UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
4330 EAST WEST HIGHWAY
BETHESDA, MD 20814

Memorandum

TO: Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences

THROUGH: Lori E. Saltzman, M.S., Director, Division of Health Sciences;
Michael A. Babich, Ph.D., Chemist, Division of Health Sciences

FROM : Leslie E. Patton, Ph.D., Toxicologist, Division of Health Sciences

SUBJECT : CPSC Staff Toxicity Review of Two Phthalates and One Phthalate Alternative for Consideration by the Chronic Hazard Advisory Panel - 2011[1]

This memo provides the U.S. Consumer Product Safety Commission's (CPSC's) Health Sciences staff assessment of the potential toxicity associated with two of the less commonly used phthalate ester compounds and one phthalate alternative, for consideration by the phthalate Chronic Hazard Advisory Panel.

CPSC staff asse[illegible] a product's potential health effects to consumers under the Federal Hazardous Subs[illegible] Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" unde[illegible] 'SA, a consumer product must satisfy a two-part definition. 15 USC 1262 (f)(1)(A). 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 C.F.R. 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 quantitative assessment of exposure and risk is performed to evaluate whether the chemical may

[1] These comments are those of the CPSC staff and have not been reviewed or approved by, and may not necessarily reflect the views of, the Commission.

be considered a “hazardous substance” under the FHSA. This memo represents the first parts of the risk assessment process, that is, the hazard identification and dose-response steps.

TABLE OF CONTENTS

TABLE OF TABLES

INTRODUCTION

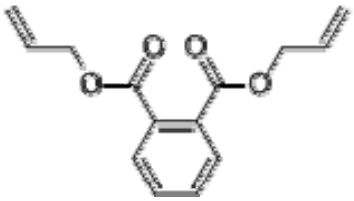
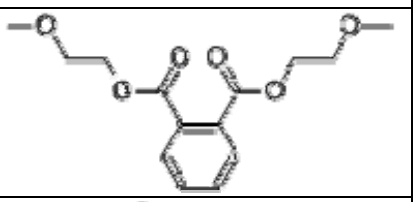
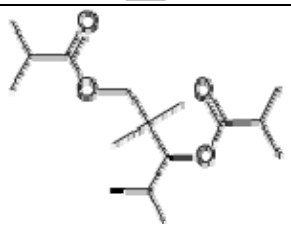
Dialkyl *ortho*-phthalates (*o*-DAPs) are a class of commercial chemicals that are used primarily as plasticizers for polyvinyl chloride (PVC) and as solvents. The general structure is a diester of 1,2-dicarboxy-benzene, where the two alkyl groups (R and R') may be similar or dissimilar; they may be branched or linear; and they may contain aromatic substituents or other functional groups. The *o-DAPs* are of particular interest due to widespread human exposure and the observation that certain *o-DAPs* induce reproductive and developmental health effects in animals. As such, chemical alternatives to phthalate esters, particularly as plasticizers, currently, are of interest.

The U.S. Consumer Product Safety Commission (CPSC) staff has written hazard summaries for six phthalates outlined in the Consumer Product Safety Improvement Act (CPSIA; 2008): butyl benzyl, dibutyl, di-(2-ethylhexyl), di-*n*-octyl, diisononyl, and diisodecyl phthalate (BBP, DBP, DEHP, DnOP, DINP, and DIDP, respectively). In addition, 17 of the less commonly used phthalate esters recently were reviewed by CPSC staff: 1,2-Benzenedicarboxylic acid, dipentyl ester (Diamyl phthalate); 1,2-Benzenedicarboxylic acid, dihexyl ester; 1,2-Benzenedicarboxylic acid, dinonyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, didecyl ester; 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13-rich; 1,2-Benzenedicarboxylic acid, dinonyl ester; 1,2-Benzenedicarboxylic acid, di-C6-10 alkyl phthalates; 1,2-Benzenedicarboxylic acid, di-C7-9-branched and linear alkyl esters; 1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters; 1,2-Benzenedicarboxylic acid, diundecyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, 2,2-dimethyl-1-(1-methylethyl)-3-(2-methyl-1-oxopropoxy)propyl phenylmethyl ester; 1,2-Benzenedicarboxylic acid, diheptyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich; 1,2-Benzenedicarboxylic acid, heptyl undecyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, nonyl undecyl ester, branched and linear; 1,2-Benzenedicarboxylic acid, mixed decyl and hexyl and octyl diesters. Five sources of information were used by CPSC staff to determine which other phthalates require investigation: (1) a TOXNET database search for publications; (2) U.S. phthalate production and consumption estimates; (3) listing as a High Production Volume (HPV) chemical by the U.S. Environmental Protection Agency (EPA); (4) information on phthalates actively being monitored in biological media; and (5) hazard, exposure, or risk reports written by other agencies.

