integrated into the endoplasmic reticulum (Dwivedi *et al.,* 1987).

Longer 10-week feed treatments with 1% DnOP following partial hepatectomy and intraperitoneal injection with the initiator diethylnitrosamine also slightly increased carnitine acetyltransferase activity, and induced a mild fatty change in the liver without a corresponding change in liver weight (DeAngelo *et al.,* 1986). This treatment strategy also increased the number of hepatic gamma-glutamyl transpeptidase (GGT) positive foci/cm^2, increased the overall foci area and percentage of the liver as foci, and GGT activity, suggesting that DnOP may have tumor promoting abilities.

DnOP-induced pathologies in Wistar rats confirmed those seen in Sprague-Dawley rats to some extent. Initial exposure to DnOP induced a hepatic centrilobular loss of glycogen and ultrastructural proliferation and dilation of smooth endoplasmic reticulum by 3, 10, and 21 days (2266, 2078, and 1906 mg/kg-day, respectively; Mann *et al.,* 1985). DnOP exposure also caused a shortening of microvilli in bile canaliculi by day 3, and a small increase in lysosomal number by day 10, but this effect was not reported in subsequent time-points. Other adverse effects such as a change in appearance to pale and greasy, increased relative liver weight, a marginal increase in the number of liver mitotic figures, a marked increase in the centrilobular accumulation of fat (and associated necrosis), accumulation of lipid droplets in hepatocytes, increased cyanide-insensitive palmitoyl-CoA oxidation, and an increase in catalase activity in the large particulate fraction of homogenates occurred later on days 10 and 21. Increased hepatomegaly, a small increase in peroxisome number and decreased 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphatase activity were only reported for day 21. In contrast to other phthalates, DnOP did not change the α-glycerophosphate dehydrogenase activity, catalase activity, cytochrome p450 induction, or nonenzymatic reducing agents at any time following exposure. Rats dosed at 2% DnOP via feed for similar times also displayed a change in liver appearance (pale and greasy) and mildly increased hepatomegaly (after 21 days), centrilobular fat accumulation, peroxisome proliferation (after 21 days), smooth endoplasmic reticulum (ER) proliferation, and loss of rough ER. A moderately increased centrilobular loss of glycogen and necrosis was also observed. No change in total glycogen loss, periportal fat accumulation, the density of inner mitochondrial matrix, or mitosis was observed following dosing in this study (Hinton *et al.,* 1986).

DnOP-induced hepatic changes in other rat strains paralleled results in Sprague-Dawley and Wistar rats. Increased relative liver weights and peroxisomal beta oxidation activity were observed following 10,000 mg/kg DnOP administration to male Fischer 344 rats in the feed for 2 weeks. Increased periportal hepatocellular replicative DNA synthesis was also observed at 2 and 4 weeks without changes in gap junction intercellular communication (Smith *et al.,* 2000). Significantly increased liver weights in male albino rats were also observed following daily intraperitoneal injection of 2500 and 5000 mg/kg-day for 5 consecutive days (Dogra *et al.,* 1985), and following 0.5 and 1.0 % DnOP dietary exposures for 26 weeks in partially hepatectomized and initiated male F344 rats (Carter *et al.,* 1992). DnOP-induced effects in this study included an increase in the volume percentage liver expressing GGT and the grams of liver expressing GGT and GST-P.

The hepatic effects of MnOP have also been studied in male Sprague-Dawley rats. Lake *et al.,* (1984) dosed rats with 715, 750, or 1000 mg/kg-day MnOP daily via oral gavage for 14 days. Treatment with MnOP (715 mg/kg-day) increased the relative liver weight, marginally increased palmitoyl-CoA oxidation, ethylmorphine N-demethylase activity, increased enoyl-CoA hydratase heat labile activity, carnitine acetyltransferase activity, lauric acid hydroxylation, reduced D-amino acid oxidase activity, but did not change the whole homogenate protein,

microsomal proteins, or cytochrome p450s. Similar treatment schedules with slightly higher doses of MnOP (750, 1000 mg/kg-day) did not result in an increased number of peroxisomes when viewed ultrastructurally. In a second study (Lake *et al.,* 1986), dosing rats with MnOP (715 mg/kg-day) once a day for 14 days resulted in increased relative liver weights, increased palmitoyl-CoA oxidation, and increased enoyl-CoA hydratase heat labile activity. No changes in liver-zinc concentration were observed at this dose level following feed exposures for 1 week (Oishi and Hiraga, 1980). The *in vitro* portion of Lake's study (1986) demonstrated that MnOP could increase carnitine acetyltransferase activity (at 20 μM) and palmitoyl-CoA oxidation (at 200 μM). These results contrast, in part, with those reported in Hinton *et al.,* (1986). Administration of 250 μM to rat hepatocytes isolated from Wistar rats increased cellular blebbing and vacuolation, marginal and central lipid accumulation, the incorporation of palmitate into triglyceride and cholesterol esters, and increased fatty acid oxidation, but did not change the cyanide-insensitive palmitoyl-CoA.

Studies suggested that mice were relatively less sensitive to hepatic effects induced by DnOP when compared to rats. Adverse effects occurred at doses higher than those presented in rat studies. CD-1 mice offspring (F_1 generation) dosed with 7500 mg/kg-day DnOP had significantly increased liver weights after 74 days of exposure via the feed (Heindel *et al.,* 1989). Increased liver weights were not observed in any dose group of parental mice of the preceding generation. These results contrasted with those reported in Smith *et al.,* (2000). In Smith's study, administration of 10,000 mg/kg-day DnOP in feed to B6C3F_1 mice for 2 or 4 weeks did not change the relative liver weight, gap junction intercellular communication, or periportal hepatocellular replicative DNA synthesis. Treatment with DnOP did, however, increase the peroxisomal beta oxidation activity at 2 weeks (500 mg/kg-day) and 2 and 4 weeks (10,000 mg/kg).

Substantial evidence of DnOP-induced hepatotoxicity has been reported in the studies reviewed. Hepatic structural and functional deficits have been observed in a variety of strains of both rats and mice. Structural alterations such as increased liver weights, centrilobular fat deposition and ultrastructural changes at the cellular level have been reported. Peroxisomal proliferation has been reported in Wistar but not Sprague-Dawley rats. Altered carnitine acetyltransferase activity suggested that fatty acid metabolism was being altered by DnOP exposures, since this function is induced in primary hepatocytes by many peroxisome proliferators (Lake *et al.,* 1986). Other enzymatic activities associated with peroxisome proliferation (i.e., cyanide-insensitive palmitoyl-CoA oxidase and enoyl-CoA hydratase activity) were marginally induced by DnOP exposures. Biochemical alterations were also observed in enzymes (i.e., lauric acid, 7-ethoxyresorufin O-deethylase, ethylmorphine N-demethylase, and 7-ethoxycoumarin O-deethylase) important in the regulation of cytochrome p450s.

Peroxisome proliferation

Peroxisome proliferator-activated receptors (PPAR) contribute to hepatic and other pathologies induced by DnOP. Bility *et al.,* (2004) demonstrated that transfected mouse and human PPARα, mouse PPARβ, and mouse and human PPARγ receptor activities were increased in a dose-dependent fashion 32.4-fold, 7.6-fold, 13.7-fold, 11-fold, and 19.1-fold, respectively, following exposure of mouse 3T3-L1 fibroblast host cells to MnOP for 24 hours. In the same report, exposure to MnOP for 48 hours induced mRNA encoding two PPARα target genes, acyl CoA oxidase – (ACOX; rat hepatoma FaO, 2.6 - 5.6-fold; human hepatoma HepG2 cells, 1.1 - 1.3-fold) and cytochrome P4504A (Cyp4A; rat hepatoma FaO cells, 3.8 - 10.9-fold). MnOP also strongly induced adipogenesis (fat production) in undifferentiated 3T3-L1 fibroblasts.

Even though mouse and human receptor PPAR activity and the induction of PPARα target genes were strongly increased by MnOP, a corresponding increase in hepatic peroxisome proliferation was not observed in most rat and mouse studies. Increased peroxisomal beta-oxidation, but not relative liver weight, gap junction intercellular communication, or periportal hepatocellular replicative DNA synthesis, has been reported in $B6C3F_1$ mice in Smith *et al.,* (2000). Increased liver weights were reported for CD-1 mice offspring (F_1 generation) dosed with 7500 mg/kg-day for 74 days (Heindel *et al.,* 1989). Increases in liver weight were not associated with ultrastructural changes. Significant changes in the activity or oxidation of rat enzymes important in the oxidation, transport, or hydration of fatty acids (palmitoyl-CoA, enoyl-CoA hydratase, carnitine acetyltransferase) and induction of microsomal CYP activity (lauric acid and ethylmorphine N-demethylase), but not peroxisomal proliferation, has also been described in Sprague-Dawley rat studies (Poon *et al.,* 1997; Lake *et al.,* 1984, 1986).

For DnOP, the discrepancy between PPAR receptor activation, gene induction, enzymatic changes and limited peroxisome proliferation has been attributed to the rapid metabolism of DnOP into lower molecular weight metabolites (Bility *et al.,* 2004). This hypothesis is supported by the observation that limited peroxisome proliferation occurs only at higher DnOP doses in Wistar rats (Mann *et al.,* 1985; Hinton *et al.,* 1986).

A lack of continual activation of downstream events following PPAR activation may also be of importance in explaining the paucity of reproductive effects induced by DnOP. Exposure to phthalates has been reported to affect PPARα, β, and γ, all of which have been described in both rat and human testes (Corton and Lapinskas, 2005). Alteration in the normal function of these receptors has been correlated to inhibition of expression of the aromatase gene, an induction of the activity of an estradiol-metabolizing enzyme 17β-HSD IV, an increase in the amount of fatty acid binding protein (Latini *et al.,* 2008), and interference in retinoic acid receptor-α signaling in mouse and rat Sertoli cells (Dufour *et al.,* 2003). In other studies, dietary exposure of PPARα-null Sv/129 mice to high levels of phthalates also resulted in increased testosterone and less severe testicular lesions when compared to normal control mice (Ward *et al.,* 1998). These studies suggested that phthalate-induced reproductive effects were partially dependent on

continued influence on PPAR receptors. The rapid metabolism of DnOP, therefore, may be one reason why PPAR-mediated reproductive events don't occur to the same extent as with other phthalates.

The above studies support the conclusion that **there is "sufficient animal evidence" for the designation of DnOP as a "probable hepatotoxicant" under the FHSA**.

Thyroid Toxicity

DnOP-induced effects on the thyroid have also been described in peer reviewed publications. Reduced thyroid follicle size in female Sprague-Dawley rats and decreased colloid density in both male and female rats were reported following the administration of 3.5 and 40.8 mg/kg-day DnOP, respectively, in feed for 13 weeks (Poon *et al.,* 1997). Ultrastructural and hormonal changes were also observed in Wistar rats exposed to 2% DnOP in feed for 3, 10, or 21 days (Hinton *et al.,* 1986). Treatment with DnOP decreased serum thyroxine (T_4) on days 3, 10, and 21 and increased triiodothyronine (T_3) on day 21. In *in vitro* tests, DnOP administration also resulted in a dose-dependent increase in iodide uptake (10^{-5} to 10^{-3} M) in the Fischer 344 rat thyroid cell line (FRTL), possibly through specific activity of the sodium-iodide symporter (NIS; Wenzel *et al.,* 2005). Ultrastructurally, DnOP also increased the number and size of lysosomes, enlarged the Golgi apparatus, and induced mitochondrial damage in thyroids (Hinton *et al.,* 1986).

DnOP-induced alterations in thyroid structure and function have been termed a "persistent hyperactive response" (Hinton *et al.,* 1986). This term is misleading in the context of thyroid function, however, since many different thyroid functions or disease states can be termed overactive or "hyperactive". Biochemically, a "hyperactive" thyroid is typified by a persistent elevation of T_3 and T_4 hormones, such as occurs in Graves' disease (Robbins *et al.,* 1994). Clearly, hormonal results following oral exposures including decreased T_4 and mildly elevated T_3 do not fit this definition, regardless of structural pathology and, in fact, are more suggestive of systemic hypothyroidism. Clinical effects that are typical of hyperthyroid patients or animals (weight loss, increased food consumption, polyuria/polydipsia) were also not observed or reported in the reviewed studies.

Pathological and ultrastructural observations are somewhat representative, however, of increased thyroid activity. DnOP-induced decrements in follicle size and colloid density as described in Poon *et al.,* (1997) are commonly seen in hyperactive thyroid follicles actively synthesizing or secreting thyroglobulin and thyroid hormones (Krstic, 1991).

DnOP-induced ultrastructural alterations including increases in the number and size of lysosomes and hypertrophy of the Golgi apparatus as described in Hinton *et al.,* (1986) are also commonly observed in active thyroids (Krstic, 1991; Krupp and Lee, 1986). The absence of increased apical vesicles (typical for hyperactive follicular cells that are synthesizing hormones; Krstic, 1991; Tsujio *et al.,* 2007) or colloid vacuoles (Nilsson *et al.,* 1988), and the presence of all other ultrastructural and pathological changes (mitochondrial damage) does not enable the determination, however, of whether biosynthetic or secretory mechanisms are primarily targeted/affected by DnOP exposures.

Substantial evidence of DnOP-induced thyroid toxicity has been presented in the studies reviewed. Structural and functional deficits have been observed both in rats and *in vitro*.

Structural alterations such as reduced thyroid follicle size and decreased colloid density were reported, as were alterations in thyroid hormones T_3 and T_4.

The above studies support the conclusion that **there is "sufficient animal evidence" for the designation of DnOP as a "probable thyroid toxicant" under the FHSA**.

Adrenal Toxicity

DnOP-induced adrenal effects were limited to a single study that described rat weight and ultrastructural changes following intraperitoneal injection of DnOP daily for 5 days a week, for 45 or 90 days (Dogra *et al.,* 1985). In these experiments, significant increases in adrenal weight were observed at five days when exposed to 2500 and 5000 mg/kg-day, and non-significant increases in weight were reported at 10,000 mg/kg-day. An increase in lipid vacuoles and a loss of arrangement of cellular columns of the zone fasciculata was also reported in the adrenal cortex following 600 mg/kg-day dosing for 90 days.

Evidence of DnOP-induced adrenal toxicity in animals is limited to only one study that used a non-traditional exposure methodology (intraperitoneal exposure).

Methodological deficiencies and a lack of additional data support the conclusion that **there is "limited or inadequate animal evidence" for the designation of DnOP as a "known or probable adrenal toxicant" under the FHSA**.

Immunotoxicity and Hematotoxicity, Clinical Chemistry

Significant immunological effects have been reported for DnOP and MnOP. Experiments suggest that DnOP interferes with the structural and functional components of immunocompetent cells and that humorally mediated immunity may also be impaired.

Dose-related decreases in the absolute weight of the thymus and lymph nodes (mesenteric, portal, and peripheral) were observed following DnOP administration to albino rats intraperitoneally (i.p.) at 100, 300, 600 mg/kg-day five days a week, for 45 or 90 days. Splenic changes (edema and paling) were also evident by 45 days at all dose levels. The high dose, 600 mg/kg-day also induced a loss of "distinction between cortex and medulla" in the thymus by 45 days and a "marked depletion" of cortical cells and medullary hyperplasia by 90 days. Splenic cellular depletion of peripheral follicles of white pulp, edema in the red pulp of the spleen, reductions in the follicle number reported in cortical regions of lymph nodes, and an increase in lipid vacuoles and a loss of arrangement of cellular columns of the zone fasciculata in the adrenal cortex were also evident by 90 days in the highest dose level. In an additional experiment, significantly increased adrenal gland and spleen weights were observed in rats injected i.p. with

2500 or 5000 mg/kg bw-day daily for five consecutive days. Marginal increases in organ weights were also reported in high dose treatment groups (10,000 mg/kg-day). Significant decreases in thymus, mesenteric lymph nodes, and portal lymph node weights and a significant increase in peripheral lymph node weights were also seen following exposure to the high dose. Splenic cells were also adversely affected by DnOP, with spleen cell responsiveness to T-lymphocyte mitogen (phytohemagglutinin - PHA) being reduced significantly in a dose-dependent manner. Responsiveness to B-lymphocyte mitogen (lipopolysaccharide - LPS) was also reduced significantly in high and medium doses of DnOP. Final experiments involving delayed hypersensitization demonstrated a DnOP-induced marginal non-significant dose-related decrease in reactivity (Dogra *et al.,* 1985).

Host resistance to virus and protozoa was also affected by exposure to DnOP (Dogra *et al.,* 1989). Dose- and time-related increases in mortality (from 0 to 70% over days 4 - 10 post-inoculation for EMCV, and 20 to 80+% over days 3 - 19 post-inoculation with *P. berghei*) and parasitemia (from 0.2 to 7% over days 5 - 14 post inoculation with *P. berghei*) were observed following gavage dosing of 650 and 2600 mg DnOP/kg-bw to Swiss albino mice daily for five days. This data suggested that DnOP impaired the host's ability to produce interferon and/or antibodies, and altered the function of white blood cells.

Immunosuppression induced by MnOP has also been investigated (Larsen *et al.,* 2001). In this study, substantial decrements were observed in IgE and IgG1 production in female Balb/c mice sensitized by subcutaneous injection of 1 µg ovalbumin (OV) and then exposed to 5.3 mg/kg-bw MnOP. An adjuvant effect for IgG1 production was also observed in 0.53, 0.053, and 0.0053 mg/kg-bw MnOP treatments.

Immunological effects do not seem to be dependent on changes in the clinical chemistry or hematology of test animals. No significant dose-related changes were reported for albumin, calcium, inorganic phosphate, total protein, white blood cell number, mean corpuscular hemoglobin and volume, or platelet count after dosing Sprague-Dawley rats with 350.1 - 402.9 mg/kg-day DnOP in feed for 13 weeks (Poon *et al.,* 1997). In addition, no changes were seen in zinc or testosterone concentration in the serum following dosing Wistar rats with 1000 mg/kg-day MnOP for one week (Oishi and Hiraga, 1980).

Data suggest that adverse immunological effects occur following DnOP exposures. Utilization of a non-traditional dosing method (intraperitoneal injection), however, makes interpretation and application of the results difficult. Substantial differences in the metabolic profile of DnOP can be expected following intraperitoneal injections when compared to oral dosing. These differences do not invalidate, however, the immunological effects reported.

The weight of evidence from the above studies supports the conclusion that **there is "sufficient animal evidence" for the designation of DnOP as a "probable immunotoxicant" under the FHSA**.

Neurotoxicity

Clinical symptoms indicative of direct neurological effects (convulsions, etc.) have not been reported for DnOP. Only one study has reported neurological signs following the gavage dosing of n-octanol, a metabolite of DnOP. In this study, clinical intoxication (neurological impairment) was observed in Wistar rat dams dosed with 650, 945, and 1300 mg/kg-day n-octanol during gestation days 6 - 15 (Hellwig and Jackh, 1997).

Classic neurological tests such as those outlined for the CPSC (CPSC, 1992), and in the "Guidelines for Neurotoxicity Risk Assessment" (EPA, 1998a) or in OPPTS 870.6200 (Neurotoxicity Screening Battery; EPA, 1998b) have not been performed for DnOP or its metabolites.

Evidence of DnOP-induced neurotoxicity is "limited or inadequate" in the animal studies reviewed. CPSC staff did not find any data on human or animal neurotoxicity of DnOP. Reported incidental behavioral or clinical effects could not be segregated from the more general toxicity posed by DnOP.

A general lack of credible data support the conclusion that **there is "limited or inadequate animal evidence" for the designation of DnOP as a "known or probable neurotoxicant" under the FHSA**.

Renal Toxicity

Investigations of DnOP-induced renal effects have largely been limited to descriptions of organ weight and ultrastructural changes. Significantly increased kidney weights have been reported following dosing of CD-1 female mice offspring (F_1 generation) with 7500 mg/kg-day DnOP in the feed for 74 days, but not in parental mice of the preceding generation treated for 105 days (Heindel *et al.,* 1989). Significant, but non-dose related, changes in kidney weight have also been described in male albino rats dosed via intraperitoneal injection with 2500 and 5000, but not 10,000 mg/kg bw-day DnOP for 5 consecutive days (Dogra *et al.,* 1985). Dose-dependent changes in kidney ultrastructure have been reported in albino rat kidney glomeruli (atrophy, cellularity), proximal tubules (swelling, luminal obliteration, desquamation degeneration of epithelium), distal tubules (dilation, hyaline casts), vasa recta (edema), and interstitium (lymphocytic infiltration) following subchronic intraperitoneal exposures to 100, 300, and 600 mg/kg-day DnOP (Khanna *et al.,* 1990). Many of these effects were retained after a 45 day recovery period. Alterations in gross kidney structure were not evident in macroscopic examinations in this study, however, even at the highest dose (600 mg/kg-day). These results contrast that seen in other species or strains. Changes in absolute or relative kidney weights were not described in male or female Sprague-Dawley rats exposed to 350.1 - 402.9 mg/kg-day DnOP in feed for 13 weeks (Poon *et al.,* 1997). In addition, kidney zinc concentrations in Wistar rats

exposed to oral doses of 1000 mg/kg-day MnOP in feed for one week were not different than controls (Oishi and Hiraga, 1980).

Data suggest that adverse effects may occur in the kidneys following DnOP exposure and that these effects may be strain or species specific. The ultrastructural study suggests that renal effects may be significant and that these changes may not be seen upon gross pathology.

The weight of evidence from the above studies supports the conclusion that **there is "sufficient animal evidence" for the designation of DnOP as a "probable renal toxicant" under the FHSA**.

Gastrointestinal Toxicity

The gastrointestinal effects of DnOP exposure have received limited examination. In one oral feeding study with male Wistar rats, no changes were reported in the appearance of the pancreas following exposure to 2% DnOP in feed for 3, 10, or 21 days (1906 – 2266 mg/kg-day; Mann *et al.,* 1985). No other studies discussing this organ system was found.

As described, limited information is available for assessing the effects of DnOP on the gastrointestinal system (oral cavity, esophagus, stomach, intestines, and pancreas). Incidental data on absorption suggest that these organs maintain their ability to transport DnOP into the bloodstream.

Genotoxicity

Overall, DnOP tested negative in bacterial mutation and direct DNA damage assays (NICNAS, 2008). In particular, DnOP (without and with S-9 activation) was negative for mutagenicity when tested with *Salmonella typhimurium* strains TA100, TA 1535, TA 1537, and TA 98 at 100 - 10,000 µg/plate (Zeiger *et al.,* 1985). Additional studies with *S. typhimurium* have reported similar findings (Florin *et al.,* 1980; Goodyear, 1981a; Sato *et al.,* 1994; Seed, 1982; Shibamoto and Wei, 1986 cited in ATSDR, 1997). In addition, DnOP (2000 µg/mL without activation, 100 - 2000µg/ml with activation) tested negative in *E. coli* DNA damage assays (Goodyear, 1981b cited in ATSDR, 1997 and NICNAS, 2008) and SOS DNA damage chromotest assays (Sato *et al.,* 1994 cited in NICNAS, 2008).

DnOP containing mixtures have also reported little potential for genotoxicity. A di-C6-C10 mixture (containing DnOP) was equivocal in a mouse lymphoma assay (no dose-response) either with or without activation (Barber *et al.,* 2000 cited in NICNAS, 2008), and negative in *S. typhimurium* bacterial mutation and CHO/HPRT locus assays (CMA, 1999 cited in NICNAS, 2008).

Data from *in vitro* and *in vivo* mammalian genetic toxicity studies were not found for DnOP. These are typically used to determine the overall potential of a chemical for inducing genotoxicity. A current genotoxicity testing complement includes: **1)** a bacterial assay (using *S. typhimurium* or *E. coli*) to detect point mutations, ***and* 2)** an *in vitro* L51784 $TK^{+/-}$- 3.7.2C mouse lymphoma cell assay to detect point mutations, chromosomal deletions, translocations, mitotic recombinations/gene conversions and aneupolidy ***or*** an *in vitro* mammalian cell (i.e., Chinese hamster fibroblasts, human, or mammalian peripheral blood lymphocytes) cytogenetic assay for structural chromosomal damage, ***and* 3)** an *in vivo* mammalian erythrocyte micronucleus test for detecting structural aberrations in erythrocyte chromosomes (FDA, 2000).

The lack of *in vitro* and *in vivo* mammalian genetic toxicity data for DnOP and primarily negative results for reviewed genotoxicity studies support the conclusion that **there is "inadequate animal or human evidence" for the designation of DnOP as a "known or probable genotoxicant" under the FHSA**.

Carcinogenicity

The CPSC staff has not found any studies directly demonstrating the carcinogenic potential of DnOP in humans or other animals. Classic carcinogenicity study designs such as those outlined for the CPSC (CPSC, 1992), and in the "Guidelines for Carcinogen Risk Assessment" (EPA, 2005) or in OPPTS 870.4200 (Carcinogenicity; EPA 1998c) have not been performed for DnOP or its metabolites.

Genetic toxicity testing in bacterial systems suggests that DnOP's ability to induce mutations is low, but *in vitro* or *in vivo* mammalian genetic toxicity studies have not been performed to confirm this observation (see Genotoxicity above).

DnOP has been reported to induce small increases in hepatic peroxisomes in some studies (Mann *et al.,* 1985; Hinton *et al.,* 1986) but not in others (Lake *et al.,* 1984; Poon *et al.,* 1997). Increases in enzymatic markers associated with peroxisome proliferation (i.e., carnitine acetyltransferase, cyanide-insensitive palmitoyl-CoA oxidase, and enoyl-CoA hydratase activity), have also been described *in vivo* in rats (Lake *et al.,* 1984) and *in vitro* with MnOP (Lake *et al.,* 1986). Peroxisome proliferation is regarded as a non-genotoxic mechanism by which hepatocellular tumors can develop, and has been demonstrated to be of importance in the carcinogenesis induced by other phthalates (i.e., DEHP; David *et al.,* 1999).

The tumor promoting activity of DnOP has also been described to a limited extent. Partially hepatectomized male Sprague-Dawley rats initiated with diethylnitrosamine (DENA) and then subsequently promoted with 1% DnOP mixed in feed for 10 weeks had a significant increase in the number of hepatic gamma-glutamyl transpeptidase (GGT)+ foci/cm^2, an increase in the overall foci area, and percent of the liver as foci (DeAngelo *et al.,* 1986). Similar results

were reported by Carter *et al.,* (1989, 1992) when using partially hepatectomized and DENA-initiated male F344 rats. In this case, 26 weeks of 0.5 and 1.0% DnOP treatment significantly increased the volume percent of liver that expressed GGT and the mass of liver expressing GGT and placental glutathione-S-transferase (GST-P). The number of GST-P+ nodules, but not GST+ altered foci, was also higher in 1% DnOP treatments.

Results from these studies suggest that DnOP may act as a promoter of preneoplastic hepatic lesions in rats. Similar mechanistic conclusions have been reported for DEHP (David *et al.,* 1999).

The use of the rat liver foci assay has been criticized by some authors as being inappropriate for estimating carcinogenic activity in peroxisome-inducing compounds (Milman and Weisburger, 1994). This is because some peroxisome proliferators (e.g., Wy-14,643) that induce altered hepatic foci and hyperplastic nodules do not have GGT+ activity in the liver (Milman and Weisburger, 1994). DnOP-induced GGT+ activity has been reported in DeAngelo *et al.,* (1986) or Carter *et al.,* (1992), suggesting that this assay criticism may be unwarranted. Use of the GGT+ foci/cm^2 (instead of foci/cm^3) has also been criticized because of biases inherent in cutting and interpreting specimens for pathological assessment. For this reason, promotion data should be considered preliminary.

Evidence of DnOP-induced carcinogenicity in animals is "limited" in the studies reviewed. CPSC staff has not found any data on the human carcinogenicity of DnOP. Genotoxicity studies in animals were uniformly negative for mutagen-inducing activity. Evidence does suggest that DnOP may have carcinogenic activity, nonetheless. Increased GGT+ foci were reported in hepatectomized and initiated rats (DeAngelo *et al.,* 1986) and suggested that DnOP may act as a tumor promoter. Increased peroxisome proliferation and associated enzymatic activity also suggested that DnOP may have the same mode of carcinogenicity as other chemicals such as DEHP.

The available reports do not fit the FHSA definition of "sufficient evidence" of carcinogenicity in animals. Even though data suggest that DnOP may function as a promoter or peroxisome proliferator, a general lack of credible data and negative genotoxicity data support the conclusion that **there is "limited or inadequate animal evidence" for the designation of DnOP as a "known or probable carcinogen" under the FHSA**.

Reproductive Toxicity

DnOP-induced changes in the reproductive system have been documented to a limited extent in some animal species but not others.

DnOP did not induce any changes in the relative testis weight, testis pathology, testis Zinc-65 concentration, or Zinc-65 excretion in Sprague-Dawley rats gavage dosed with 2800 mg/kg-day once a day for four days (Foster *et al.,* 1980). Similarly, no significant changes in absolute or relative testis weight, the incidence of seminiferous tubule atrophy, or bilateral reduction in sperm density were observed following dosing Sprague-Dawley rats with 350.1 mg/kg-day DnOP in feed for 13 weeks (Poon *et al.,* 1997).

In the Wistar rat, the relative testicular weight was decreased following exposure to 2078 mg/kg-day DnOP in feed for 10 days. Decrements in relative testicular weight in this study may have been due to slight changes in body weight. Similar doses (2266 and 1906 mg/kg-day) did not induce any changes in testicular parameters at 3 and 21 days following exposure (Mann *et al.,* 1985). DnOP also did not alter Leydig cells. No DnOP-induced changes to seminiferous tubular structure, Leydig cell morphology, or various aspects of Leydig cell ultrastructure (i.e., mitochondrial swelling, degeneration, SER focal dilatation, vesiculation, and peroxisomes) were found following daily oral gavage with 2000 mg/kg for two days (Jones *et al.,* 1993).

Even though testicular changes have not been described in a robust fashion for the Wistar rat, DnOP has been shown to alter mitochondrial function in testes. *In vivo* gavage treatments with 2000 mg/kg DnOP decreased the mitochondrial respiration control ratio and state-3 oxygen consumption, but did not alter the pyruvate or lactate concentration in testes cells isolated from the rat (Oishi, 1990). *In vitro* DnOP treatments also decreased mitochondrial oxygen consumption in the high dose group (1.3 µmole/ml; Oishi, 1990) of similar cells.

In a continuous breeding protocol study involving CD-1 mice, dietary DnOP administration did not affect reproductive parameters (mating index, fertility index, litters/pair, live (M & F) pups/litter, proportion of pups born alive, sex of pups born alive, live pup weight) in the parental F_0 mice or in matings of the high dose (8640 mg/kg-day for 26 weeks; Morrissey *et al.,* 1989) F_1 generation (allowed to grow for an additional 74 days post-weaning). In the F_1 generation, DnOP treatment also had no effect on the testis, epididymis, or prostate weight, epididymal sperm concentration, percentage of motile sperm, or average estrous cycle length (Heindel *et al.,* 1989; Morrissey *et al.,* 1989).

In the Wistar rat, MnOP did not induce any changes in absolute or relative testis weight, zinc or testosterone concentration in the testes following exposure to 1000 mg/kg-day in feed for one week (Oishi and Hiraga, 1980). These findings contrast that with Jones *et al.,* (1993) in which gavage doses of 2000 mg/kg MnOP once a day for two days resulted in significant alterations in Leydig cell cytoplasmic ultrastructure (such as mitochondrial swelling, degeneration, smooth endoplasmic reticulum (SER) focal dilatation, increased number and length of filipodia, and peroxisomes). These findings were replicated in *in vitro* experiments in which Leydig cell primary cultures were treated with 1000 µM MnOP. In this experiment, incubation with MnOP decreased the LH-stimulated secretion of testosterone and produced ultrastructural changes (increase in filopodial proliferation from the basal lamellar processes and

the cell soma, SER dilatation, mitochondrial swelling, mitochondrial degeneration and numeric reduction, and vesiculation of the Golgi apparatus). MnOP-induced germ cell detachment and disruption of Sertoli-cell monolayers in co-cultures of Sertoli-germ cells have also been reported (NICNAS, 2008).

Additional cellular changes have also been described for MnOP-treated cells. In testis cells isolated from Wistar rats, treatment of mitochondria with MnOP induced a dose-dependent decrease in mitochondrial state 3 oxygen consumption (over 0.065, 0.13, 0.325, 0.65, and 1.30 µmole/ml; Oishi, 1990). Uncoupled mitochondrial oxidative phosphorylation has also been reported following *in vitro* MnOP treatment (Takahashi, 1977 and Melnick and Schiller, 1985 cited in Oishi, 1990).

The reproductive effects of DnOP and its metabolites have not been thoroughly investigated in female animals. Of the studies assessing reproductive impacts, female data was only available for a continuous breeding and a receptor binding study. The breeding study determined that DnOP exposure did not affect fertility or mating for mlaes or females, and that the average estrous cycle length remained unaffected in females. The receptor study demonstrated that DnOP did not induce estrogenic responses *in vivo* or *in vitro* and had no direct binding affinity for human estrogen receptors (NICNAS, 2008). Neither study investigated potential pathologies to reproductive organs, however, as has been done in numerous other studies involving male animals. Both structural and functional data are important when determining a lack of effects following chemical exposures.

Evidence of DnOP-induced reproductive toxicity in animals is "limited" in the studies reviewed. CPSC staff have not found any data on the reproductive toxicity of DnOP in humans. No dose-dependent changes in testes, the epididymis, prostate, Leydig cells, seminiferous tubules sperm structure or function, or average estrous cycle length have been reported in the reviewed animal studies. In continuous breeding animal studies, no effects have been reported for reproductive parameters (mating, fertility, live pups) in F_0 or F_1 generations. Similar findings were considered by the NTP CERHR Phthalates Expert Panel prior to their conclusion that "existing data do not suggest that DnOP is a potent developmental or reproductive toxicant in rodents" (NTP, 2003).

Comparatively little evidence supports the impression that DnOP can induce reproductive effects. Alterations in testis mitochondrial function have been reported following exposure to both DnOP and MnOP at doses that did not induce tissue pathologies. Further, exposures to MnOP induced changes to Leydig cell ultrastructure without changing testes in a gross manner. These changes were not reported, however, following exposures to DnOP.

The available data do not demonstrate that there is "sufficient evidence" of reproductive toxicity in animals. Even though data suggest that DnOP may alter testicular energy regulation, these effects were not translated into tissue pathologies. This fact and the availability of

numerous published studies that report no DnOP-induced alterations in reproductive tissue structure or function, support the conclusion that **there is "limited or inadequate animal evidence" for the designation of DnOP as a "known or probable reproductive toxicant" under the FHSA**.

Prenatal and Postnatal Toxicity

The prenatal and postnatal toxicity effects of DnOP have been investigated to a limited extent in a number of animal models.

DnOP induced significant decreases in average fetus weights and a significant increase in the incidence of gross fetal malformations in Sprague-Dawley rats (5 per group) dosed intraperitoneally on gestation days 5, 10, and 15 with 0, 4890, and 9780 mg/kg-day (Singh *et al.,* 1972; Table 5.4). Although not specified for individual phthalates, gross abnormalities consisted of tail absence, anophthalmia, twisted hind legs and hematomas/hemangiomas.

Table 5.4 Developmental Toxicity Data in Sprague-Dawley Rats Injected with DnOP (Singh *et al.,* 1972)

Volume injected (ml/kg) on Gd 5, 10, and 15	Average Fetus Weight (avg grams ± SE)	Number of Gross Abnormalities (% gross abnormalities based on total number of fetuses)
0 (cottonseed oil)	4.45 ± 0.17	1 (1.9%)
5 (4890 mg/kg-day)	4.00 ± 0.18 ($P \leq 0.01$)	8 (15.7%)
10 (9780 mg/kg-day)	3.40 ± 0.13 ($P \leq 0.01$)	15 (27.3%)

Malformation data from this study contrast with those reported in female CD-1 mice when exposed to 7460 - 8640 mg/kg-day DnOP in feed for 74 days (Heindel *et al.,* 1989, Morrissey *et al.,* 1989) or 9780 mg/kg-day DnOP via oral gavage once a day during gestation days (Gd) 6 - 13 (Hardin *et al.,* 1987). In Hardin and Morrissey's studies, external developmental malformations or variations were not observed on gross pathology in the F_1 or F_2 generations of CD-1 mice. Hardin *et al.*'s abbreviated prenatal assay documented a reduction in the number of live neonates born per litter and a reduction in weight gain in the pups when measured from PNd 1 to 3 (Table 5.5). Changes in the number of viable litters, the percent survival of live born neonates, or the birth weight of pups were not observed. Reductions in the number of pups born per litter and weight gain may have been related to exceptionally high control values for this test.