This document reviews the current toxicity datasets for two additional phthalate esters, plus one phthalate alternative. These are 1,2-benzenedicarboxylic acid, di-2-propenyl ester (diallyl phthalate), bis(2-methoxyethyl) phthalate (DMEP), and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB). This assessment was prepared from peer-reviewed literature, including review articles, and unpublished studies carried out by industry. The literature search was executed in October–November 2010, and included online databases, such as Toxnet (which indexes databases such as ChemIDPlus, DART, HSDB, and Toxline), TSCATS, and IPCS INCHEM. Toxicological reviews and robust summaries from groups such as the Australian government (National Industrial Chemicals Notification and Assessment Scheme, the European Chemicals Bureau (ECB), and the U.S. E.P.A.'s HPV program are referenced throughout this report.

The remainder of this report describes toxicity, use, and exposure data identified for each of the three compounds listed below in Table 1. Compounds are divided by section, and descriptions of data are followed by summary tables for each compound.

Table 1: Compounds Reviewed in this Document

Chemical Name	CAS Number	Molecular Formula	MW	Structure
1,2-Benzenedicarboxylic acid, di-2-propenyl ester (Diallyl phthalate)	131-17-9	$C_{14}H_{14}O_4$	*246.3*	
Bis(2-methoxyethyl) phthalate	117-82-8	$C_{14}H_{18}O_6$	*282.3*	
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	$C_{16}H_{30}O_4$	*286.4*	

1,2-BENZENEDICARBOXYLIC ACID, DI-2-PROPENYL ESTER (CAS # 131-17-9)

1,2-Benzenedicarboxylic acid, di-2-propenyl ester, commonly known as diallyl phthalate or DAP, is a linear *ortho*-phthalate diester comprising two three-carbon backbones. Diallyl phthalate is manufactured commercially, using allyl alcohol with free phthalic acids, acid anhydrides, or acid chlorides in the presence of an aromatic sulfonic acid catalyst. Alternatively, diallyl phthalate can be made from the reaction of allyl chloride and phthalic anhydride in the presence of sodium hydroxide, sodium carbonate, tertiary amines, or quaternary ammonium salts (HSDB 2010). The major use of this phthalate is as a cross-linking agent during the manufacture of other polymers like polyvinyl chloride (PVC). These polymers are used for finished consumer products, such as window frames, insulating varnish for coil and wire, and sheet molded compound for ship bodies. In addition, DAP commonly is used as a reactive plasticizer; and unlike other phthalate plasticizers, it can bond covalently to the polymer matrix, after the curing process, to produce rigid thermoset plastics. DAP is consumed during this process and is not typically used to plasticize the final product (OECD 2004).

Approximately one-half of the DAP produced is used as a monomer to form the DAP prepolymer (OECD 2004). DAP prepolymer is used for impregnated paper-decorated particle boards for wall materials or furniture, printing in UV curable ink, grindstone, coil bobbin, and hot stamping foil. The residual DAP (<2% by weight in) in the prepolymer bonds covalently to be incorporated into the polymer matrix during completion of the polymerization to produce finished products.

The U.S. EPA's Inventory Update Report (IUR) listed U.S. production/importation volume of diallyl phthalate to be between 50 million and 100 million pounds in 1986, and 1to10 million pounds in the surveys conducted every four years from 1990–2006 (U.S. EPA 2006). Because its production exceeds one million pounds per year, diallyl phthalate qualifies as a High Production Volume material according to the EPA.

Exposure

At workplaces where diallyl phthalate is used or produced, occupational exposure may occur through inhalation of aerosols or dermal contact (HSDB 2010). In the NOES 1981–1983 survey, NIOSH estimated that 8784 workers (2027 of which were female) were exposed to diallyl phthalate. In a Japanese survey of occupational exposure, workers were found to be exposed to $\leq$0.11 mg/m^3 DAP during manufacture of DAP and 0.02-0.96 mg/m^3 during manufacture of the polymer (OECD 2004). These are below the UK, German, Irish, and New Zealand Occupational Exposure Limit, Time-Weighted Average (OEL-TWA) value of 5 mg/m.^{3}

The general population may be exposed via dermal contact with consumer products containing this compound (HSDB 2010). However, because DAP is a chemical intermediate, exposure to consumers is anticipated to be low, because little residual DAP remains in the final consumer product. DAP prepolymer exposure is also expected to be low, because it is incorporated into the polymer matrix of the consumer product and is not likely to be released. A study reported by

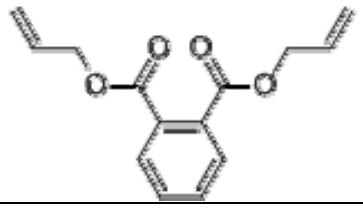
the OECD (2004) showed that the DAP-emission rate from decorative laminate boards (DAISO DAP®) was less than 0.011 $\mu g/m^2$/hour (DAISO 2003).