Table 5.5 Toxicity Data in CD-1 Mice Gavage Dosed with DnOP (Hardin *et al.,* 1987)

Volume administered on Gd 6 - 13 (ml/kg)	Weight Gain from PNd 1 - 3 (g/pup)	Live born per litter
0 (corn oil)	0.7 ± 0.2	11.5 ± 1.7
10 (9780 mg/kg-day)	0.6 ± 0.1 ($P < 0.05$)	10.2 ± 2.8 ($P < 0.05$)

One study has also assessed the developmental toxicity of n-octanol, a precursor used in the synthesis of DnOP (Hellwig and Jackh, 1997). As with results in CD-1 mice, no fetal effects such as changes in weight, viability, or an increase in malformations/variation/retardations were observed in Wistar rats gavage dosed with 130 mg/kg-day during gestation days 6 - 15.

The lack of details on maternal toxicity in the Singh publication limits the utility of presented data for determining whether developmental effects are induced by DnOP. Rat maternal mortality, weight, weight gain, food and water consumption, clinical signs, gestation length, and other parameters indicative of maternal toxicity were not reported in this study. Information on maternal toxicity is critical to understand, because developmental or fetal effects can result indirectly from maternal toxicity (EPA, 1993). In fact, restricted feed consumption (as occurs following toxic insult) has been correlated to prenatal and postnatal body weight decrements in rat fetuses and pups (Wolterbeek *et al.,* 2004).

Adequate descriptions of adverse developmental effects were also missing in Singh's study. Developmental variations and malformations were not reported for each treatment litter and phthalate in this study, but were instead lumped into a summary sentence. The litter is typically used as the unit of interpretation when reviewing developmental studies, and developmental differences from controls are subdivided into malformations and variations (EPA, 1993). Summary consideration of all "abnormalities" for all tested phthalates, therefore, does not describe the data in enough detail for credible interpretation.

The intraperitoneal route of dose administration in Singh's study was also problematic. In the study, DnOP doses approximately equivalent to one-fifth of the LD_{50} were injected into female rats on three widely-spaced separate occasions during gestation. Dosing strategies such as intraperitoneal injection bypass absorptive and metabolic functions normally encountered through oral routes of exposure. This route of exposure has also been shown to be less acutely toxic than with oral dosing (Singh *et al.,* 1972; Goldemberg and Safrin, 1977; Lawrence *et al.,* 1975). Intermittent dosing instead of daily administration is also not encouraged for compounds that are metabolized relatively quickly (i.e., DnOP). This dosing strategy results in large periods of developmental time where blood concentrations of the chemical may be below critical effect levels. Instead of intraperitoneal exposures, the preferred route and duration of exposure for most developmental studies includes daily gavage dosing from implantation through the major period of embryonic organogenesis or just before term necropsy (EPA, 1991, 1993; Tyl, external reviewer communication, 2009).

As with the Singh study, the "abbreviated [developmental] assay" performed by Hardin *et al.,* (1987) also had issues that limited its utility. Hardin *et al.*'s study was a screening level approach for prioritization of chemicals for conventional testing. Portions of a typical developmental toxicity study, such as implantation, corpora lutea, resorptions, postimplantation loss, affected implants, and external/visceral/skeletally altered offspring (EPA, 1993) were not reported in the study, limiting conclusions to the basic variables of weight changes and viability.

The dosing strategy, oral gavage, was the preferred method for dose delivery in developmental studies, but the dose of DnOP (10 mL/kg) was three times higher than recommended volumes (3 - 4 mL/kg). Higher volumes can induce diarrhea in animals and potentially limit chemical absorption by enhancing excretion. The dose timing and duration were also abbreviated (Gd 6 - 13) when compared to typical developmental assays for mice (implantation on Gd 4 - 5 through major organogenesis on Gd 6 - 15 or term necropsy; EPA, 1991, 1993; Tyl, external reviewer communication, 2009).

Evidence of DnOP-induced developmental toxicity in animals was "limited" in the studies reviewed. CPSC staff have not found any data on the human developmental toxicity of DnOP. Data from rat and mouse studies suggested that DnOP can induce moderate decrements in weight or weight gain in fetuses or neonates. Decrements in weight gain may have been associated, however, with higher than normal control values or maternal toxicity. Malformations and decrements in the number of live born per litter were also observed in one study, but not in others. Malformations were described in a general way, however, limiting the utility of these observations.

The available data do not demonstrate that there is "sufficient evidence" of prenatal or postnatal (developmental) toxicity in animals. Even though data suggest that DnOP may induce fetal weight decrements and malformations or variations, these effects are difficult to interpret in light of the lack of data on maternal toxicity, and the distribution of malformations. Additional methodological deficiencies in the presented studies also support the conclusion that **there is "limited or inadequate animal evidence" for the designation of DnOP as a "known or probable developmental toxicant" under the FHSA**.

Organ-specific Hazard Endpoint Selections

Sufficient data have been presented to select organ-specific hazard endpoints. Selection of these endpoints was performed without regard for the species type, gender, or sufficiency of information in the respective organ systems (Table 5.6). These endpoints can be used in ranking relative effects on the organ systems or exposure durations. Overall, acute studies had higher concentration hazard endpoints than subchronic studies. Further, liver and thyroid endpoints had lower effect concentrations when compared to other organ systems.

Table 5.6 Organ-specific Hazard Endpoint Selections

Species (Gender)	*Exposure Route*	*Dose*	*Dose Duration*	*Toxicological Endpoint*	*Toxicological Basis*	*Citation*	*Organ System*
[1]Wistar rats (F)	Oral gavage (n-octanol)	130, 650, 945, and 1300 mg/kg-day	Once daily for Gd 6 - 15	Maternal LOAEL = 650 mg/kg-day; NOAEL = 130 mg/kg-day	Clinical intoxication (nervous system effects such as lateral and abdominal position, unsteady gait, salivation, piloerection, nasal discharge, and pneumonia)	Hellwig and Jackh, 1997	General
Albino rats (M)	Oral gavage	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Reduction in the follicle number in cortical regions of the lymph nodes	Dogra *et al.*, 1985	Lymph nodes
Albino rats (M)	Oral gavage	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Loss of distinction between cortex and medulla, depletion of cortical cells and medullary hyperplasia by 90 days	Dogra *et al.*, 1985	Thymus
Albino rats (M)	Oral gavage	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 100 mg/kg-day	Washed out appearance and edema; severe cellular depletion of peripheral follicles of the white pulp and edema in the red pulp by 90 days	Dogra *et al.*, 1985	Spleen
Albino mice (M, 100g)	Intraperitoneal injection	0, 100, 300, 600 mg/kg-day (10 rats per group)	Once daily, five days a week for 90 days (with a 45 day recovery period)	LOAEL = 100 mg/kg-day	Dose-dependent changes in the glomerulus (atrophy, cellularity), proximal tubules (swelling, luminal obliteration, desquamation degeneration of epithelium), distal tubules (dilation, hyaline casts), vasa recta (edema), and interstitium (lymphocytic infiltration) with many effects retained after a 45 day recovery period	Khanna *et al.*, 1990	Kidney
Albino rats (M)	Oral gavage	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Derangement of the cellular columns of the zona fasciculate and increase in the lipid vacuoles by 90 days	Dogra *et al.*, 1985	Adrenal glands
Crj; Wistar rats (M)	Oral gavage	2000 mg/kg	Once	LOAEL = 2000 mg/kg	Decreased mitochondrial state 3 oxygen consumption, decreased respiration control ratio	Oishi, 1990	Testis
CD-1 mice (F)	Oral gavage	9780 mg/kg-day	Once daily for Gd 6 - 13	Fetal LOAEL= 9780 mg/kg-day	Reduced number of live neonates per litter and weight gain in the pups	Hardin *et al.*, 1987	Fetus
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 350.1 – 402.9 mg/kg-day; NOAEL = 36.8 - 40.8 mg/kg-day	Increased ethoxyresorufin-O-deethylase activity, moderate accentuation of zonation (M), anisokaryosis (M), nuclear hyperchromicity (M), perivenous cytoplasmic vacuolation, and endothelial prominence (M)	Poon *et al.*, 1997	Liver
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 40.8 mg/kg-day; NOAEL = 4.1 mg/kg-day	Decreased colloid density (F), reduced follicle size (F)	Poon *et al.*, 1997	Thyroid

[1] Red text highlights studies that are acute in exposure duration (0 - 14 days). Blue text highlights studies that have subchronic exposure durations (14 - 364 days).

Overall Uncertainty

The hazard database for DnOP consists of approximately 20 robust studies and a variety of other less well described studies. Exposure durations in these studies are primarily acute (single or short-term repeated dose, typically a maximum of 5 days) and intermediate/subchronic. Significant data gaps exist when considering acute exposures (single dose), chronic exposures (and carcinogenicity), and exposures occurring during and after pregnancy (prenatal and postnatal exposures). In most of the studies reviewed, the routes of exposures were primarily oral. Significant immunological effects were determined using the intraperitoneal route of exposure.

Overall Acceptable Daily Intakes

Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" due to chronic effects and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers.

Short-term oral exposures – general population

For acute oral short-duration exposures, the dose LOAEL of 1000 mg/kg-day (Lake *et al.,* 1984, 1986) was chosen as the representative overall hazard endpoint. This endpoint is derived from two studies in which male Sprague-Dawley rats were gavage dosed with DnOP once daily for 14 days. In these studies, DnOP doses of 1000 mg/kg-day (LOAEL) increased the relative liver weight and other biochemical functions associated with the liver (i.e., palmitoyl-CoA oxidation, enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, ethylmorphine N-demethylase activity). These changes occurred at a lower dose than that used to induce other pathologies in acute studies (Dogra *et al.,* 1985; Mann *et al.,* 1985; Hinton *et al.,* 1986).

The LOAEL of 1000 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 1000 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations, 10X for conversion of a LOAEL to a NOAEL). The 1000-fold "safety factor" is typically applied by CPSC to the lowest LO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). Other federal agencies such as ATSDR have also historically used 10X factors each for intraspecies, interspecies, and LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995). This magnitude of factor for LOAEL to NOAEL extrapolation has also been shown to be protective for 99% of the responses to mild adverse effects from other routes of exposure (inhalation; Alexeeff *et al.,* 2002). The relative mildness of the adverse effect in this case (liver), however, does encourage the future consideration of lower uncertainty factors for LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995; Dourson *et al.,* 1996). **The acute oral ADI was calculated to be 1.0 mg/kg-day.**

Intermediate-term oral exposures – general population

For intermediate-duration oral exposures, the NOAEL of 36.8 mg/kg-day (Poon *et al.,* 1997) was chosen as the representative overall hazard endpoint. This endpoint is derived from a study in which male Sprague-Dawley rats were dosed with DnOP in feed for 13 weeks. DnOP doses of 350.1 mg/kg-day (LOAEL) induced liver changes including increased ethoxyresorufin-

O-deethylase activity, moderate accentuation of zonation, anisokaryosis, nuclear hyperchromicity, perivenous cytoplasmic vacuolation, and endothelial prominence. These histopathologic and biochemical changes occurred at doses lower than that needed to induce an increase in liver weight. Selection of the liver as the overall target endpoint was supported, however, by additional hepatic pathologies observed at slightly higher doses in acute (Lake *et al.,* 1984, Lake *et al.,* 1986; LOAEL = 1000 mg/kg-day) and subchronic (DeAngelo *et al.,* 1986; LOAEL = 500 mg/kg-day) studies. In addition, this endpoint is lower than those determined in other organ systems such as the lymph nodes, thymus, and adrenal glands (Dogra *et al.,* 1985; LOAEL = 600 mg/kg-day, NOAEL = 300 mg/kg-day). Other lower NOAELs (i.e., 0.4 - 4.1 mg/kg-day for liver and thyroid effects) portrayed in the data were not chosen as hazard thresholds because of a lack of supporting evidence from additional studies.

The NOAEL of 36.8 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). **The subchronic oral ADI was calculated to be 0.368 mg/kg-day.**

ADIs for inhalation or dermal exposures, for chronic exposures, and for reproductive and developmental toxicity were not calculated for DnOP because of the paucity of available or qualified studies.

Other regulatory levels

Calculated acute and subchronic ADIs were comparable to Minimal Risk Levels (MRLs) generated by the Agency for Toxic Substances and Disease Registry (ATSDR, 1997). The ATSDR generated an acute oral MRL of 3 mg/kg-day based on a LOAEL of 1000 mg/kg-day for liver effects (Lake *et al.,* 1986; uncertainty factor of 300) and an intermediate oral MRL of 0.4 mg/kg-day based on a LOAEL of 350 mg/kg-day for liver effects (Poon *et al.,* 1997; NOAEL = 40.8 mg/kg-day; uncertainty factor of 100).

Summary

The FHSA defines a "hazardous substance" as a substance that satisfies both parts of a two-part test. To be a hazardous substance, a product must first present one or more of the hazards enumerated in the statute (i.e., it must be toxic, corrosive, flammable, an irritant, or a strong sensitizer, or generate pressure through decomposition, heat, or other means). Secondly, the product must have the potential to cause substantial personal injury or substantial illness during or as a result of any customary or reasonably foreseeable handling or use, including reasonably foreseeable ingestion by children. This hazard summary reviews evidence to support conclusions for the toxicity, corrosivity, irritancy, and sensitizing potential of DnOP.

Overall, animal data are sufficient to support the conclusion that DnOP is probably not acutely toxic following single dose oral exposures under the FSHA. Acute oral toxicities (LD_{50}'s) for DnOP in at least four reported studies were ≥ 6513 mg/kg. This is in excess of the oral LD_{50} range (50 - 5000 mg/kg) necessary to be termed acutely toxic under the FHSA.

Sufficient animal data and limited human data also support the conclusion that DnOP is probably not corrosive or an ocular or dermal irritant under the FHSA.

In terms of sensitization, contrasting results in animal (negative) and human (negative and positive) data support the conclusion that there is "limited or inadequate human and animal evidence" for the designation of DnOP as a "sensitizer" under the FHSA.

Sufficient animal data exist to support the conclusion that DnOP probably has subchronic toxicity in a variety of organs under the FHSA. DnOP-induced adverse effects were noted in test subject's thyroid, immune system, kidney, and liver in two, three, three, and eight published studies, respectively.

Inadequate or limited animal data exist when considering DnOP-induced effects as: 1) a carcinogen, 2) a neurotoxicant, 3) a reproductive or developmental toxicant, 4) a dermal or inhalation toxicant, 5) a respiratory irritant, or 6) a genotoxicant. Adverse effects were not observed or key evidence was missing in the majority of studies presented, limiting the overall conclusions to "possibly toxic" under the FHSA.

In evaluating the potential hazards presented by DnOP, the Commission has appropriately followed the definitions for toxicity (both acute and chronic), irritancy, and sensitization under the FHSA and its implementing regulations, 16 CFR §1500. At this time, there is insufficient data for the CPSC staff to conduct the second part of the analysis to determine what potential exposures to DnOP would be if used in children's toys, child care articles, or other consumer products.

In summary, data support the conclusion that DnOP can be considered “toxic” under the FHSA due to its probable acute (repeat-dose) and subchronic toxicity. This conclusion is based on sufficient evidence in animals of DnOP-induced toxicity to the liver, kidney, thyroid, and immune system. Products that contain DnOP may be considered ‘hazardous’ under the FHSA if short-duration (acute) or intermediate-duration (subchronic) exposures during “reasonably foreseeable handling and use” exceed the acute or subchronic ADIs of 1.0 or 0.368 mg DnOP/kg bw-day, respectively.

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Appendix 1. Summary Table of DnOP-induced Effects [1,2]

Species (Gender)	*Exposure Route*	*Dose (No. of animals per dose group)*	*Dose Duration*	*Toxicological Endpoint*	*Toxicological Basis*	*Citation*	*Organ System*
Mortality							
Albino rats (M)	Oral gavage	NA	Once	LD_{50} = 53,700 mg/kg-bw	Mortality	Dogra *et al.*, 1987	Mortality
Swiss albino mice (?)	Oral gavage	NA	Once	LD_{50} = 13,000 mg/kg-bw	Mortality	Dogra *et al.*, 1989	Mortality
Sprague-Dawley rats (F)	Oral gavage	NA	Once	LD_{50} > 48,900 mg/kg	Mortality	Singh *et al.*, 1972	Mortality
Mice	Oral gavage	NA	Once	LD_{50} > 12,800 mg/kg	Mortality	Eastman Kodak Company, 1978 cited in ATSDR, 1997	Mortality
Albino rats	NA	NA	Once	LD_{50} = 47,000 mg/kg	Mortality	Balynina and Berezovskaia, 1976	Mortality
CD-1 mice (F)	Oral gavage	9780 mg/kg-day	Once daily for Gd 6 - 13	Maternal NOAEL= 9780 mg/kg-day	No change in maternal mortality	Hardin *et al.*, 1987	Mortality
Wistar rats (F)	Oral gavage (n-octanol)	130, 650, 945, and 1300 mg/kg-day	Once daily for Gd 6 - 15	Maternal LOAEL = 650 mg/kg-day; NOAEL = 130 mg/kg-day	Mortality in 2/10 dams versus 0/10 control dams	Hellwig and Jackh, 1997	Mortality
General							
BALB/cJ mice (F)	Subcutaneous injection (MnOP)	1, 10, 100, 1000 ug/mL (0.0053, 0.053, 0.53, 5.3 mg/kg-bw)	Once	NOAEL = 1000 ug/mL (5.3 mg/kg-bw)	No change in body weight	Larsen *et al.*, 2001	General
Sprague-Dawley rats (M)	Oral gavage	2800 mg/kg-day	Once daily for 4 days	NOAEL = 2800 mg/kg-day	No change in body weight, food consumption	Foster *et al.*, 1980	General
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	NOAEL = 10,000 mg/kg-day	No change in body weight	Dogra *et al.*, 1985	General
CD-1 mice (F)	Oral gavage	9780 mg/kg-day	Once daily for Gd 6 - 13	Maternal NOAEL= 9780 mg/kg-day	No change in maternal body weight	Hardin *et al.*, 1987	General
Wistar rats (F)	Oral gavage (n-octanol)	130, 650, 945, and 1300 mg/kg-day	Once daily for Gd 6 - 15	Maternal LOAEL = 650 mg/kg-day; NOAEL = 130 mg/kg-day	Clinical intoxication (nervous system effects such as lateral and abdominal position, unsteady gait, salivation, piloerection, nasal discharge, and pneumonia)	Hellwig and Jackh, 1997	General
Wistar rats (M)	Oral feeding (MnOP)	0, 2% (1000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 7 days	LOAEL = 1000 mg/kg-day	Decreased final body weight/gain and food consumption	Oishi and Hiraga, 1980	General