The OECD (2004) reported on a Japanese survey of DAP concentrations ranging from 7.1 to 134.5 ng/m^3 inside houses in Japan. In contrast, no DAP was detected in the indoor air of 45 rooms in 23 houses, and 12 offices in 12 office buildings in Japan between 1999 and 2001. Particulate DAP in four Japanese houses ranged from below the limit of detection (1.7 ng/m^3) to 6.2 ng/m.[3] In gaseous form, levels ranging from <1.7 ng/m^3 (the limit of detection) to 12.5 ng/m^3 were reported, with the highest level found in a new home.

Physicochemical Properties

Some physical and chemical properties of diallyl phthalate are summarized below in Table 2.

Table 2: Physicochemical Properties of Diallyl Phthalate

Identification	**Information**
Chemical Name	Diallyl phthalate
Synonyms	1,2-Benzenedicarboxylic acid, di-2-propenyl ester; Allyl phthalate; Dapon 35; Dapon R; Di-2-propenyl 1,2-benzenedicarboxylate; Phthalic acid, diallyl ester; o-Phthalic acid, diallyl ester; 1,2-Benzenedicarboxylic acid, 1,2-di-2-propen-1-yl ester
CAS Number	131-17-9
Structure	
Chemical Formula	$C_{14}H_{14}O_4$
Molecular Weight	246.261
Physical State	Liquid
Color	Colorless
Melting Point	-70 ° C
Boiling Point	158-165° C @ 4 mm Hg
Vapor Pressure	1.16E-03 mm Hg @25°C
Water Solubility	182 mg/L @20°C
Log K_{ow}	3.23
Flashpoint	166°C
Source: HSDB 2010	

Toxicokinetics

Absorption
DAP is well absorbed in mice and rats and, like other phthalates, depends on the hydrolysis of the diester (Eigenberg et al. 1986).

Distribution
Eigenberg et al. (1986) administered intravenous (iv) doses of 10 mg/kg ^{14}C-labeled DAP to Fischer 344 rats and B6C3F1 mice. Blood was sampled at early time points in rats (5, 10, 15, 20, and 30 minutes post-dosing) and mice (5, 10, and 15 minutes). Animals were sacrificed at 0.5, 1, 2, 4, 8, 12, or 24 hours after dosing; and samples were taken of urine, blood, intestinal contents, brain, lung, liver, kidney, spleen, testes, small intestine, thigh muscle, and abdominal skin. Results showed that DAP cleared rapidly from the blood of both species, with a half-life of approximately two minutes. No DAP was detected in the blood, liver, kidney, muscle, skin, or small intestine 30 minutes after dosing. In rats, 6 to 7 percent of the total ^{14}C remained in tissue 24 hours after iv administration, and in mice 1–3 percent remained. The highest levels of radioactivity were found in the small intestine, liver, dermis, muscles, blood, and kidneys.

Metabolism
The metabolic pathway suggested for DAP begins with hydrolysis of the diester to monoallyl phthalate (MAP) and allyl alcohol (Bingham et al. 2001). The half-life of DAP following iv administration is two minutes (Eigenberg et al. 1986). Allyl alcohol can be oxidized to acrolein and acrylic acid and further metabolized to CO_2. Allyl alcohol and acrolein can also react with reduced glutathione to form 3-hydroxypropylmercapturic acid, or they can be oxidized to the epoxides glycidol and glycidaldehyde. These epoxides, in turn, can be hydrolyzed to glycerin and glyceraldehyde or conjugated with reduced glutathione (Bingham et al. 2001).

MAP, allyl alcohol, 3-hydroxypropylmercapturic acid (HPMA), and an unidentified polar metabolite were detected in the urine of both rats and mice who had received gavage doses of DAP (see Excretion section below) (Eigenberg et al. 1986). The polar metabolite was thought to be a metabolite of allyl alcohol because it was also detected when animals were fed allyl alcohol by itself. MAP had a half-life in blood of 32, and 9 minutes in rats and mice, respectively. No MAP was detected in blood, liver, kidney, skin, muscle, or small intestine within four hours of dosing rats, and two hours of dosing mice. Compared to rats, mice excreted more MAP (39 vs. 33%), 3-hydroxypropylmercapturic acid (28 vs. 17%), and polar metabolite (20 vs. 8%), but the same quantity of allyl alcohol. These results, coupled with those from an acute dose-response study described in the Acute Toxicity section below, indicate that the toxicity of DAP results from its metabolite, allyl alcohol, and the extent of DAP toxicity is related to the amount of glutathione conjugation with allyl alcohol or acrolein, an active metabolite of allyl alcohol (Eigenberg et al. 1986).

Excretion
Eigenberg et al. (1986) administered ^{14}C-labeled diallyl phthalate to Fischer 344 rats and B6C3F1 mice via 1-mL gavage doses of 1, 10, or 100 mg/kg body weight (bw). Urine, feces, volatile metabolites, and $^{14}CO_2$ were collected for the next 24 hours. At the end of this time, animals were sacrificed, and selected tissues were removed for analysis. In mice, 8 percent of the ^{14}C was excreted as carbon dioxide, and 91 percent was excreted in the urine. Rats excreted

30 percent of the radioactivity as CO_2, and 60 percent appeared in the urine. The route of elimination, as a percentage of administered dose, was not altered significantly by the dose level of DAP in rats. However, in mice, the quantity of $^{14}CO_2$ excreted significantly was higher at the highest dose. Study authors determined that the $^{14}CO_2$ excreted by mice and rats after dosing was a product of free allyl alcohol oxidation.

Irritation and Sensitization

Undiluted DAP was applied to abraded and intact sites on six female New Zealand white rabbits and covered with an occlusive dressing for 24 hours. The animals were examined 24 and 72 hours after removal of the chemical. Some slight irritation was noted in the study, but DAP is not classifiable as a skin irritant (OECD 2004). Another primary irritation study showed that 0.5 grams of DAP was a moderate skin irritant on intact and abraded rabbit skin (score of 2.29 out of 8.0) (FMC Corporation 1978).

Irritation of DAP was tested on the intact skin of six male albino rabbits (sex and compound purity not specified) (Ethyl Corporation 1979). DAP doses of 0.5 mL were applied to shaved skin and covered with gauze and a rubber dam material to prevent evaporation. After four hours, skin was examined and washed, then examined again after 48 hours. DAP was classified as not irritating under these conditions.

In ocular irritation testing, DAP rated 1 on a scale of 1–10 in rabbit eyes after a 24–hour exposure (HSDB 2010). The Ethyl Corporation (1979) performed a Draize eye test in which 0.1mL of undiluted DAP was instilled into the right eye of 6 rabbits according to FSHA 16 CFR 1500. The animals were examined after one and four hours and then daily on days 1, 2, 3, 4, and 7. Examination failed to show any ocular redness or chemosis in any rabbits. Two other studies of rabbits produced similar results (OECD 2004). Therefore, DAP is not considered an eye irritant.

In a mouse local lymph node assay, groups of CBA/Ca female mice received 25 µL topical applications of DAP solution on the surface of the ear at concentrations of 0, 0.5, 5, or 50 percent (w/v) on three consecutive days. The Stimulation Index was 3.23 at 5 percent (w/v) and 10.74 at 50 percent (w/v). Therefore, DAP was considered to be a skin sensitizer. There were no clinical signs of toxicity during the study (OECD 2004).

No further data on ocular, dermal, or respiratory irritation or sensitization of diallyl phthalate were identified in the relevant literature.

Acute Toxicity In Vivo

Acute toxicity data for DAP are summarized below in Table 3.

Oral

Oral LD_{50} values of 656–896 mg diallyl phthalate/kg bw have been established in rats (ChemIDPlus 2010, FMC Corporation 1978, NTP 1985). FMC Corporation (1978) reported an oral LD_{50} value of 896 $^{+}$/-202 mg/kg bw for 99percent-pure DAP in Wistar rats. In a National

Toxicology Program study (NTP 1985), Fischer 344 rats (5 animals/dose) were dosed by gavage with DAP at 464-1470 mg/kg bw (male) or 316-1470 mg/kg bw (females). All high-dose animals (1470 mg/kg bw) died during the study after experiencing diarrhea, inactivity, hunched posture, hyperpnoea, and watery oral and nasal secretions. At necropsy, hemorrhagic lesions were apparent in the urinary bladder, and the lungs appeared dark in animals receiving the highest dose. These clinical signs occurred less frequently at the next lowest dose (1000 mg/kg bw); however, darkened lungs still were frequent at this dose and at some of the lower doses (681 and 464 mg/kg bw). Also, at 1000 mg/kg bw, there was fluid in the thoracic cavity, and the intestines appeared to be reddened in two females that died early. The LD_{50} was 891 mg/kg bw in males and 656 mg/kg bw in females.

In rabbits, an oral LD_{50} of 1700 mg/kg bw was determined (no further details available) (ChemIDPlus 2010). In B6C3F1 mice, five animals/sex/dose were administered 681-2150 mg/kg bw (males) or 1000-3160 mg/kg bw (females) (NTP 1983). There were no deaths in the group of females receiving the lowest dose, but all other dose groups had at least one death. At necropsy, no evidence of treatment-related lesions was observed. The LD_{50} for male mice was 1070 mg/kg bw and 1690 mg/kg bw for female mice.