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2266, 2078, and 1906 mg/kg-day	Increased food consumption and body weight at days 3, 10, and 21	Mann *et al.*, 1985	General
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change body weight, food consumption, or clinical toxicity	Poon *et al.*, 1997	General
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, 5.0, and 10.0% (0.0, 750, 1875, 3750, 7500, 15,000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks	NOAEL = 10% (15,000 mg/kg-day)	No change in the weight gain or food consumption	Heindel *et al.*, 1989	General
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1875, 3750, 7500 or 8640 (F_1) mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks (F_0) and subsequent 10 - 11 weeks (F_1)	NOAEL = 5.0% (7500 or 8640 mg/kg-day)	No change in the weight gain or food consumption of F_0 or F_1 generations	Heindel *et al.*, 1989	General
Integument/Behavioral							
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, 5.0, and 10.0% (0.0, 750, 1875, 3750, 7500, 15,000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks	LOAEL = 10% (15,000 mg/kg-day); NOAEL = 5.0% (7500 mg/kg-day)	Rough hair coat	Heindel *et al.*, 1989	Integumentary/Behavioral
Clinical chemistry/Hematology							
Wistar rats (M)	Oral feeding (MnOP)	0, 2% (1000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 7 days	NOAEL = 1000 mg/kg-day	No change serum zinc or testosterone concentration	Oishi and Hiraga, 1980	Clinical chemistry

Species (Gender)	*Exposure Route*	*Dose (No. of animals per dose group)*	*Dose Duration*	*Toxicological Endpoint*	*Toxicological Basis*	*Citation*	*Organ System*
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change in albumin, calcium, inorganic phosphate, or total protein	Poon *et al.*, 1997	Clinical chemistry
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change in white blood cell number, mean corpuscular hemoglobin and volume, or platelet count	Poon *et al.*, 1997	Hematology
Immune/Lymph nodes/Thymus/Spleen							
BALB/cJ mice (F)	Subcutaneous injection (MnOP)	1, 10, 100, 1000 ug/mL (0.0053, 0.053, 0.53, 5.3 mg/kg-bw)	Once	LOAEL = 1000 ug/mL (5.3 mg/kg-bw); NOAEL = 100 ug/mL (0.53 mg/kg-bw)	Immunosuppression of IgE and IgG1	Larsen *et al.*, 2001	Immune
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 5000 mg/kg-day; NOAEL = 2500 mg/kg-day	Reduction in delayed hypersensitivity response	Dogra *et al.*, 1985	Immune
Swiss albino mice (?)	Oral gavage	0, 650, 2600 mg/kg-bw (40 mice per dose group)	Once daily for 5 days	LOAEL = 650 mg/kg-bw	Increase in mortality co-associated with viral and parasitic burden, reduced mean survival time	Dogra *et al.*, 1989	Immune
Swiss albino mice (?)	Oral gavage	0, 650, 2600 mg/kg-bw (40 mice per dose group)	Once daily for 5 days	LOAEL = 650 mg/kg-bw	Increase in % parasitemia co-associated with viral and parasitic burden	Dogra *et al.*, 1989	Immune
Albino rat	Oral gavage	NA	Once daily for 5 days	Male LOAEL = 2685 mg/kg-day	Reduced antibody synthesis	Dogra *et al.*, 1987 cited in ATSDR, 1997	Immune
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 10,000 mg/kg-day; NOAEL = 5000 mg/kg-day	Decrease in absolute portal and mesenteric lymph node weight	Dogra *et al.*, 1985	Lymph node
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 10,000 mg/kg-day; NOAEL = 5000 mg/kg-day	Increase in absolute peripheral lymph node weight	Dogra *et al.*, 1985	Lymph node
Albino rats (M)	Intraperitoneal injection	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Reduction in the follicle number in cortical regions of the lymph nodes	Dogra *et al.*, 1985	Lymph node
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 10,000 mg/kg-day; NOAEL = 5000 mg/kg-day	Decrease in absolute thymus weight	Dogra *et al.*, 1985	Thymus

Species (Gender)	*Exposure Route*	*Dose (No. of animals per dose group)*	*Dose Duration*	*Toxicological Endpoint*	*Toxicological Basis*	*Citation*	*Organ System*
Albino rats (M)	Intraperitoneal injection	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Loss of distinction between cortex and medulla, depletion of cortical cells and medullary hyperplasia by 90 days	Dogra *et al.*, 1985	Thymus
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 2500 mg/kg-day	Increase in absolute spleen weight	Dogra *et al.*, 1985	Spleen
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 10,000 mg/kg-day; NOAEL = 5000 mg/kg-day	Decrease in spleen cell responsiveness to PHA	Dogra *et al.*, 1985	Spleen
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 5000 mg/kg-day; NOAEL = 2500 mg/kg-day	Decrease in spleen cell responsiveness to LPS	Dogra *et al.*, 1985	Spleen
Albino rats (M)	Intraperitoneal injection	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 100 mg/kg-day	Washed out appearance and edema; severe cellular depletion of peripheral follicles of the white pulp and edema in the red pulp by 90 days	Dogra *et al.*, 1985	Spleen
Kidney/Adrenal glands							
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 2500 mg/kg-day	Increase in absolute kidney weight	Dogra *et al.*, 1985	Kidney
Wistar rats (M)	Oral feeding (MnOP)	0, 2% (1000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 7 days	NOAEL = 1000 mg/kg-day	No change in kidney zinc concentration	Oishi and Hiraga, 1980	Kidney
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change in absolute or relative kidney weight	Poon *et al.*, 1997	Kidney
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1875, 3750, 7500 or 8640 (F_1) mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks (F_0) and subsequent 10 - 11 weeks (F_1)	LOAEL = 5.0% (8640 mg/kg-day)	Increased absolute kidney weight (F, F_1)	Heindel *et al.*, 1989	Kidney

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Albino mice (M, 100g)	Intraperitoneal injection	0, 100, 300, 600 mg/kg-day (10 rats per group)	Once daily, five days a week for 90 days (with a 45 day recovery period)	NOAEL = 600 mg/kg-day	No macroscopic evidence of change in kidneys.	Khanna *et al.*, 1990	Kidney
Albino mice (M, 100g)	Intraperitoneal injection	0, 100, 300, 600 mg/kg-day (10 rats per group)	Once daily, five days a week for 90 days (with a 45 day recovery period)	LOAEL = 100 mg/kg-day	Dose-dependent changes in the glomerulus (atrophy, cellularity), proximal tubules (swelling, luminal obliteration, desquamation degeneration of epithelium), distal tubules (dilation, hyaline casts), vasa recta (edema), and interstitium (lymphocytic infiltration) with many effects retained after a 45 day recovery period	Khanna *et al.*, 1990	Kidney
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 2500 mg/kg-day	Increase in absolute adrenal gland weight	Dogra *et al.*, 1985	Adrenal glands
Albino rats (M)	Intraperitoneal injection	100, 300, 600 mg/kg bw-day	Five days a week for 45 or 90 days	LOAEL = 600 mg/kg-day; NOAEL = 300 mg/kg-day	Derangement of the cellular columns of the zona fasciculate and increase in the lipid vacuoles by 90 days	Dogra *et al.*, 1985	Adrenal glands
Testis/Reproduction/Fetus							
Crj; Wistar rat testes cells	*In vitro* (MnOP)	0.065, 0.13, 0.325, 0.65, 1.30 µmole/ml	Once	LOAEL = 0.065 µmole/ml	Decreased mitochondrial state 3 oxygen consumption	Oishi, 1990	Testis
Crj; Wistar rat testes cells	*In vitro*	0.065, 0.13, 0.325, 0.65, 1.30 µmole/ml	Once	LOAEL = 1.3 µmole/ml	Decreased mitochondrial state 3 oxygen consumption	Oishi, 1990	Testis
Crj; Wistar rats (M)	Oral gavage	2000 mg/kg	Once	LOAEL = 2000 mg/kg	Decreased mitochondrial state 3 oxygen consumption, decreased respiration control ratio	Oishi, 1990	Testis
Crj; Wistar rats (M)	Oral gavage	2000 mg/kg	Once	NOAEL = 2000 mg/kg	No change in pyruvate or lactate concentration in testes, or phosphorylative activity ratio in testes mitochondria	Oishi, 1990	Testis

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Wistar rat Leydig cells	*In vitro* (MnOP)	1000µm	Once for various time points	LOAEL = 2000 mg/kg-day	Decreased the LH-stimulated secretion of testosterone, increased the filopodial proliferation from the basal lamellar processes and the cell soma, SER dilatation, mitochondrial swelling, mitochondrial degeneration and numeric reduction, and vesiculation of the Golgi apparatus.	Jones *et al.*, 1993	Testis
Wistar rats (M)	Oral gavage	2000 mg/kg	Once daily for 2 days	NOAEL = 2000 mg/kg-day	No change in seminiferous tubular structure, Leydig cell morphology, or various aspects of Leydig cell ultrastructure (i.e., mitochondrial swelling, degeneration, SER focal dilatation, vesiculation, and peroxisomes	Jones *et al.*, 1993	Testis
Wistar rats (M)	Oral gavage (MnOP)	2000 mg/kg	Once daily for 2 days	LOAEL = 2000 mg/kg-day	Significant alterations in Leydig cell cytoplasmic ultrastructure (such as mitochondrial swelling, degeneration, SER focal dilatation, increased number and length of filipodia, and peroxisomes)	Jones *et al.*, 1993	Testis
Sprague-Dawley rats (M)	Oral gavage	2800 mg/kg-day	Once daily for 4 days	NOAEL = 2800 mg/kg-day	No change in relative testis weight, testis pathology, Zinc-65 content, Zinc-65 excretion	Foster *et al.*, 1980	Testis
Wistar rats (M)	Oral feeding (MnOP)	0, 2% (1000 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 7 days	NOAEL = 1000 mg/kg-day	No change in absolute and relative testis weight or testis zinc or testosterone concentration	Oishi and Hiraga, 1980	Testis
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2078 mg/kg-day	Decreased relative testicular weight on day 10	Mann *et al.*, 1985	Testis
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change in absolute or relative testis weight, the incidence of seminiferous tubule atrophy, or bilateral reduction in sperm density	Poon *et al.*, 1997	Testis

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1875, 3750, 7500 or 8640 (F_1) mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks (F_0) and subsequent 10 - 11 weeks (F_1)	NOAEL = 5.0% (7500 or 8640 mg/kg-day)	No change in reproductive parameters (mating index, fertility index, litters/pair, live pups/litter, proportion of pups born alive, live pup weight) in the parental F_0 mice or in matings of the high dose F_1 generation, no effect on the testis, epididymis, or prostate weight, epididymal sperm concentration, percentage of motile sperm, or average estrous cycle length (F_1 generation)	Heindel *et al.*, 1989; Morrissey *et al.*, 1989	Testis/Reproduction
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1875, 3750, 7500 or 8640 (F_1) mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks (F_0) and subsequent 10 - 11 weeks (F_1)	LOAEL = 5.0% (8640 mg/kg-day)	Decreased seminal vesicle weight (F_1)	Heindel *et al.*, 1989; Morrissey *et al.*, 1989	Testis/Reproduction
CD-1 mice (F)	Oral gavage	9780 mg/kg-day	Once daily for Gd 6 - 13	Fetal LOAEL= 9780 mg/kg-day	Reduced number of live neonates per litter and weight gain in the pups	Hardin *et al.*, 1987	Fetus
CD-1 mice (F)	Oral gavage	9780 mg/kg-day	Once daily for Gd 6 - 13	NOAEL= 9780 mg/kg-day	No change in the number of viable litters, the percent survival of live born neonates, or the birth weight of pups	Hardin *et al.*, 1987	Fetus
Wistar rats (F)	Oral gavage (n-octanol)	130, 650, 945, and 1300 mg/kg-day	Once daily for Gd 6 - 15	LOAEL = 650 mg/kg-day; NOAEL = 130 mg/kg-day	No change in body weight, viability, malformations in low dose animals	Hellwig *et al.*, 1997	Fetus
Sprague-Dawley rats (F)	Intraperitoneal	0, 5, and 10 ml/kg (0, 4890, and 9780 mg/kg-day; 5 rats per group)	Once on Gd 5, 10, and 15	LOAEL = 4890 mg/kg-day	Significant decrease in average fetal weights, and a significant increase in the incidence of gross fetal malformations	Singh *et al.*, 1972	Fetus
Liver/Pancreas							
Sprague-Dawley rat hepatocytes (M)	In vitro (MnOP)	20, 50, 100, 200 µM	Once	LOAEL = 200 µM	Increased palmitoyl-CoA oxidation	Lake *et al.*, 1986	Liver
Sprague-Dawley rat hepatocytes (M)	*In vitro* (MnOP)	20, 50, 100, 200 µM	Once	LOAEL = 20 µM	Increased carnitine acetyltransferase	Lake *et al.*, 1986	Liver
Wistar rat hepatocytes	*In vitro* (MnOP)	0.25 mM	Once	NOAEL = 0.25 mM	No change in cyanide-insensitive palmitoyl-CoA oxidation, no increase in cell death	Hinton *et al.*, 1986	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Wistar rat hepatocytes	*In vitro* (MnOP)	0.25 mM	Once	LOAEL = 0.25 mM	Increased blebbing and vacuolation, increased marginal and central lipid accumulation, increased incorporation of palmitate into triglyceride and cholesterol esters, increased fatty acid oxidation	Hinton *et al.*, 1986	Liver
Albino rats (M)	Intraperitoneal injection	(0, 2500, 5000, 10,000 mg/kg bw-day)	Once daily for 5 days	LOAEL = 2500 mg/kg-day	Increase in absolute liver weight	Dogra *et al.*, 1985	Liver
Sprague-Dawley rats (M)	Oral gavage	2000 mg/kg-day	Once daily for 14 days	NOAEL = 2000 mg/kg-day	No change in peroxisome number (appeared to have)	Lake *et al.*, 1984	Liver
Sprague-Dawley rats (M)	Oral gavage (MnOP)	715 mg/kg-day	Once daily for 14 days	NOAEL = 715 mg/kg-day	Increased relative liver weight, marginally increased palmitoyl-CoA oxidation, ethylmorphine N-demethylase activity, increased enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, reduced D-amino acid oxidase activity	Lake *et al.*, 1984	Liver
Sprague-Dawley rats (M)	Oral gavage (MnOP)	750, 1000 mg/kg-day	Once daily for 14 days	NOAEL = 750, 1000 mg/kg-day	No change in peroxisome number (appeared to have)	Lake *et al.*, 1984	Liver
Sprague-Dawley rats (M)	Oral gavage (MnOP)	715 mg/kg-day	Once daily for 14 days	NOAEL = 715 mg/kg-day	No change in the whole homogenate protein, microsomal proteins, or cytochrome p450s	Lake *et al.*, 1984	Liver
Sprague-Dawley rats (M)	Oral gavage	1000 mg/kg-day	Once daily for 14 days	NOAEL = 1000 mg/kg-day	No change in the whole homogenate protein, cytochrome p450s, microsomal protein, or 7-ethoxycoumarin O-deethylase activity	Lake *et al.*, 1984	Liver
Sprague-Dawley rats (M)	Oral gavage	1000 mg/kg-day	Once daily for 14 days	LOAEL = 1000 mg/kg-day	Increased relative liver weight, marginally increased palmitoyl-CoA oxidation, marginally increased enoyl-CoA hydratase heat labile and ethylmorphine N-demethylase activity, and reduced lauric acid 11-12 hydroxylation and 7-ethoxycoumarin O-deethylase activity	Lake *et al.*, 1986	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Sprague-Dawley rats (M)	Oral gavage (MnOP)	715 mg/kg-day	Once daily for 14 days	LOAEL = 715 mg/kg-day	Increased relative liver weight, increased palmitoyl-CoA oxidation, increased enoyl-CoA hydratase heat labile activity	Lake *et al.*, 1986	Liver
Sprague-Dawley rats (M)	Oral gavage	1000 mg/kg-day	Once daily for 14 days	LOAEL = 1000 mg/kg-day	Increased relative liver weight, marginally increased palmitoyl-CoA oxidation, increased enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, ethylmorphine N-demethylase activity, and reduced D-amino acid oxidase activity, 7-ethoxyresorufin O-deethylase, and 7-ethoxycoumarin O-deethylase	Lake *et al.*, 1984	Liver
Wistar rats (M)	Oral feeding (MnOP)	0, 2% (1000 mg/kg-day; ATSDR, 1997)	*Ad libitum* for 7 days	NOAEL = 1000 mg/kg-day	No change in liver zinc concentration	Oishi and Hiraga, 1980	Liver
Wistar rats (M)	Oral feeding	0, 2% (2000 mg/kg-day)	*Ad libitum* for 3, 10, or 21 days	Male LOAEL = 2000 mg/kg-day	A changed appearance to pale and greasy, mildly increased hepatomegaly (after 21 days), centrilobular fat accumulation, peroxisome proliferation (after 21 days), smooth endoplasmic reticulum (ER) proliferation, and loss of rough ER, moderate centrilobular loss of glycogen and necrosis	Hinton *et al.*, 1986	Liver
Wistar rats (M)	Oral feeding	0, 2% (2000 mg/kg-day)	*Ad libitum* for 3, 10, or 21 days	Male NOAEL = 2000 mg/kg-day	No change in total glycogen loss, periportal fat accumulation, the density of inner mitochondrial matrix, or mitosis	Hinton *et al.*, 1986	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2078, and 1906 mg/kg-day	Change in appearance to pale and greasy, increased relative liver weight, a marginal increase in the number of liver mitotic figures, a marked increase in the centrilobular accumulation of fat (and associated necrosis), accumulation of lipid droplets in hepatocytes, increased cyanide-insensitive palmitoyl CoA oxidation, catalase activity in the large particulate fraction of homogenates on days 10 and 21	Mann *et al.*, 1985	Liver
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2266, 2078, and 1906 mg/kg-day	Centrilobular loss of glycogen, ultrastructural proliferation and dilation of smooth endoplasmic reticulum on days 3, 10, and 21 (marked on days 10 and 21)	Mann *et al.*, 1985	Liver
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2266 mg/kg-day	Shortening of microvilli in bile canaliculi by day 3	Mann *et al.*, 1985	Liver
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 2078mg/kg-day	Small increase in lysosomal number by day 10	Mann *et al.*, 1985	Liver
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	LOAEL = 1906 mg/kg-day	Increased hepatomegaly, small increase in peroxisome number and decreased 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphatase activity by day 21	Mann *et al.*, 1985	Liver
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	NOAEL = 2266, 2078, and 1906 mg/kg-day	No change in α-glycerophosphate dehydrogenase, catalase activity, cytochrome p450 induction, or nonenzymatic reducing agents on days 3, 10, or 21	Mann *et al.*, 1985	Liver
Fischer 344 rats (M)	Oral feeding	0, 1000, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	LOAEL = 10,000 mg/kg; NOAEL = 1000 mg/kg	Increased relative liver weight and peroxisomal beta oxidation activity at 2 weeks	Smith *et al.*, 2000	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Fischer 344 rats (M)	Oral feeding	0, 1000, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	LOAEL = 10,000 mg/kg; NOAEL = 1000 mg/kg	Increased periportal hepatocellular replicative DNA synthesis at 2 and 4 weeks	Smith *et al.*, 2000	Liver
Fischer 344 rats (M)	Oral feeding	0, 1000, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	NOAEL = 10,000 mg/kg	No change in gap junction intercellular communication at 2 and 4 weeks	Smith *et al.*, 2000	Liver
B6C3F1 mice (M)	Oral feeding	0, 500, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	LOAEL = 10,000 mg/kg; NOAEL = 500 mg/kg	Increased peroxisomal beta oxidation activity at 2 and 4 weeks	Smith *et al.*, 2000	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 500, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	LOAEL = 500 mg/kg	Increased peroxisomal beta oxidation activity at 4 weeks	Smith *et al.*, 2000	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 500, 10,000 mg/kg	*Ad libitum* for 2 and 4 weeks	NOAEL = 10,000 mg/kg	No change in relative liver weight, gap junction intercellular communication, or periportal hepatocellular replicative DNA synthesis at 2 and 4 weeks	Smith *et al.*, 2000	Liver
Sprague-Dawley rats (M)	Oral feeding preceded with a partial hepatectomy and then an IP dose of diethylnitrosamine	1% (500 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 10 weeks	LOAEL = 1% DnOP (500 mg/kg-day)	Mild fatty change, increased gamma glutamyl-transpeptidase + foci, increase in the overall foci area, % of the liver as foci, GGT activity, and a slight increase in carnitine acetyltransferase activity	DeAngelo *et al.*, 1986	Liver
Sprague-Dawley rats (M)	Oral feeding preceded with a partial hepatectomy and then an IP dose of diethylnitrosamine	1% (500 mg/kg-day, ATSDR, 1997)	*Ad libitum* for 10 weeks	NOAEL = 1% DnOP (500 mg/kg-day)	No change in liver weight	DeAngelo *et al.*, 1986	Liver
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 – 402.9 mg/kg-day	No change in absolute or relative liver weight, aminopyrine-N-demethylase activity, aniline hydroxylase activity, or the number or % area of peroxisomes	Poon *et al.*, 1997	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 350.1 – 402.9 mg/kg-day; NOAEL = 36.8 - 40.8 mg/kg-day	Increased ethoxyresorufin-O-deethylase activity, moderate accentuation of zonation (M), anisokaryosis (M), nuclear hyperchromicity (M), perivenous cytoplasmic vacuolation, and endothelial prominence (M)	Poon *et al.*, 1997	Liver
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 40.8 mg/kg-day; NOAEL = 4.1 mg/kg-day	Moderate accentuation of zonation (F), and endothelial prominence (F)	Poon *et al.*, 1997	Liver
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 0.4 mg/kg-day	Moderate nuclear hyperchromicity (F)	Poon *et al.*, 1997	Liver
Fischer 344 rats (M)	Oral feeding preceded with a partial hepatectomy and then an IP dose of diethylnitrosamine	0, 0.5, and 1%	*Ad libitum* for 26 weeks	LOAEL = 1%; NOAEL = 0.5%	Increased dose-dependent relative liver weight (correlated to the loss in body weights), increased the volume % liver expressing GGT and the grams of liver expressing GGT and GST-P, and increased the number of GST-P+ nodules in 1% DnOP treatments.	Carter *et al.*, 1992	Liver
Fischer 344 rats (M)	Oral feeding preceded with a partial hepatectomy and then an IP dose of diethylnitrosamine	0, 0.5, and 1%	*Ad libitum* for 26 weeks	NOAEL = 1%	No change in absolute liver weight or the number of GST+ altered foci	Carter *et al.*, 1992	Liver
CD-1 mice (M & F)	Oral feeding	0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1875, 3750, 7500 or 8640 (F_1) mg/kg-day, ATSDR, 1997)	*Ad libitum* for 26 weeks (F_0) and subsequent 10 - 11 weeks (F_1)	LOAEL = 5.0% (8640 mg/kg-day)	Increased absolute liver weight (M & F, F_1)	Heindel *et al.*, 1989	Liver