Three male and two female dogs received a single oral dose of 800 mg/kg bw, equivalent to the rat oral LD_{50} (FMC Corporation 1989a). DAP quickly elicited vomiting in all five dogs. Two dogs died, one from a pulmonary embolism and one from severe gastrointestinal bleeding and possible jaundice. The three surviving animals showed signs of hepatotoxicity based on elevated plasma concentrations of aspartate aminotransferase, alanine aminotransferase (ALT), and serum alkaline phosphatase (SAP). Elevated SAP indicated possible obstructive jaundice and intrahepatic cholestasis. Based on hematology results and urinalysis, dogs returned to normal within one week. The LD_{50} value was considered to be ca. 800 mg/kg bw.

Eigenberg et al. (1986) exposed Fischer 344 rats and B6C3F1 mice orally to a single dose of 300–900 mg/kg bw DAP or 25–200 mg/kg bw allyl alcohol, a metabolite of DAP thought to be responsible for its toxicity. After 24 hours, ALT activity in the blood was analyzed, and livers underwent histopathological analysis. Rats receiving 300 mg/kg bw DAP experienced periportal inflammation, and at 400-900 mg/kg bw, 80 percent of rats developed periportal hemorrhagic and coagulative necrosis. ALT levels were elevated in the plasma of rats receiving 400–600 mg/kg bw, and in mice only at the highest dose of 900 mg/kg bw. Two of four mice receiving this dose also showed signs of periportal necrosis. In other dose groups, no effects of DAP on mice were noted. Allyl alcohol was also associated with periportal necrosis and elevated ALT activity in both species, the severity increasing with dose. Mice given 100 mg/kg bw or more allyl alcohol did not survive; while lower doses did not cause any hepatotoxicity or mortality. Nine of twelve rats across the range of allyl alcohol doses had periportal necrosis. Taken together, these data suggest that the toxicity of DAP results from its metabolite, allyl alcohol. Furthermore, authors postulated that the differential toxicity of DAP is related to the extent of glutathione conjugation with allyl alcohol or its active metabolite acrolein (Eigenberg et al. 1986).

Dermal
Ten rabbits/dose (sex and strain not identified) received 200, 2000, or 5000 mg/kg bw DAP (FMC Corporation 1978). Two weeks following application, there were deaths in all three groups (3, 4, and 6 deaths, respectively). The LD_{50} was reported to be 3300 mg/kg bw, which was in agreement with other acute dermal toxicity studies (OECD 2004).

In rabbits, a subcutaneous LD_{Lo} of 1000 mg/kg was determined (ChemIDPlus 2010). Clinical observations included changes in lungs, thorax, and respiration, and diffuse hepatocellular necrosis.

The corrosiveness of DAP was tested on the intact skin of six male albino rabbits (sex, strain, and compound purity not specified) (Ethyl Corporation 1979). DAP doses of 0.5 mL were applied to shaved skin and covered with gauze and a rubber dam material to prevent evaporation. After four hours, skin was examined and washed, then examined again after 48hours. Skin corrosion was not evident under the conditions of this assay.

Inhalation
Male and female Sprague-Dawley rats (5/sex/dose) received whole body exposure to aerosol doses of 940, 3090, 5390, 6660, 8080, 9170, or 9710 mg/m^3 DAP for one hour under FIFRA guidelines (FMC Corporation 1982). Clinical observations included salivation, crusty eye, nose, and muzzle, damp fur, poor coat quality, irregular breathing, and yellow/brown stained fur. At necropsy, abnormalities were noted in the stomach, spleen, large intestines, kidney, and eyes among the test rats. Study authors determined a combined sex LC_{50} of 8300 mg/m,^{3} a male LC_{50} value of 10,310 mg/m^3 and 5200 mg/m^3 for females.

In another acute inhalation study, 10 Sprague-Dawley rats were exposed to a nominal concentration of 67,200 mg/m^3 DAP for four hours (FMC Corporation 1980). The mean airborne concentration was 4,470 mg/m.^{3} Exposure produced immediate signs of irritation, discoloration of the nasal turbinates and lungs, and 100 percent mortality on Day 1.