Species (Gender)	***Exposure Route***	***Dose (No. of animals per dose group)***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Wistar albino rats (M)	Oral feeding	0, 2% (2266, 2078, and 1906 mg/kg-day, respectively for days 3, 10, and 21)	*Ad libitum* for 3, 10, and 21 days	NOAEL = 2266, 2078, and 1906 mg/kg-day	No gross changes in appearance or pathology	Mann *et al.*, 1985	Pancreas
Thyroid							
Wistar rats (M)	Oral feeding	0, 2% (2000 mg/kg-day)	*Ad libitum* for 3, 10, or 21 days	Male LOAEL = 2000 mg/kg-day	Decreased serum thyroxine (T_4) on days 3, 10, and 21, increased triiodothyronine (T_3) on day 21, increased number and size of lysosomes, enlarged Golgi apparatus, mitochondrial damage	Hinton *et al.*, 1986	Thyroid
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	NOAEL = 350.1 mg/kg-day	No change in follicle size (M)	Poon *et al.*, 1997	Thyroid
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 3.5 mg/kg-day; NOAEL = 0.4 mg/kg-day	Decreased colloid density (M)	Poon *et al.*, 1997	Thyroid
Sprague-Dawley rats (M & F)	Oral feeding	5, 50, 500, and 5000 ppm (male - 0.0, 0.4, 3.5, 36.8, 350.1; female – 0.0, 0.4, 4.1, 40.8, 402.9 mg/kg-day)	*Ad libitum* for 13 weeks	LOAEL = 40.8 mg/kg-day; NOAEL = 4.1 mg/kg-day	Decreased colloid density (F), reduced follicle size (F)	Poon *et al.*, 1997	Thyroid

[1] Gray highlighted areas have been chosen as toxicological endpoints and used in the calculation of both acute and subchronic ADIs.

[2] Red text highlights studies that are acute in exposure duration (0-14 days). Blue text highlights studies that have subchronic exposure durations (14-365 days). No chronic studies were retrieved or reviewed for DnOP.

Appendix 2. Critical Study Reviews

Bility *et al.* (2004) studied the activation of mouse and human peroxisome proliferator-activated receptors (PPAR) by MnOP. Mouse 3T3-L1 fibroblasts were transfected with chimeric mouse or human PPAR α-LBD/Gal4-DBD-, PPAR β-LBD/Gal4-DBD, and PPAR γ-LBD/Gal4-DbD-Gal4 luciferase reporter plasmids, placed in multiwall plates, and exposed to MnOP (3, 10, 30, 100, 200 μM). Twenty-four hours following treatment the cells were lysed and luciferase activity and protein content measured. In another experiment, rat hepatoma FaO and human hepatoma HepG2 cells were cultured, treated with 10 and 100 μM MnOP for 48 hours, total RNA harvested and electrophoresed, and analyzed via primers for PPAR target gene activity. In the last experiment, mouse 3T3-L1 fibroblasts were cultured and then induced to undergo "adipogenesis" by various treatments including 50 μM MnOP. After 6 days, cell were fixed and stained with Oil Red O, and microscopically photographed. Overall, a significant dose-related increase was seen in the activity of mouse and human PPAR α (LOAEL = 10 μM; 32.4 and 7.6-fold increase, respectively), mouse PPAR β (LOAEL = 100 μM; 13.7-fold increase), and mouse (LOAEL = 10 μM; 11-fold increase) and human PPAR γ (LOAEL = 100 μM; 19.1-fold increase). MnOP also induced the creation of mRNA encoding two PPAR α target genes (acyl CoA oxidase - ACOX and cytochrome P4504A – Cyp4A). In FaO cells, MnOP induced the creation of ACOX (2.6-fold (10 μM) and 5.6-fold (100 μM) and Cyp4A (3.8-fold (10 μM) and 10.9-fold (100 μM)). In HepG2 cells, MnOP slightly induced the creation of ACOX (1.1-fold (10 μM) and 1.3-fold (100 μM), but not Cyp4A (0.6-fold (10 μM) and 0.6-fold (100 μM)). In the final histopathologic experiment, MnOP appeared to be a strong inducer of *in vitro* adipogenesis.

Carter *et al.* (1989) determined the effects of chronic administration of DnOP in the diet on the rat liver. Male Fischer 344 rats were placed on a 1% DnOP feed diet for 65 weeks, sacrificed, and then histopathologically evaluated. All animals treated with DnOP had hepatomegaly (mass not described) and "numerous liver nodules". Up to a 3-fold increase was also reported in N-acetyl-β-glucosaminidase, β-galactosidase, α-mannosidase, and aryl sulfatase. Cathepsin D and β-glucuronidase were also increased. Hepatic acid phosphatase and the expression of GGTase were not elevated in treated rats, but GGTase activity was increased in liver nodules. The authors postulated that glycosidic degradation of the carbohydrates in the extracellular matrix may contribute to the mechanism of tumor induction. No further details were given for this study.

Carter *et al.* (1992) investigated the ability of DnOP to promote the expression of GGT and placental glutathione-S-transferase (GST-P). Male Fischer 344 rats were partially hepatectomized and then injected with a single intraperitoneal shot of diethylnitrosamine (DENA). Ten days after injection the rat diets were switched to include 0, 0.5 and 1.0% DnOP. Twenty-six weeks later, rats were sacrificed and livers weighed and analyzed for pathologies via

histochemistry. Administration of DnOP did not affect absolute liver weight, but did increase relative liver weight in a dose-dependent fashion (because of the loss in body weights). DnOP also significantly increased the volume % liver expressing GGT and the grams of liver expressing GGT and GST-P. The number of GST+ altered foci was not different than controls. In contrast, the number of GST-P+ nodules was higher in 1% DnOP treatments.

DeAngelo *et al.* (1986) studied the ability of 1% DnOP to promote the development of preneoplastic lesions in male Sprague-Dawley rats that had been partially hepatectomized and then injected intraperitoneally with the initiator DENA. Rats were subsequently fed with 1% DnOP mixed with feed for 10 weeks. Treated rats had a significant increase in the number of hepatic gamma-glutamyl transpeptidase (GGT)+ foci/cm^2, an increase in the overall foci area, and % of the liver as foci, GGT activity, a slight increase in carnitine acetyltransferase activity, and a mild fatty change in the liver without a corresponding change in liver weight. GGT+ foci have been closely linked to the development of neoplastic nodules and hepatomas.

Dogra *et al.* (1985) exposed three groups of young male albino rats to DnOP via intraperitoneal injection (0.002, 0.006, 0.012x LD_{50}; 100, 300, 600 mg/kg bw-day) daily at 5 days a week for 45 or 90 days. At both 45 and 90 days, two animals from each dose were sacrificed and the thymus, spleen, adrenal, and lymph nodes (portal, lumbar, popliteal, tracheobronchial, and axial) examined histopathologically after fixation and staining. Exposure to DnOP resulted in a dose-related decrease in the absolute weight of the thymus and lymph nodes (mesenteric, portal, and peripheral) without affecting the body weights. Low and middle doses did not alter the structure of the thymus at any time point. The high dose, however, induced a loss of "distinction between cortex and medulla" by 45 days and a "marked depletion" of cortical cells and medullary hyperplasia by 90 days. Splenic changes (edema and paling) were evident by 45 days at all dose levels and cellular depletion of peripheral follicles of white pulp and edema in the red pulp by ninety days in the highest dose level. Lymph nodes were also adversely affected in high dose rats, with reductions in the follicle number reported in cortical regions of lymph nodes. High dose effects such as an increase in lipid vacuoles and a loss of arrangement of cellular columns of the zone fasciculate were also seen in the adrenal cortex. In the same publication, 4 groups of young male albino rats were also exposed to DnOP via intraperitoneal injection (0.0, 0.05, 0.1, 0.2xLD_{50}; 0, 2500, 5000, and 10,000 mg/kg bw-day) daily for 5 consecutive days. After the fifth day, rats were allowed to recover for two additional days prior to weighing, sacrifice, collection of peripheral blood for hematology, collection of spleen, thymus, and lymph node cells for estimates of cellularity, and collection of other organs for pathology. In this portion of the study, significant increases in liver, kidney, adrenal, and spleen weight were observed at the two lower doses. Non-significant increases in organ weights were reported at the high dose. Significant decreases in thymus, mesenteric lymph nodes, and portal lymph node weights and a significant increase in peripheral lymph node weights were seen following exposure to the high dose of DnOP. A "lowering of cell populations in various lymphoid organs in a dose dependent manner" was also reported, but no data was given to verify

this conclusion. *In vitro* tests of lymphoproliferative ability were also assessed in excised splenic cell cultures. Overall, spleen cell responsiveness to T-lymphocyte mitogen (PHA) was reduced significantly in a dose-dependent manner and responsiveness to B-lymphocyte mitogen (LPS) was reduced significantly in high and medium doses of DnOP. In the same publication, an additional four groups of young male albino rats were exposed to DnOP via intraperitoneal injection (0, 2500, 5000, and 10,000 mg/kg bw-day) daily for 5 consecutive days and then sensitized with $1x10^8$ sheep red blood cells. Five days following the induction, rats were challenged with a second dose of SRBCs. Twenty-four hours after challenge, the delayed hypersensitization reaction was assessed by measuring foot pad thickness. A marginal non-significant dose-related decrease in reactivity was observed with DnOP.

Dogra *et al.* (1989) also investigated how DnOP altered a mouse host's resistance to virus and protozoa. In this study, groups of Swiss albino mice (40 per group) were exposed to 0.0, 0.05, and $0.2xLD_{50}$ (0, 650, and 2600 mg DnOP/kg-bw), respectively, via oral gavage daily for 5 days. Forty-eight hours after the last dose of DnOP, mice were exposed intraperitonally to encephalomyocarditis (EMCV; 0.5 ml of a 10^{-6} dilution of EMCV), or *Plasmodium berghei* (10^3 infected syngenic erythrocytes per mouse). Mortality and parasitemia were observed over time. DnOP dose- and time-related increases in mortality (from 0 to 70% over days 4-10 post-inoculation for EMCV, and 20 to 80+% over days 3-19 post-inoculation with *P. berghei*, Table A2.1) and parasitemia (from 0.2 to 7% over days 5-14 post inoculation with *P. berghei*) were observed in association with both EMCV and *P. berghei* (Table A2.2). This data suggested that DnOP impaired the host's ability to produce interferon and/or antibodies, and altered the function of white blood cells.

Table A2.1 Approximate Cumulative % Mortality in DnOP-exposed Mice (Dogra *et al.*, 1989)

Treatment	Days post Inoculation with EMCV or *Plasmodium berghei*											
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**14**	**16**	**19**
PMCV oil control	-	0	-	10	-	10	-	20	-	-	20	-
650 mg/kg-day	-	0	-	20	-	40	-	-	-	-	40	-
2600 mg/kg-day	-	10	-	30	-	40	-	70	-	-	70	-
***P.b.* oil control**	0	20	20	-	20	20	20	-	25	35	-	55
650 mg/kg-day	0	25	25	-	25	25	25	-	25	45	-	70
2600 mg/kg-day	25	65	65	-	70	70	70	-	70	75	-	80

Table A2.2 Malaria Parasitemia (15%) in DnOP-exposed Mice (Dogra *et al.*, 1989)						
Treatment	**Days post Inoculation with *Plasmodium berghei***					
	5	**7**	**8**	**9**	**11**	**14**
Oil control	0.0537 ± 0.047 (16)	0.6813 ± 0.067 (16)	2.187 ± 0.963 (16)	3.187 ± 1.459 (16)	3.312 ± 1.436 (15)	4.400 ± 1.681 (13)
650 mg/kg-day	0.11 ± 0.17 (15)	1.27 ± 0.80 (15) P < 0.05	2.09 ± 0.85 (15)	2.60 ± 0.93 (15)	3.35 ± 1.12 (15)	5.23 ± 2.62 (11)
2600 mg/kg-day	0.0214 ± 0.0348 (7)	1.3833 ± 0.8953 (6) P < 0.01	3.1667 ± 1.1255 (6) P < 0.05	4.00 ± 1.3784 (6) P < 0.05	4.8333 ± 1.8619 (6) P < 0.05	7.00 ± 2.5495 (5) P < 0.01

Foster *et al.* (1980) investigated the effects of DnOP on Sprague-Dawley rat testicular structure and function. Oral gavage doses of 0 and 2800 mg/kg-day were administered once a day for 4 days. Food consumption, body weights, testicular parameters, and the liver and kidney weights were determined. Overall, no changes in weight gain or food consumption, relative testis weight, testis pathology, testis Zinc-65 concentration, kidney Zinc-65 concentration, or Zinc-65 excretion were induced at a dose of 2800 mg/kg-day.

Hardin *et al.* (1987) investigated the effect of DnOP on fetal toxicity and development in an abbreviated prenatal assay. Female CD-1 mice were exposed to 9780 mg/kg-day DnOP via oral gavage once a day during gestation days 6 - 13 and then allowed to deliver litters. Maternal variables (number dead/treated, weight change, and viable litters) were reported following birth. DnOP treatment did not change the number maternal deaths, body weight change or viable litters. Malformations were not reported for either live or dead pups. DnOP treatment reduced the number of live neonates per litter and weight gain in the pups, but did not change the number of viable litters, the percent survival of live born neonates, or the birth weight of pups (Table A2.3). Changes in fetal parameters were not related to maternal deaths or changes in body weight, but may have been related to exceptionally high control values for this test.

Table A2.3 Developmental Toxicity Data in CD-1 Mice Gavage Dosed with DnOP (Hardin *et al.*, 1987)		
Volume administered on Gd 6-13 (ml/kg)	Weight Gain (PNd 1-3) (g/pup)	Live born per litter
0 (corn oil)	0.7 ± 0.2	11.5 ± 1.7
10 (9780 mg/kg-day)	0.6 ± 0.1 (P < 0.05)	10.2 ± 2.8 (P < 0.05)

Heindel *et al.* (1989; and Morrissey *et al.*, 1989) investigated the effect of DnOP on fertility in a continuous breeding protocol study involving CD-1 mice. Both male and female mice were predosed for 7 days with DnOP in feed and then also dosed during the subsequent 98 days of cohabitation. Reproductive function was assessed during cohabitation and pathological endpoints were determined following sacrifice at the studies end. Dose selection was determined in a 14 day pilot study initially using 0.0, 0.5, 1.25, 2.5, 5.0, and 10.0% (0.0, 750, 1800, 3600,

7500, and 15,000 mg/kg-day, ATSDR, 1997) DnOP in feed. In this pilot study, only the incidence of rough hair coats was increased when observing clinical signs, weight gain or feed consumption. This pilot study led to the subsequent dose selection of 0.0, 0.5, 1.25, 2.5, and 5.0% (0.0, 750, 1800, 3600, 7500 mg/kg-day, ATSDR, 1997) DnOP in feed for the 26 week study. Treatment with DnOP had no effect on any physical appearance, body weight gain, or food consumption in either the F_0 or F_1 generations, but significantly increased liver weight in the high dose F_1 generation (allowed to mature for an additional 74 days post-birth), and significantly increased kidney weight in the female high dose F_1 generation. Treatment with DnOP had no effect on reproductive parameters (mating index, fertility index, litters/pair, live (M & F) pups/litter, proportion of pups born alive, sex of pups born alive, live pup weight) in the parental F_0 mice or in matings of the high dose (8640 mg/kg-day, Morrissey *et al.,* 1989) F_1 generation (allowed to grow for an additional 74 days post-weaning). In the F_1 generation, DnOP treatment also had no effect on the testis, epididymis, or prostate weight, epididymal sperm concentration, percentage of motile sperm, or average estrous cycle length, but did significantly decrease seminal vesicle weight and marginally reduced the percentage of morphologically abnormal sperm.

Hellwig and Jackh (1997) investigated the prenatal effects of n-octanol, a precursor alcohol used in the synthesis of DnOP. In the study, virgin Wistar rats (Chbb/THOM; 214-233 g) were oral gavage dosed with 0, 130, 650, 975, and 1300 mg/kg-day n-octanol (8-10 animals per group) during post-coitum (Gd) days 6 - 15. Both n-octanol-induced maternal and fetal parameters (i.e., food consumption, body weights, clinical signs, uterine weight, corpora lutea, implantations, resorptions, live and dead fetuses, fetal malformations/variations/retardations, etc) were determined in this study. Maternal mortality increased in medium and high dose treatments. Maternal toxicity also increased, but in a dose-related fashion (Table A2.4). Clinical signs of toxicity included lateral and abdominal positioning, unsteady gait, salivation, piloerection, nasal discharge, and pneumonia increased in severity. This increase was correlated with a non-significant slight decrease in food consumption and body weight at 650, 975, and 1300 mg/kg-day. No significant effects were reported when observing uterus weights, corpora lutea/dam, implantation sites/dam, live fetuses/dam, resorptions/dam, fetal weights/deaths, and malformations-variations-retardations. Litters with cheliochisis (1 at 1300 mg/kg-day), anopthalmia (1 at 1300 mg/kg-day), and bipartite thoracic vertebral bodies (2 at 975 mg/kg-day) were thought to be coincidental and not biologically significant.

Table A2.4 Maternal Lethality and Clinical Signs Following the Administration of n-octanol (Hellwig and Jackh, 1997)

Adverse Effect	Gavage Dose (mg/kg-day)					
	0 (control group)	0 (control group 2)	130	650	975	1300
Maternal Lethality	-	-	-	2/10	2/10	2/10
Clinical signs	-	-	+ equivocal	+	+	++

Hinton *et al.* (1986) administered 2% DnOP via feed to male Wistar rats *ad libitum* for 3, 10, or 21 days in order to determine potential alterations in liver structure and function. Rats were sacrificed, blood drawn, and organs removed for analysis after respective time-points were reached. *In vitro* experiments involving rat hepatocytes and 0.25 mM MnOP were also conducted in order to determine enzymatic and ultrastructural effects. Livers from treated rats displayed a variety of alterations such as a changed appearance to pale and greasy, mildly increased; hepatomegaly (after 21 days), centrilobular fat accumulation, peroxisome proliferation (after 21 days), smooth endoplasmic reticulum (ER) proliferation, and loss of rough ER, and moderately increased; centrilobular loss of glycogen and necrosis. Even so, there were no changes in total glycogen loss, periportal fat accumulation, the density of inner mitochondrial matrix, or mitosis. *In vitro* experiments revealed MnOP-induced increases in blebbing and vacuolation, increased marginal and central lipid accumulation, increased incorporation of palmitate into triglyceride and cholesterol esters, and increased fatty acid oxidation, without a change in cyanide-insensitive palmitoyl CoA oxidation or increase in cell death. Treatment with DnOP also decreased serum thyroxine (T_4) on days 3, 10, and 21 and increased triiodothyronine (T_3) on day 21. Ultrastructurally, DnOP increased the number and size of lysosomes, enlarged Golgi apparatus, and induced mitochondrial damage in thyroids. Ultrastructural damage was suggestive of thyroid hyperactivity.

Jones *et al.* (1993) determined the effect of DnOP and MnOP on Leydig cells in the testes and Leydig cell primary cultures of male Wistar rats. *In vivo* tests featured rats dosed via oral gavage with 2000 mg/kg DnOP or MnOP once daily for 2 days. One day post-dosing, rats were sacrificed and the testes removed and fixed for histopathology. Leydig cell primary cultures were isolated from rats, purified, raised in culture, and then treated with 1000 μM DnOP or MnOP in time-course experiments. Leydig cell functional integrity and testosterone production were assessed following the appropriate incubation periods. DnOP treatments did not alter the seminiferous tubular structure, Leydig cell morphology, or various aspects of Leydig cell ultrastructure (i.e., mitochondrial swelling, degeneration, SER focal dilatation, vesiculation, and peroxisomes). MnOP treatments, in contrast, produced significant alterations in Leydig cell cytoplasmic ultrastructure (such as mitochondrial swelling, degeneration, SER focal dilatation, increased number and length of filipodia, and peroxisomes) in *in vivo* experiments. In *in vitro* experiments, incubation of Leydig cells with MnOP decreased the LH-stimulated secretion of testosterone. Ultrastructurally, MnOP produced a large increase in filopodial proliferation from the basal lamellar processes and the cell soma, SER dilatation, mitochondrial swelling, mitochondrial degeneration and numeric reduction, and vesiculation of the Golgi apparatus.

Khanna *et al.* (1990) investigated the subchronic effects of DnOP on rat kidneys. Groups of 10 young male albino rats (100 g) were injected intraperitoneally with 0.0, 0.002, 0.006, and 0.012 x LD_{50} DnOP (0, 100, 300, and 600 mg/kg) once daily, 5 days a week for 90 days. Following 90 days, approximately half of the rats in each group were sacrificed and necropsied. During necropsy, the kidneys were removed and preserved in 10% formal saline. Following

fixation the kidneys were paraffin embedded, sectioned, and stained with hematoxylin and eosin for histopathological examination. The remaining rats in each group were then maintained for 45 more days (recovery), sacrificed, necropsied, and histopathologically evaluated in a manner similar to that of original test groups. Overall, there was little macroscopic evidence of change in kidneys of rats dosed with DnOP. Microscopically, the low dose of DnOP induced glomerular atrophy and increased cellularity, a large Bowman's space, cloudy swelling and luminal obliteration in the proximal tubules, dilation and hyaline casts in the distal tubules, and mild edema in the vasa recta. Glomerular atrophy was retained after a recovery period of 45 days. The mid-dose of DnOP induced a marked rarefaction of glomerular tufts, widely dilated capillaries, decreased cellularity, marked desquamation degeneration of the proximal collecting tubule epithelium, hyaline casts in the distal tubules, and prominent edema in the vasa recta. Glomerular damage and interstitial accumulation of mononuclear cells persisted following 45 days of recovery. The high dose of DnOP induced marked tubular and glomerular changes in the kidney cortices, widespread desquamation of the lining of Henle's loop cells and collecting ducts in the kidney medulla, and vascular bundle shrinkage and obliteration in the vasa recta. Irreversible tubule damage, edema in the vasa recta, and infiltration of lymphocytes into the interstitium persisted following 45 days of recovery. These results suggest that a dose-dependent irreversible nephrotoxicity occurred following subchronic intraperitoneal exposures to DnOP.

Lake *et al.* (1984) determined the effects of DnOP and MnOP on rat liver structure and function. In the study, male Sprague-Dawley rats were exposed to 1000 or 2000 mg/kg-day DnOP or 715, 750, or 1000 mg/kg-day MnOP daily via oral gavage for 14 days (6 animals per group). Following the last dose, rats were starved overnight, sacrificed, and the livers removed for structural and biochemical analysis. DnOP treatment (1000 mg/kg-day) increased the relative liver weight, marginally increased palmitoyl-CoA oxidation, increased enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, ethylmorphine N-demethylase activity, and reduced D-amino acid oxidase activity, 7-ethoxyresorufin O-deethylase, and 7-ethoxycoumarin O-deethylase, but didn't change the whole homogenate protein, cytochrome p450s, microsomal protein, or 7-ethoxycoumarin O-deethylase activity (Table A2.5).

Table A2.5 Liver Toxicity Data in Sprague-Dawley Rats Gavage Dosed with DnOP (Lake *et al.*, 1984)		
Liver Parameter	Control (Corn oil)	1000 mg/kg-day DnOP (percent of control)
Relative liver weight (g liver/100 g body weight±SEM)	3.6 ± 0.1	4.2 ± 0.1 (115; P < 0.01)
Enoyl-CoA Hydrolase – heat labile activity (µmol/min/mg homogenate protein)	1.6 ± 0.1	2.6 ± 0.3 (165)
Carnitine acetyltransferase (nmol/min/mg homogenate protein)	4.1 ± 0.4	12.6 ± 2.4 (305)
Lauric acid hydrolase (nmol/hr/mg microsomal protein)	170 ± 8	214 ± 31 (125)
Palmitoyl-CoA oxidation (nmol/min/mg homogenate protein)	5.6 ± 0.3	6.9 ± 0.5 (125)
Ethylmorphine N-demethylase (nmol/hr/mg microsomal protein)	660 ± 71	811 ± 25 (125)
D-amino oxidase (nmol/hr/mg microsomal protein)	121 ± 5	68 ± 3 (55; P < 0.01)
7 ethoxyresorufin O-deethylase (nmol/hr/mg microsomal protein)	8.4 ± 1.2	4.5 ± 0.6 (55; P < 0.01)
7 ethoxycoumarin O-deethylase (nmol/hr/mg microsomal protein)	140 ± 6	97 ± 11 (70; P < 0.05)

In the same study, treatment with MnOP (715 mg/kg-day) increased the relative liver weight, marginally increased palmitoyl-CoA oxidation, ethylmorphine N-demethylase activity, increased enoyl-CoA hydratase heat labile activity, carnitine acetytransferase activity, lauric acid hydroxylation, reduced D-amino acid oxidase activity, but did not change the whole homogenate protein, microsomal proteins, or cytochrome p450s. Similar treatment schedules with slightly higher doses of DnOP (2000 mg/kg-day) or MnOP (750, 1000 mg/kg-day) did not result in increased number of peroxisomes when viewed ultrastructurally.

Lake *et al.* (1986) followed initial studies with DnOP and MnOP with additional testing on primarily rat liver function. Male Sprague-Dawley rats were dosed with DnOP (1000 mg/kg-day) or MnOP (715 mg/kg-day) once a day for 14 days. The rats were sacrificed 12-24 hours later and the livers were weighed and homogenized for biochemical assays. An *in vitro* portion of the study created hepatocyte cultures, treated the cultures with 20, 50, 100, and 200 µM MnOP and then biochemically assayed for various enzymatic activity. Overall, *in vivo* treatment with DnOP increased relative liver weight, marginally increased palmitoyl-CoA oxidation, marginally increased enoyl-CoA hydratase heat labile and ethylmorphine N-demethylase activity, and reduced lauric acid 11-12 hydroxylation and 7-ethoxycoumarin O-deethylase activity. *In vivo* treatments with MnOP were similar and increased the relative liver weight, increased palmitoyl-CoA oxidation, and increased enoyl-CoA hydratase heat labile activity. *In vitro* treatments increased palmitoyl-CoA oxidation (at 200 µM) and also increased carnitine acetyltransferase activity (at 20 µM).

Larsen *et al.* (2001) determined the extent of immunosuppression in female Balb/c mice induced by MnOP, a metabolite of DnOP. Animals were sensitized by subcutaneous injection of 1 µg ovalbumin (OV) and administered 1, 10, 100, and 1000 µg/ml of MnOP (0.0053, 0.053,

0.53, 5.3 mg/kg-bw). This was boosted ten and fifteen days later with 0.1 µg OV. Four days after each booster, blood was collected, centrifuged, and the serum saved until analysis by ELISA for antibody levels. Significant body weight decrements were not observed in any animal. This finding was in contrast to the substantial decrements observed in IgE and IgG1 production in high dose groups following both booster shots. The altered levels of IgE and IgG1 were used to establish a LOAEL at 1000 µg/mL (5.3 mg/kg) and a NOAEL of 100 µg/mL (0.53 mg/kg) for immunosuppressive effects. As an aside, an adjuvant effect for IgG1 production was observed in 100, 10, and 1 ug/mL DnOP treatments.

Mann *et al.* (1985) investigated the effects of DnOP on liver structure and function. Effects to additional organs such as the testis and pancreas were also reported. In the study, male Wistar rats were exposed to 2% DnOP in feed (*ad libitum*) for 3, 10, or 21 days, then sacrificed for organ pathology and enzyme analysis. Exposure doses for the various time-points were calculated to be 2266, 2078, and 1906 mg/kg-day for days 3, 10, and 21, respectively (ATSDR, 1997). Overall, DnOP exposure resulted in increased food consumption and body weight at days 3, 10, and 21. In terms of the liver, DnOP induced a hepatic centrilobular loss of glycogen and ultrastructural proliferation and dilation of smooth endoplasmic reticulum at all time-points following exposure. DnOP exposure also caused a shortening of microvilli in bile canaliculi by day 3, and a small increase in lysosomal number by day 10, but this effect was not reported in subsequent time-points. Other attributes such as a change in appearance to pale and greasy, increased relative liver weight, a marginal increase in the number of liver mitotic figures, a marked increase in the centrilobular accumulation of fat (and associated necrosis), accumulation of lipid droplets in hepatocytes, increased cyanide-insensitive palmitoyl CoA oxidation, and an increase in catalase activity in the large particulate fraction of homogenates occurred later on days 10 and 21. Increased hepatomegaly, a small increase in peroxisome number and decreased 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphatase activity were reported only for the last time-point, day 21. In contrast to other phthalates, DnOP did not change the α-glycerophosphate dehydrogenase activity, catalase activity, cytochrome p450 induction, or nonenzymatic reducing agents at any time following exposure. DnOP exposure did not change the overall appearance of the pancreas in any of the sample groups, but decreased the relative testicular weight on day 10, but not day 3 or 21. Testicular effects may have been due to body weight decrements.

Oishi and Hiraga (1980) investigated the effects of MnOP on Wistar rat testicular structure and function. Rats were exposed to oral doses of 0 and 2% MnOP in feed (0, 1000 mg/kg-day) for 7 days. Food consumption, body weights, testicular parameters were determined. Overall, body weights and food consumption were significantly decreased after 7 days. Even so, there were no changes in the zinc concentration in the liver or kidney, zinc or testosterone concentration in the serum, the absolute or relative testis weight, and zinc or testosterone concentrations in the testes.

Oishi (1990) studied the pharmacokinetics and mitochondrial respiration in male Crj;Wistar rat testes following the administration of 2000 mg/kg DnOP via oral gavage. In the same study, isolated testes mitochondria were treated *in vitro* with DnOP and respiration parameters determined. In pharmacokinetic experiments, administration of 2000 mg/kg DnOP resulted in the creation of a peak blood and testis concentrations of MnOP at around 3 and 6 hours, respectively, after administration. Other pharmacokinetic parameters associated with the administration were described for blood ($t_{½}$= 3.3h, Area under the concentration time curve (AUC)=1066 µg-h/mL, Mean residence time (MRT) = 5.4 h, and Variance of residence time (VRT) = 19.5 h^2) and the testes ($t_{½}$= 5.0, AUC=358 µg-h/mL, MRT = 6.2 h, and VRT = 21.7 h^2). *In vitro* treatment of mitochondria with MnOP induced a dose-dependent decrease in mitochondrial state 3 oxygen consumption over 0.065, 0.13, 0.325, 0.65, and 1.30 µmole/ml treatments (LOAEL = 0.065 µmole/ml). Similar treatment with DnOP decreased mitochondrial oxygen consumption only at the 1.3 µmole/ml treatment. *In vivo* treatments with DnOP decreased the mitochondrial respiration control ratio and state 3 oxygen consumption in rat testes, but did not alter the pyruvate or lactate concentration. In two other studies cited by Oishi, MnOP was reported to uncouple mitochondrial oxidative phosphorylation *in vitro* (Takahashi, 1977; Melnick and Schiller, 1985).