Table 3: Acute Toxicity of DAP

Route	Species (Strain)	Endpoint	Dose	Reference
Oral	Mouse (B6C3F1)	LD_{50}	1070 mg/kg (M); 1690 mg/kg (F)	NTP 1983
	Rat (Fischer 344)	LD_{50}	891 mg/kg (M); 656 mg/kg (F)	NTP 1985
	Rat (Wistar)	LD_{50}	896 mg/kg (Combined)	FMC Corp. 1978
	Dog	LD_{50}	~800 mg/kg (Combined)	FMC Corp. 1989a
	Rabbit	LD_{50}	1700 mg/kg	ChemIDPlus 2010
Dermal	Rabbit	LD_{50}	3300 mg/kg	FMC Corp. 1978
	Rabbit	LD_{50}	3360 mg/kg	OECD 2004
	Rabbit	LD_{Lo}	3140 mg/kg	OECD 2004
	Rabbit	LD_{50}	3808 mg/kg	OECD 2004
Subcutaneous	Rabbit	LD_{Lo}	1000 mg/kg	ChemIDPlus 2010
Inhalation	Rat (Sprague-Dawley)	LC_{50}	8300 mg/m^3 (Combined); 10,310 mg/ m^3 (M); 5200 mg/ m^3 (F)	FMC Corp. 1982
	Rat (Sprague-Dawley)	LC_{100}	4470 mg/m^3	FMC Corp. 1980

In Vitro Toxicity

Lagente et al. (1979) measured the effect of phthalate ester exposure on lecithin/cholesterol acyltransferase activity in human cells *in vitro*. Diallyl phthalate concentrations ranging from 1–25 μM reduced enzyme activity. At 5 μM, inhibition was between 40 and 59 percent. Enzyme activity decreased in a dose-dependent manner and then leveled off. Further details were not included. Comparing results across phthalates, there appeared to be a trend where enzyme inhibition decreased with increasing phthalate molecular weight.

In a study of the human mammary carcinoma cell line (MCF-7), DAP, along with six other phthalate compounds (dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-n-octyl phthalate (DnOP), dinonyl phthalate (DNP), diisodecyl phthalate (DIDP), and ditridecyl phthalate (DTP)) were evaluated for cell proliferation potency/efficiency. DAP's proliferative effect was 80 percent that of 17β-estradiol's, and was considered insignificant. In this assay, only BBP and DBP showed significant relative proliferative effect. None of the phthalates demonstrated significant relative proliferative potency compared to estradiol. The competitive inhibition of these seven phthalate compounds versus estradiol was also evaluated in this study. The relative binding affinity (RBA) for the human estrogen receptor was insignificant for DAP (0.00002 %) compared to estradiol (RBA: 100%) (Kim and Ryu 2006). However, in another *in vitro* study, isomers of diallyl phthalate were able to displace E2 in a dose-dependent manner from an estrogen receptor expressed on the Sf9 vaculovirus (Nakai et al. 1999). Still, compared to estradiol, DAP was active only weakly.

Ekwall et al. (1982) tested the cytotoxicity of DAP to HeLa cells in the previously-validated Metabolic Inhibition Test (MIT-24) system. After a 24-hour exposure, there was partial cell inhibition at 11 mg/mL and total inhibition at 55 mg/mL. After seven days, partial and total inhibition was obtained at 11 and 25 mg/mL, respectively. The seven-day IC_{50} was determined to be 7.5 mg/mL, approximately equivalent to dibutyl phthalate, diethyl phthalate, and dimethyl phthalate.

Repeat Dose Toxicity

An *in vivo* uterotrophic assay was carried out on four phthalates: DAP, BBP, DBP, and DTP. Doses of estradiol (0.3, 3, or 30 μg/kg), or phthalate (20, 200, or 2000 mg/kg bw), were administered to Sprague-Dawley rats subcutaneously. Estradiol significantly increased uterine wet weights at each dose level, while none of the four phthalates induced an increase in uterine wet weight at any given dose (Kim and Ryu 2006). These results suggest that DAP is not estrogenic and that the estrogenic activity measured *in vitro* by Nakai et al. (1999) may not be relevant to the action of DAP in phthalate-exposed humans

The NTP (1985) performed a 14-day and a 13-week study of DAP using F344/N rats. In the first, five rats/sex/dose received 0, 50, 100, 200, 400, or 600 mg/kg DAP by gavage for 14 consecutive days. No histology was performed for this study. All rats in the high-dose group and 4/10 rats died in the 400 mg/kg group died. These animals showed macroscopic abnormalities of the lungs and stomachs. At 200 mg/kg, enlarged cecums and spleens were

observed in males and females. This effect persisted in males at doses down to 50 mg/kg. At 200 mg/kg and higher, the liver appeared abnormal: enlarged, dark, mottled, and yellowish-spotted. Less severe mottling was also seen on livers of males at 50 and 100 mg/kg doses.

Based on results of the 14-day study, male and female Fischer 344 rats (10/sex/dose) received gavage doses of 0, 25, 50, 100, 200, or 400 mg/kg bw/day for five days/week for 13 weeks (NTP 1985). At the high dose, 8 out of 10 male rats either died or were humanely sacrificed during the study. Body weight gain in this group was depressed 12 percent relative to that of the vehicle controls and clinical signs such as diarrhea, rough hair coat, or alopecia around the head, hunched posture, and general emaciation were observed. These clinical signs were less frequent, but still present, at 200 mg/kg bw/day in both sexes.

The eight rats that died early showed gross liver abnormalities with enlarged, mottled and pale, rough, granular or pitted surfaces. In most rats, darkened or bright red lungs were found. The two surviving high-dose males, most high-dose females, and half of the 200 mg/kg/day males had enlarged livers with mottled, yellow blotches on the surface, and were pale, rough, granular, or pitted. The severity was dose-related and more prevalent in males than in females. Periportal lesions of hepatic lobules, necrosis, fibrosis, bile duct hyperplasia, and hepatocellular hyperplasia occurred in males and females at 200 and 400 mg/kg bw/day. Necrosis, fibrosis, and biliary hyperplasia were observed at the two top doses, whereas hepatocellular alterations in the periportal region were observed at doses as low as 50 mg/kg bw/day in males and 100 mg/kg bw/day in females. Livers from the lowest dose group (25 mg/kg bw/day) were not examined based on the presence of only minimal hepatic changes at the next highest dose.

Acute necrotizing colitis characterized by loss of surface and glandular epithelium, varying degrees of mucosal and submucosal edema, and acute inflammatory cell infiltration were found in 7 out of 8 high-dose early death males. In addition, three of these male rats exhibited multifocal renal cortical tubular necrosis. Greenish-brown-kidney was observed in females at 400 mg/kg bw/day. The NOAEL in this study was 50 mg/kg bw/day for females based on the liver changes described above. The NOAEL and LOAEL for males were not determined because no histopathological examination of the liver at 25 mg/kg bw/day was performed.

The NTP (1983) also performed 14-day and a 13-week studies of DAP in B6C3F1 mice. In the shorter study, five mice/dose/sex were administered 0, 50, 100, 200, 400 or 600 mg/kg bw/day. Mortality occurred in the two highest dose groups. No other treatment related effects were noted, including mean body weight gain changes or the appearance of lesions. In the 13-week study 10 mice/dose/sex received DAP by gavage at 0, 25, 50, 100, 200, or 400 mg/kg bw five days/week. Mortalities were not associated with DAP treatment. No statistically significant effects of DAP were found in this study. The NOAEL for males and females was 400 mg/kg bw/day.

The OECD screening information dataset on diallyl phthalate (OECD 2004) reported a reproductive/developmental toxicity screening test performed by the DAP Consortium (2004) under OECD test guideline 421, which included systemic effects that will be discussed in this section. Sprague-Dawley rats (10 /dose/sex) receiving doses of 0, 16.7, 50, or 150 mg/kg bw/day DAP by gavage for up to 54 days showed liver effects at the high dose, including periportal

hepatocellular fibrosis, necrosis, enlargement and basophilia, and bile duct proliferation. Stomach ulcerations were also noted, although this was thought to be a portal of entry effect, rather than a true treatment-related effect. The systemic NOAEL was determined to be 50 mg/kg bw/day in both males and females based on the liver effects.

Reproductive and Developmental Toxicity

Reproductive

In the reproductive/developmental toxicity screening test described in the previous section, male and female Sprague-Dawley rats were gavaged with 16.7, 50, or 150 mg/kg bw/day DAP from 14 days prior to mating to four days post-partum. Three females in the high-dose group died or were killed *in extremis,* compared to no deaths at the other doses. The three presented with signs of stress near parturition, like pilo-erection, pallor of the extremities, abdominal discomfort, and vaginal bleeding, which indicated dystocia, as well as the histological finding presented above. DAP produced no significant effects on fertility of male or female rats as measured by pregnancy rate and distribution of precoital intervals. As noted above, only 7out of 10 high-dose females lived to give normal parturition. No effects of DAP on number of corpora lutea, implantations, or live births were discerned, although effects on newborns and live newborns were not evaluated in the three rats that died or were killed. There were no treatment-related effects on offspring viability, growth and development from conception to early lactation, or morphology. The NOAEL for reproductive effects in the study was 50 mg/kg/day based on dystocia, and 150 mg/kg/day for developmental effects. The parental/systemic NOAEL was also 50 mg/kg/day based on adverse liver changes.