Poon *et al.* (1997) investigated the effects of DnOP on liver, thyroid, and testicular structure and function. Male and female Sprague-Dawley rats were exposed to 0, 5, 50, 500, 5000 mg/kg DnOP (0, 0.4 - 0.4, 3.5 - 4.1, 36.8 - 40.8, 350.1 - 402.9 mg/kg-day for M - F, respectively, 10 rats per sex per group) in feed for 13 weeks. Following exposures, rats were sacrificed and blood and organs analyzed for altered enzyme levels and histopathology. In general, no changes to bodyweight, food consumption, or clinical toxicity were observed for the any of the dose levels. In the liver, DnOP induced an increase in the ethoxyresorufin-O-deethylase activity and moderately accentuated zonation (M), anisokaryosis (M), nuclear hyperchromicity (M), perivenous cytoplasmic vacuolation, and endothelial prominence (M) in livers in the high dose group. A moderate accentuation of zonation, and endothelial prominence and moderate nuclear hyperchromicity were also seen in female rats exposed to a lower doses of DnOP (40.8 and 0.4 mg/kg-day, respectively; Table A2.6). No changes were reported for absolute or relative liver weight, aminopyrine-N-demethylase activity, aniline hydroxylase activity, or the number or % area of peroxisomes at any of the test dose levels. For the thyroid, DnOP induced a reduced follicle size in female, but not male rats (40.8 mg/kg-day) and decreased colloid density in both male and female rats, but at different dose levels (3.5 and 40.8 mg/kg-day). These changes were observed even though no significant dose-related changes were reported in clinical chemistry or hematology (albumin, calcium, inorganic phosphate, total protein, white blood cell number, mean corpuscular hemoglobin and volume, or platelet count) at the highest test dose. In addition, no changes were observed in the absolute or relative kidney weights at the highest test dose or in absolute or relative testis weight, the incidence of seminiferous tubule atrophy, or bilateral reduction in sperm density at any of the test doses.

Table A2.6 DnOP-induced Liver Effects in Male and Female Sprague-Dawley Rats (Poon *et al.*, 1997)					
Adverse Effect	**Dose (mg/kg-day) in Feed**				
	0 (corn oil)	**0.4**	**3.5 - 4.1**	**36.8 - 40.8**	**350.1 - 402.9**
Absolute liver weight in male rats, g ± stddev (% body weight)	17.1 ± 1.7 (3.31)	18.5 ± 2.1 (3.42)	18.4 ± 2.1 (3.39)	18.6 ± 3.4 (3.49)	18.6 ± 1.5 (3.49)
Absolute liver weight in female rats, g ± stddev (% body weight)	9.83 ± 1.04 (3.32)	9.66 ± 1.31 (3.15)	9.83 ± 0.88 (3.26)	10.25 ± 1.16 (3.20)	10.30 ± 1.36 (3.52)
Male Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	0.120	0.126	0.131	0.150	0.375 (P < 0.05)
Female Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	0.163	0.165	0.165	0.198	0.345 (P < 0.05)
Male liver accentuation of zonation (Average Severity Index; ASI)[1]	1 (0.1)	2 (0.2)	1 (0.1)	1 (0.1)	10 (3.1)
Male liver anisokaryosis (ASI)[2]	1 (0.1)	0	4 (0.3)	5 (0.4)	9 (1.9)
Male liver nuclear hyperchromaticity (ASI)[3]	0	0	2 (0.3)	4 (0.4)	5 (1.0)
Male liver perivenous cytoplasmic vacuolation (ASI)[4]	0	0	0	0	9 (2.7)
Male liver endothelial prominence (ASI)[5]	0	0	0	0	7 (1.1)
Female liver accentuation of zonation (ASI)[1]	5 (0.4)	6 (0.4)	9 (0.7)	10 (0.8)	10 (1.6)
Female liver anisokaryosis (ASI)[2]	9 (1.5)	10 (2.0)	10 (2.3)	10 (2.5)	10 (3.0)
Female liver nuclear hyperchromaticity (ASI)[3]	3 (0.6)	10 (2.1)	9 (1.6)	10 (1.9)	10 (2.0)
Female liver perivenous cytoplasmic vacuolation (ASI)[4]	0	0	0	0	5 (1.2)
Female liver endothelial prominence (ASI)[5]	0	0	5 (0.5)	9 (0.9)	10 (1.5)

Rowland *et al.* (1977) investigated the hydrolysis of phthalates in the gastrointestinal tract of adult male Wistar rats (250 g). Rat stomach, small intestine, or cecum content suspensions were centrifuged, the supernatants filtered through 45 µm filters, and then incubated with ^{14}C-labeled DnOP for 16 hours. Following incubations, supernatants were assayed for DnOP and metabolites using thin layer chromatography and scintillation counting. The rate of metabolism was fastest in the small intestine (Table A2.7), with no metabolism being apparent after 30 minutes, but slight metabolism occurring by 16 hours. Metabolism in other phthalates was not affected by antibiotics, was affected by pre-incubation of the supernatant at 37C for 90 minutes. Further, with other phthalates, younger rats had slower rates of metabolism than older rats, and adult female rats had slower rates of metabolism than older male rats. The authors hypothesized that hydrolysis of DnOP is probably catalyzed by enzymes derived from mammals and bacteria.

Table A2.7 Metabolism of DnOP by Gastrointestinal Contents (Rowland *et al.*, 1977)			
Phthalate	% of DnOP Metabolized in 16 Hours (± SEM)		
	Stomach	Small Intestine	Cecum
DnOP	4.2 ± 2.2	11.1 ± 0.6	0.7 ± 0.1

Sato *et al.* (1994) investigated the ability of DnOP to induce mutagenicity in the Ames test, the SOS chromotest, and also it ability to enhance the mutagenicity of amino acid pyrolysates (4-nitroquinoline-1-oxide, 2-nitrofluorene, benzo-a-pyrene, and others). Overall, DnOP mutagenicity ratios were not significantly different than negative controls in the Ames test. Similarly, DnOP by itself did not induce the SOS response with or without S9 activation. DnOP enhanced the mutagenicity of 3-amino-1,4-dimethyl-5H-pyrido-[4,3]indole (Trp-1) and 3-amino-1-methyl-5H-pyrido-[4,3-b]indole (Trp-2) 2-fold over controls in the Ames test, but did not enhance the number of survivors. The mutagenicity of Trp-1 in the SOS chromotest was suppressed somewhat by DnOP.

Singh *et al.* (1972) determined the effect of DnOP on the development of Sprague-Dawley rats (200-250 g) dosed intraperitoneally on gestation days 5, 10, and 15 with 0, 5 and 10 mL/kg (0, 4890, and 9780 mg/kg-day; 5 rats each group) DnOP. DnOP-induced effects were estimated by observing changes at the fetus, not litter, level, in this study. In rats, treatment with DnOP significantly decreased the average weight of the fetus and increased the number of gross abnormalities (Table A2.8). Gross abnormalities for the phthalates consisted of tail absence, anophthalmia, twisted hind legs and hematomas/hemangiomas. No significant DnOP-induced changes were seen in the number of corpora lutea, number of resorptions, number of dead fetuses, number of live fetuses, and number of skeletal abnormalities.

Table A2.8 Developmental Toxicity Data in Sprague-Dawley Rats Injected with DnOP (Singh *et al.*, 1972)

Volume injected (ml/kg) on Gd 5, 10, and 15	Average Fetus Weight (avg. grams ± SE)	Number of Gross Abnormalities (% gross abnormalities based on total number of fetuses)
0 (cottonseed oil)	4.45 ± 0.17	1 (1.8%)
5 (4890 mg/kg-day)	4.00 ± 0.18 ($P \leq 0.01$)	8 (15.7%)
10 (9780 mg/kg-day)	3.40 ± 0.13 ($P \leq 0.01$)	15 (27.3%)

Smith *et al.* (2000) investigated the effects of DnOP on liver function in male Fischer 344 rats and B6C3F_1 mice. In the study, rats and mice were administered DnOP in feed (0, 1000, and 10,000 and 0, 500, and 10,000 mg/kg for rats and mice respectively) *ad libitum* for 2 and 4 weeks. Following exposures, the animals were sacrificed and livers removed for biochemical analysis. In rats, treatment with high doses of DnOP for 2 weeks increased the relative liver weights and peroxisomal beta oxidation activity. Increased periportal hepatocellular replicative DNA synthesis was also observed at 2 and 4 weeks. No changes in gap junction intercellular communication were reported for any time point or treatment. In mice, treatment with high doses of DnOP increased peroxisomal beta oxidation activity at both 2 and 4 weeks. Low dose treatments also increased peroxisomal beta oxidation activity at 4 weeks. Neither high nor low dose treatments changed the relative liver weight, gap junction intercellular communication, or periportal hepatocellular replicative DNA synthesis at 2 and 4 weeks.

Takeuchi *et al.* (2005) determined the effect of DnOP on the human estrogen receptor α and β, and the human androgen receptor using *in vitro* reporter gene assays. Transfected cells were dosed with DnOP and co-incubated with 17β-estradiol (E_2) or 5α-dihydrotestosterone for 24 hours, then assayed for activity. DnOP had neither agonistic nor antagonistic activity with estrogen receptors α and β. In addition, no antiandrogenic effects were reported for DnOP.

Toda *et al.* (2004) investigated the binding affinity of DnOP to estrogen receptors. Test chemicals dissolved in DMSO (10^{-3} to 10^{-8} M) were incubated with estrogen receptor α and β and 17β-estradiol (E_2) for 1 hour at 4C, and then incubated on microtiter plates coated with E_2 for 1 hour at 4C. Following incubation, the reaction was stopped and the microplate read on a microplate reader to determine the percent inhibition when compared to a diethylstilbesterol (DES) positive control. In the study, DnOP did not bind to estrogen receptor α or β (10^{-3} to 10^{-5} M), but when hydroxylated bound to both estrogen receptor α and β (10^{-4} to 10^{-8} M). DnOP's relative binding affinity to estrogen receptor α was highest of the phthalates tested and only 20 times less than the positive control diethylstilbestrol (Table A2.9). Phthalate monoesters and ring-hydroxylated monoesters were negative in ER binding assays in this study.

Table A2.9 Binding Affinity of DnOP to Estrogen Receptor α or β (Toda *et al.*, 2004)

Compound	ERα IC_{50} μM	Relative Binding Affinity (RBA)	ERβ IC_{50} μM	Relative Binding Affinity (RBA)
DnOP-OH	0.58 ± 0.08	4.9 ± 0.6	1.87 ± 0.17	1.7 ± 0.1
DES	0.028 ± 0.002	100	0.031 ± 0.002	100

Zeiger *et al.* (1985) determined the mutagenicity of DnOP in *Salmonella typhimurium*. DnOP was tested in a Salmonella plate incorporation assay with strains TA1535, TA1537, TA98, and TA100 with and without activation. In the assays, DnOP was not mutagenic for Salmonella either without or with metabolic activation (rat or hamster liver S-9).

Appendix 3. Phthalate Chemical Product List

CAS Number	Phthalate Chemical Name	CAS Number	Phthalate Chemical Name
21395-09-5	(+/-)-mono-2-octylphthalate	27554-26-3	diisooctyl phthalate
68296-97-9	(±)-2-octyl hydrogen phthalate		diisophentyl phthalate
23276-77-9	(1-ethylhexyl) hydrogen phthalate	27253-26-5	diisotridecyl phthalate
67939-28-0	(butylstannylidyne)tris(thioethylene) triisooctyl triphthalate	96507-86-7	diisoundecyl phthalate
68928-78-9	(dibutylstannylene)bis(thioethylene) diisooctyl diphthalate	85507-79-5	diisoundecyl phthalate
1322-94-7	(dimethylcyclohexyl) hydrogen phthalate	17840-25-4	dilithium isophthalate
84473-57-4	[2-[bis(2-hydroxyethyl)amino]ethyl] hydrogen phthalate	15968-00-0	dilithium phthalate
55334-51-5	[4-(methoxycarbonyl)phenyl]methyl methyl terephthalate	14309-54-7	dimethyl 1,4-Cyclohexadiene-1,2-dicarboxylate; dimethyl 3,6-Dihydrophthalate
26761-40-0	1,2-benzenedicarboxylic acid diisodecyl ester; diisodecyl phthalate	18014-00-1	dimethyl 2,5-dibromoterephthalate
2055-00-7	1,2-ethanediyl dimethyl phthalate	3293-89-8	dimethyl 2,5-dichloroterephthalate
118-99-0	1,3-diphenylguanidinium phthalate	5292-51-3	dimethyl 2,5-difluoroterephthalate
40139-96-6	1-[2-(methacryloyloxy)-1-methylethyl] hydrogen sulphophthalate	35636-63-6	dimethyl 2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]terephthalate
60728-41-8	1-methyl 2-aminoterephthalate	67906-31-4	dimethyl 2-[[2-[(2-methoxyphenyl)amino]-2-oxo-1-(1,4,5,6-tetrahydro-4,6-dioxo-1,3,5-triazin-2-yl)ethyl]azo]terephthalate
65859-45-2	1-methyl-2-[(2-methyl-1-oxoallyl)oxy]ethyl hydrogen phthalate	5292-47-7	dimethyl 2-fluoroterephthalate
20566-35-2	2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetabromophthalate	14186-60-8	dimethyl 2-methylterephthalate
2202-98-4	2-(2-hydroxyethoxy)ethyl hydrogen phthalate	55447-98-8	dimethyl 2-sulphoterephthalate
51986-91-5	2,2'-diethyl dihydrogen 4,4'-carbonylbisphthalate	52656-24-3	dimethyl 4-[(3-aminophenyl)hydroxymethyl]phthalate
43135-99-5	2,2-dimethylpropane-1,3-diyl dihexahydrophthalate	51832-31-6	dimethyl 4-aminophthalate
35512-59-5	2,2-dimethylpropane-1,3-diyl phthalate	39617-05-5	dimethyl 4-dimethylaminophthalate
85851-76-9	2,5-dimethylheptyl 4-methyloctyl phthalate	5985-24-0	dimethyl 4-hydroxyisophthalate
85851-77-0	2,5-dimethylheptyl 6-methyloctyl phthalate	59340-47-5	dimethyl 4-iodophthalate
85391-52-2	2,5-dimethylheptyl nonyl phthalate	22955-73-3	dimethyl 4-methoxyisophthalate
54380-33-5	2-[(2-methyl-1-oxoallyl)oxy]ethyl hydrogen 3-chloro-2-hydroxypropylphthalate	23038-61-1	dimethyl 4-methylisophthalate
41284-31-5	2-[[4-(2,2-dicyanovinyl)-3-methylphenyl]ethylamino]ethyl methyl terephthalate	20116-65-8	dimethyl 4-methylphthalate
38056-88-1	2-acryloyloxyethyl 2-hydroxyethyl phthalate	610-22-0	dimethyl 4-nitrophthalate
30697-40-6	2-acryloyloxyethyl hydrogen phthalate	3748-70-7	dimethyl 5-(1-hydroxy-N-octadecyl-2-naphthamido)isophthalate
61827-64-3	2-ethylhexyl 2-methylpropyl phthalate	70364-24-8	dimethyl 5-(N-tosylsulphamoyl)isophthalate; potassium salt
85661-32-1	2-ethylhexyl 3-methoxypropyl phthalate	29920-31-8	dimethyl 5-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azoterephthalate
89-13-4	2-ethylhexyl 8-methylnonyl phthalate	51760-21-5	dimethyl 5-bromoisophthalate
53272-22-3	2-ethylhexyl isodecyl phthalate	20330-90-9	dimethyl 5-chloroisophthalate
85851-92-9	2-ethylhexyl isononyl phthalate	13036-02-7	dimethyl 5-hydroxyisophthalate; dimethyl 5-hydroxybenzene-1,3-dicarboxylate~5-hydroxyisophthalic acid dimethyl ester

98072-28-7	2-ethylhexyl isotridecyl phthalate	51839-15-7	dimethyl 5-iodoisophthalate
98088-96-1	2-ethylhexyl isoundecyl phthalate	57052-99-0	dimethyl 5-nitroisophthalate
63468-13-3	2-ethylhexyl methyl terephthalate	138-25-0	dimethyl 5-sulphoisophthalate
85851-86-1	2-ethylhexyl nonyl phthalate	18643-86-2	dimethyl bromoterephthalate
85391-47-5	2-ethylhexyl undecyl phthalate	1687-29-2	dimethyl cis-1,2-cyclohexanedicarboxylate; dimethyl cis-hexahydrophthalate
17689-42-8	2-hydroxyethyl hydrogen phthalate	36928-64-0	dimethyl dihydrogen 4,4'-carbonylbisphthalate
46828-05-1	3,5-bis(methoxycarbonyl)phenylisocyanate; dimethyl-5-(isocyanato)isophthalate	5292-45-5	dimethyl nitroterephthalate; nitroterephthalic acid dimethyl ester
97692-55-2	3-ethylheptyl 2,5-dimethylheptyl phthalate	131-11-3	dimethyl phthalate
85851-79-2	3-ethylheptyl 4-methyloctyl phthalate	120-61-6	dimethyl terephthalate
85851-80-5	3-ethylheptyl 6-methyloctyl phthalate	3965-55-7	dimethyl-5-sulfoisophthalate sodium salt; DMSIP
13365-26-9	3-nitrophthalic acid dimethyl ester; dimethyl-3-nitrophthalate	27987-25-3	dimethylcyclohexyl-phthalate; DMCHP
25333-72-6	4,5alpha-epoxy-14-hydroxy-3-methoxy-17-methyl-6-oxomorphinan hydrogen terephthalate	84-74-2	di-n-butyl phthalate = DBP; phthalic acid di-n-butyl ester
22479-95-4	4-hydroxy phthalic acid dimethyl ester; dimethyl-4-hydroxy phthalate	4654-26-6	di-n-octyl terephthalate; dioctyl terephthalate
85851-78-1	4-methyloctyl 6-methyloctyl phthalate	131-18-0	di-n-pentyl phthalate; dipentylphthalate
85391-53-3	4-methyloctyl nonyl phthalate	14117-96-5	dioctadecyl phthalate
152699-63-3	5-aminoisophthalic acid dibenzyl ester; dibenzyl 5-amino isophthalate	137-89-3	dioctyl isophthalate
119-05-1	6-methylheptyl 8-methylnonyl phthalate	10578-33-3	dioleoyl phthalate
85391-54-4	6-methyloctyl nonyl phthalate	1539-04-4	diphenyl terephthalate
85409-84-3	8-methylnonyl phenylmethyl phthalate	17573-13-6	diphenylguanidine phthalate
3179-56-4	acetyl cyclohexane sulfonyl peroxide; 29% in phthalate plasticizer	18824-74-3	dipotassium 3,4,5,6-tetrabromophthalate
3814-58-2	allyl 2,3-epoxypropyl phthalate	15968-02-2	dipotassium 5-tert-butyliso phthalate
13654-74-5	aluminium tetrabromophthalate (3:2)	4409-98-7	dipotassium phthalate
67952-97-0	aluminium tridecyl phthalate (1:3:3)	4409-98-7	dipotassium phthalate
85959-15-5	ammonium dihydrogen 3-sulphonatophthalate		dipropargyl isophthalate
65229-11-0	ammonium dihydrogen 4-sulphonatophthalate		dipropargyl phthalate
83968-68-7	ammonium sodium hydrogen 5-sulphonatoisophthalate; compound with hexane-1,6-diamine (2:1)		dipropargyl terephthalate
50930-79-5	aniline hydrogen phthalate; 99%	68003-45-2	disodium 2-dodecyl 4-sulphonatophthalate
15656-86-7	barium phthalate	53566-35-1	disodium 4-hydroxyisophthalate
16883-83-3	benzyl 3-isobutyryloxy-1-isopropyl-2,2-dimethylpropyl phthalate	68189-35-5	disodium dodecyl 4-sulphonatophthalate
26386-42-5	benzyl butyl terephthalate	51821-29-5	disodium hydrogen sulphonatophthalate
27215-22-1	benzyl isooctyl phthalate	10028-70-3	disodium terephthalate
1248-43-7	benzyl octyl phthalate	25357-79-3	disodium tetrabromophthalate
21578-94-9	bis(1,1-dimethylethyl) dioxyterephthalate	2155-71-7	di-tert-butyl diperoxyphthalate
117-85-1	bis(2-(2-ethoxyethoxy)ethyl) phthalate	43039-86-7	di-tert-butyl peroxyhexahydroterephthalate
62240-27-1	bis(2,2,2-trifluoroethyl) phthalate	30448-43-2	di-tert-butyl phthalate

7415-86-3	bis(2,3-dibromopropyl) phthalate	119-06-2	ditridecyl phthalate; bis(tridecyl) Phthalate
97890-18-1	bis(2,3-epoxypropyl) 3,4,5,6-tetrabromophthalate	3648-20-2	diundecyl phthalate
97890-17-0	bis(2,3-epoxypropyl) 3,4,5,6-tetrachlorophthalate	21577-80-0	dodecyl hydrogen phthalate
7195-43-9	bis(2,3-epoxypropyl) isophthalate	85-71-2	ethoxycarbonylmethyl methyl phthalate
7195-45-1	bis(2,3-epoxypropyl) phthalate	29092-13-5	ethyl hydrogen tetrabromoterephthalate
7195-44-0	bis(2,3-epoxypropyl) terephthalate	4196-98-9	ethylene phthalate
57376-95-1	bis(2,4,6-tribromophenyl) terephthalate	51834-16-3	hexadecyl hydrogen phthalate
85391-48-6	bis(2,5-dimethylheptyl) phthalate	64084-40-8	hexadecyl octadecyl phthalate
605-54-9	bis(2-ethoxyethyl)phthalate	75673-16-4	hexyl 2-ethylhexyl phthalate
7299-89-0	bis(2-ethylbutyl) phthalate	61702-81-6	hexyl isodecyl phthalate
70152-36-2	bis(2-ethylheptyl) phthalate	71850-12-9	hexyl isooctyl phthalate
85409-66-1	bis(2-ethylhexyl) 4-(isopropyl)-5-methylphthalate	85851-89-4	hexyl isotridecyl phthalate
26040-51-7	bis(2-ethylhexyl) tetrabromophthalate	61827-62-1	hexyl octyl phthalate
6422-86-2	bis(2-ethylhexyl)terephthalate; 98.5%; DOTP	9050-31-1	hydroxypropyl methyl cellulose phthalate; methyl hydroxypropyl cellulose phthalate
85851-82-7	bis(2-ethylnonyl) phthalate	9050-31-1	hydroxypropyl methyl cellulose phthalate; hydroxypropyl methyl cellulose phthalat
85851-81-6	bis(2-ethyloctyl) phthalate	9050-31-1	hypromellose phthalate
7259-89-4	bis(2-hydroxyethyl) 5-nitroisophthalate	52118-12-4	iron phthalate (2:3)
84-73-1	bis(2-hydroxyethyl) phthalate	30833-53-5	isobutyl hydrogen phthalate
959-26-2	bis(2-hydroxyethyl)terephthalate; terephthalic acid bis(2-hydroxyethyl)ester	85168-75-8	isodecyl isononyl phthalate
117-82-8	bis(2-methoxyethyl)phthalate; di(2-methoxyethyl) phthalate	42343-35-1	isodecyl isooctyl phthalate
85851-83-8	bis(2-methyldecyl) phthalate	85168-77-0	isodecyl isotridecyl phthalate
70857-56-6	bis(2-methyloctyl) phthalate	94979-22-3	isodecyl isoundecyl phthalate
84787-86-0	bis(2-methylpropyl) 4-(dimethylamino)phthalate	85851-91-8	isodecyl nonyl phthalate
117-83-9	bis(2-n-butoxyethyl)phthalate; phthalic acid bis(2-butoxyethyl) ester	1330-96-7	isodecyl octyl phthalate
101012-82-2	bis(2-oxo-2-phenylethyl) phthalate	96507-81-2	isodecyl undecyl phthalate
53306-54-0	bis(2-propylheptyl) phthalate	96532-79-5	isononyl isooctyl phthalate
85851-84-9	bis(2-propylhexyl) phthalate	85168-76-9	isononyl isotridecyl phthalate
85851-85-0	bis(2-propyloctyl) phthalate	85168-79-2	isononyl isoundecyl phthalate
37832-65-8	bis(3,3,5-trimethylcyclohexyl) phthalate	98088-97-2	isononyl nonyl phthalate
14103-61-8	bis(3,5,5-trimethylhexyl) phthalate	85851-88-3	isononyl octyl phthalate
85409-67-2	bis(3-cyclohexylpropyl) phthalate	96507-82-3	isononyl undecyl phthalate
85391-51-1	bis(3-ethylheptyl) phthalate	67907-16-8	isooctyl 2-mercaptoethyl phthalate
85661-30-9	bis(3-methoxypropyl) phthalate	72512-75-5	isooctyl 2-phenoxyethyl terephthalate
20198-64-5	bis(3-phenylpropyl) phthalate	30849-48-0	isooctyl hydrogen phthalate
146-50-9	bis(4-methyl-2-pentyl)phthalate	94979-21-2	isooctyl isotridecyl phthalate

85391-50-0	bis(4-methyloctyl) phthalate	96532-80-8	isooctyl isoundecyl phthalate
85391-49-7	bis(6-methyloctyl) phthalate	96507-85-6	isooctyl nonyl phthalate
89-16-7	bis(8-methylnonyl) phthalate	96507-84-5	isooctyl undecyl phthalate
159852-53-6	bis(hexafluoroisopropyl)terephthalate	1459-93-4	isophthalic acid dimethyl ester; dimethyl isophthalate
82001-21-6	bis(pentabromobenzyl) tetrabromophthalate	744-45-6	isophthalic acid diphenyl ester; diphenyl isophthalate
94441-98-2	bis(pentabromobenzyl) tetrabromoterephthalate	1877-71-0	isophthalic acid monomethyl ester; monomethyl isophthalate
57212-63-2	bis(pentabromophenyl) terephthalate	93843-14-2	isotridecyl hydrogen phthalate
93951-36-1	bis[(1-methyl-1-phenylethyl)phenyl] isophthalate	85168-78-1	isotridecyl isoundecyl phthalate
3388-01-0	bis[(tetrahydrofuran-2-yl)methyl] phthalate	85851-90-7	isotridecyl nonyl phthalate
36388-36-0	bis[[1,4a-dimethyl-7-(1-methylethyl)tetradecahydrophenanthryl]methyl] phthalate	98072-29-8	isotridecyl undecyl phthalate
57569-40-1	bis[2-(1,1-dimethylethyl)-6-[[3-(1,1-dimethylethyl)-2-hydroxy-5-methylphenyl]methyl]-4-methylphenyl] terephthalate	96507-78-7	isoundecyl nonyl phthalate
32741-83-6	bis[2-(azidoformyloxy)ethyl] isophthalate	96507-79-8	isoundecyl undecyl phthalate
94088-05-8	bis[2-[(2-methyl-1-oxoallyl)oxy]ethyl] 2,5-bis(chloroformyl)terephthalate	93839-98-6	lead 3-(acetamido)phthalate
94088-04-7	bis[2-[(2-methyl-1-oxoallyl)oxy]ethyl] 4,6-bis(chloroformyl)isophthalate	60580-60-1	lead 5-nitroterephthalate
33374-28-6	butoxyethyl butyl phthalate	38787-87-0	lead isophthalate
85-69-8	butyl 2-ethylhexyl phthalate	6838-85-3	lead phthalate
85-68-7	butyl benzyl phthalate	16183-12-3	lead phthalate
84-64-0	butyl cyclohexyl phthalate	17976-43-1	lead phthalate (dibasic)
42597-49-9	butyl hydrogen tetrabromophthalate	42596-02-1	lithium terephthalate
24261-19-6	butyl hydrogen tetrachlorophthalate	68123-44-4	magnesium 4,4'-carbonylbisphthalate (2:1)
17851-53-5	butyl isobutyl phthalate	78948-87-5	magnesium bis(monoperoxyphthalate) hexahydrate; monoperoxyphthalic acid magnesium salt hexahydrate
42343-36-2	butyl isodecyl phthalate	84665-66-7	magnesium monoperoxyphthalate hexahydrate; monoperoxyphthalic acid magnesium salt 6H2O
3461-31-2	butyl nonyl phthalate	549-14-4	magnesium phthalate
84-78-6	butyl octyl phthalate	67801-55-2	methyl (4-methylphenyl)methyl terephthalate
89-19-0	butyl-n-decyl phthalate	39973-15-4	methyl hydrogen 4-(m-aminobenzoyl)phthalate
94275-93-1	cadmium (1-ethylhexyl) phthalate (1:2:2)	23843-86-9	methyl hydrogen 4-[(3-aminophenyl)hydroxymethyl]phthalate
94247-16-2	cadmium isooctyl phthalate (1:2:2)	6725-72-0	methyl phenyl terephthalate
94275-94-2	cadmium octyl phthalate (1:2:2)	4376-18-5	monomethyl phthalate; methyl hydrogen phthalate
5064-27-7	cadmium phthalate	131-70-4	n-butyl hydrogen phthalate; butyl hydrogen Phthalate
23239-68-1	calcium dibenzyl diphthalate	39020-35-4	nitroterephthalate dimethyl ester
94248-52-9	calcium dichlorophthalate	119-07-3	n-octyl-n-decyl phthalate
94275-90-8	calcium hydrogen 3,4,5,6-tetrachlorophthalate	24539-59-1	nonyl hydrogen phthalate
84681-97-0	calcium octadecyl phthalate (1:2:2)	65185-89-9	nonyl undecyl phthalate
5793-85-1	calcium phthalate	17181-26-9	octadecyl hydrogen phthalate
16130-76-0	calcium terephthalate	5393-19-1	octyl hydrogen phthalate

9004-38-0	cellulose acetophthalate; cellulose acetate phthalate	523-31-9	phthalic acid dibenzyl ester; dibenzyl phthalate
6732-01-0	cholesterol hydrogen phthalate	84-66-2	phthalic acid diethyl ester; diethyl phthalate ; DEP
51084-32-3	cobalt methyl terephthalate (1:2:2)	84-69-5	phthalic acid diisobutyl ester; di-isobutyl phthalate
34262-88-9	cobalt terephthalate	84-76-4	phthalic acid di-n-nonyl ester; di-n-nonyl phthalate
68123-45-5	copper 4,4'-carbonylbisphthalate (2:1)	117-84-0	phthalic acid di-n-octyl ester; di-n-octylphthalate
5423-38-1	copper dibutyl diphthalate	25053-15-0	poly(diallyl phthalate); average MW 65000 (gpc)
10027-30-2	copper phthalate	25038-59-9	poly(ethylene terephthalate); polyethylene terephthalate = PET
6190-36-9	cotarnine phthalate	29382-68-1	polyvinyl hydrogen phthalate
1169-98-8	cyclohexyl 2-ethylhexyl phthalate	10433-41-7	potassium dimethyl 5-sulphonatoisophthalate
5334-09-8	cyclohexyl isobutyl phthalate	877-24-7	potassium hydrogen phthalate; phthalic acid monopotassium salt~potassium biphthalate
71486-48-1	cyclohexyl isooctyl phthalate		potassium monomethyl terephthalate
85391-46-4	decyl 2-ethylhexyl phthalate	29801-94-3	potassium phthalate (2:1)
25724-58-7	decyl hexyl phthalate	97552-48-2	propane-1,3-diyl isophthalate
24539-60-4	decyl hydrogen phthalate	32657-12-8	S,S-bis[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]methyl] terephthalate
96507-83-4	decyl isononyl phthalate	24066-77-1	sodium (2,3-dihydroxypropyl) phthalate
53363-96-5	decyl isooctyl phthalate	83249-61-0	sodium 2-[(1-oxooctadec-9-enyl)amino]ethyl phthalate
98072-27-6	decyl isotridecyl phthalate	25425-73-4	sodium 2-ethylhexyl phthalate
96507-80-1	decyl isoundecyl phthalate	20259-91-0	sodium decyl phthalate
96507-76-5	decyl nonyl phthalate	73309-51-0	sodium diethyl 2-[(2-amino-8-hydroxy-6-sulphonatonaphthyl)azo]terephthalate
19295-82-0	decyl undecyl phthalate	33562-89-9	sodium dihydrogen 4-sulphonatophthalate
94023-12-8	D-glucitol phthalate; cyclic	66687-30-7	sodium dihydrogen 5-(3-sulphonatopropoxy)isophthalate
117-81-7	di-(2-Ethylhexyl)phthalate; dioctylphthalate	31352-31-5	sodium dimethyl 5-(3-sulphonatopropoxy)phthalate
62736-00-9	di(D-glucitol) phthalate	83781-01-5	sodium hydrogen 3(or 4)-sulphophthalate
110-22-5	diacetyl peroxide; 25% solution in dimethyl phthalate	68966-32-5	sodium hydrogen 3-chlorophthalate
13846-31-6	diallyl hexahydrophthalate	93762-14-2	sodium isobutyl phthalate
1087-21-4	diallyl isophthalate	94248-71-2	sodium isooctyl phthalate
131-17-9	diallyl phthalate	94108-00-6	sodium nonyl phthalate
131-71-9	diallyl phthalate practical	827-27-0	sodium phthalate
1026-92-2	diallyl terephthalate	15596-76-6	sodium terephthalate
7495-85-4	diallyl tetrahydrophthalate	94108-01-7	sodium tridecyl phthalate
523-24-0	diammonium phthalate	94248-20-1	strontium dichlorophthalate
523-31-9	dibenzyl phthalate; phthalic acid dibenzyl ester	94275-91-9	strontium hydrogen 3,4,5,6-tetrachlorophthalate (1:2)
19851-61-7	dibenzyl terephthalate	636-09-9	terephthalic acid diethyl ester; diethyl terephthalate
3126-90-7	dibutyl isophthalate	173550-97-5	terephthalic acid mono(2-bromoethyl) ester; 2-bromoethyl hydrogen terephthalate
1962-75-0	dibutyl terephthalate	33693-84-4	tert-butyl hydrogen phthalate

3015-66-5	dibutyl tetrachlorophthalate	15042-77-0	tert-butyl monoperphthalate
68515-51-5	di-C6-10-Phthalate	49693-09-6	tetrabromo phthalic acid diallyl ester; diallyl tetrabromo phthalate
68515-41-3	di-C7-9-Phthalate	49693-09-	tetrabromophthalic acid diallyl ester; diallyl tetrabromo phthalate
68515-43-5	di-C9-11-Phthalate; 1,2-benzoldicarbonsäure di-9-11-verzweigte und lineare Alkylester	68123-46-6	tetracesium 4,4'-carbonylbisphthalate
131-15-7	dicapryl phthalate	67846-42-8	tetraethyl 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis(phthalate)
84-61-7	dicyclohexyl phthalate; DCHP	35395-64-3	tetrahydrofurfuryl hydrogen phthalate
68443-43-6	didecyl 2-ethyl-2-[[(2-methyl-1-oxoallyl)oxy]methyl]propane-1,3-diylphthalate	68226-87-9	tetralithium 4,4'-carbonylbisphthalate
84-77-5	didecyl phthalate; di-n-decyl phthalate	68516-73-4	tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate
2432-90-8	didoceyl phthalate; 98%	85050-00-6	tetramethyl 5,5'-[(1,4-dioxo-1,4-butanediyl)diimino]bisisophthalate
65701-07-7	diethyl 4,4'-carbonylbis(hydrogen phthalate); compound with benzene-m-diamine	79723-02-7	tetramethylammonium hydrogen phthalate
65701-06-6	diethyl 4,4'-carbonylbis(hydrogen phthalate); compound with p,p'-methylenedianiline (1:1)	56585-48-9	tetrapotassium 4,4'-carbonylbisphthalate
64139-21-5	diethyl 4-hydroxyphthalate	68123-47-7	tetrarubidium 4,4'-carbonylbisphthalate
636-53-3	diethyl isophthalate; DEIP	67892-57-3	tetrasodium 4,4'-[(1-methylethylidene)bis(1,4-phenyleneoxy)]bisphthalate
3648-21-3	diheptyl phthalate; di-n-heptyl phthalate	68123-48-8	tetrasodium 4,4'-carbonylbisphthalate
13372-18-4	dihexadecyl phthalate	68226-88-0	tricesium hydrogen 4,4'-carbonylbisphthalate
84-75-3	dihexyl phthalate	61886-60-0	tridecyl isodecyl phthalate
605-50-5	diisoamyl phthalate; DIAP	67907-14-6	triisooctyl (methylstannylidyne)tris(thioethylene) triphthalate
70969-58-3	diisobutyl hexahydrophthalate	71686-04-9	trilithium 5-sulphonatoisophthalate
18699-48-4	diisobutyl terephthalate	68226-92-6	trilithium hydrogen 4,4'carbonylbisphthalate
90937-19-2	diisoheptyl phthalate	68226-90-4	tripotassium hydrogen 4,4'-carbonylbisphthalate
71850-09-4	diisohexyl phthalate	68226-89-1	trirubidium hydrogen 4,4'-carbonylbisphthalate
68515-50-4	diisohexyl phthalate	68226-91-5	trisodium hydrogen 4,4'-carbonylbisphthalate
68515-48-0	diisononyl phthalate; DINP	52642-40-7	trisodium sulphonatophthalate
28553-12-0	di-"isononyl" phthalate; diisononyl phthalate	51622-03-8	undecyl hydrogen phthalate
67907-15-7	diisooctyl (dimethylstannylene)bis(thioethylene)phthalate	60580-61-2	zinc 5-nitroisophthalate
71850-11-8	diisooctyl isophthalate	2880-85-5	zinc phthalate

*Source: Chemos GmbH Chemical Product list: http://www.chemos-group.com