Developmental

Pregnant Sprague-Dawley rats received 0, 100, 150, 200, or 250 mg/kg/day DAP by gavage (5ml/kg) on Gestational Days (GD) 6-20 (Saillenfait et al. 2008). Body weights and food consumption were recorded regularly throughout. On GD 21, females were killed and necropsied; uterine contents and fetuses were examined. Maternal weight gain and food consumption were reduced at the top two doses at varying time points. One dam from the high-dose group died on GD 21 for unknown reasons. Necropsy revealed liver lesions in dams receiving 150 mg/kg/day DAP and above. The maternal NOAEL was 100 mg/kg/day based on these results. There was no significant increase in the incidence of resorptions or malformations at any dose, nor was there teratogenicity or an apparent effect on fertility. However, DAP did cause fetal toxicity at doses that also produced maternal effects. Fetal body weight was reduced significantly at 200 and 250 mg/kg/day. At the highest dose, there was a significant increase in the percent of litters and fetuses with skeletal variations and the mean percent of fetuses per litter with skeletal variations. Significantly delayed bone ossification (e.g. in forelimb and hindlimb phalanges, metatarsals, caudal vertebrae) was also noted at 250 mg/kg/day. At the second highest dose, there was a significant increase in the percent of fetuses with skeletal variations, although specific variations were not significantly different from controls, and no dose-dependent trend was observed. The developmental NOAEL was 150 mg/kg/day based on fetal body weight changes and increased incidence of fetal skeletal variations.

Genotoxicity

In vitro

Diallyl phthalate has been tested for mutagenicity in a number of independent bacterial cell assays. Results were largely negative (Zeiger et al. 1985; Ethyl Corp. 1979; FMC Corporation 1977; Seed 1982; Sato et al. 1994), but included some reproducible positive results as well. Zeiger et al. (1985) did not observe a significant increase in reverse histidine mutations in the presence or absence of metabolic activation in *Salmonella typhimurium* tester strains TA98**,** TA100, TA1535, and TA1537 at DAP concentrations up to 10,000 µg/plate (purity = 98.9%). In another set of assays performed under OECD guidelines 471 and 472, five strains of *S. typhimurium* and one strain of *Escherichia coli* were exposed to DAP at 0, 1.22, 4.88, 19.5, 78.1, 313, 1250, or 5000 µg/plate, both with and without exogenous metabolic activation (Japan MOL 2000, cited in OECD 2004). Cytotoxicity was observed at the two highest doses for *S. typhimurium* strains in the presence of exogenous metabolic activation, and the three highest doses in the absence of metabolic activation. DAP was weakly mutagenic in *E. coli* strain WP2uvrA/pKM101 with metabolic activation. The other non-cytotoxic doses were not mutagenic. In another Ames assay, a 1 percent solution of DAP in dioxane was tested for point mutations at concentrations of 0-100 µL/plate with and without metabolic activation. *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were not mutagenic under the conditions of this assay (FMC Corporation 1977). The same authors tested DAP (purity >98%) for mutagenicity at concentrations of 50–1000 µg/plate with activation and 150–6000 µg/plate without (FMC Corporation 1989c). A reproducible positive response was observed in tester strain TA1535 in the absence of metabolic activation. In the other strains of *S. typhimurium* (TA98, TA100, TA1537, and TA1538), no positive responses were observed either with or without added activation, and DAP was not cytotoxic.

Sato et al. (1994) determined that DAP did not enhance the mutagenicity of the genotoxin 3-amino-1-methyl-5H-pyrido-[4,3-b]indole to *S. typhimurium* tester strain TA98.

Compared to bacterial reverse mutation, results in mammalian cells more clearly pointed to a conclusion of mutagenicity. In the mouse lymphoma assay, mutagenic responses were noted in the absence of metabolic activation at doses of 60 µg/mL and higher and in the presence of metabolic activation at doses as low as 12.5 µg/mL (Myhr and Caspary 1991). DAP concentrations of 200–300 µg/mL weakly but consistently induced chromosomal aberrations in Chinese Hamster Ovary (CHO) cells *in vitro* in the presence of metabolic activation (Gulati et al. 1989). No significant increase in chromosomal aberrations was observed in the absence of activation at DAP concentrations of 50–500 µg/mL (Gulati et al. 1989). A similar pattern of results was obtained with respect to metabolic activation in the sister chromatid exchange assay in CHO cells. Positive results were obtained when metabolic activation was present at higher ($\geq$160 µg/mL) DAP concentrations. Finally, DAP induced the formation of micronuclei in Chinese Hamster Liver (CHL) IU cells on short-term treatment with 11 µg/mL and higher in the presence of metabolic activation. Continuous treatment with DAP did not have this effect in the absence of activation (OECD 2004).

In vivo

In a sex-linked recessive lethal (SLRL) assay, no increase in these mutations was seen in *Drosophila melanogaster* following oral DAP doses of 100 or 140 ppm (Valencia et al. 1985).

B6C3F1 mice were administered 0, 43.8, 87.5, or 175 mg/kg/day DAP for three consecutive days. There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated mice compared to the control group (Shelby et al. 1993). In a chromosome aberration assay, B6C3F1 mice received a single IP injection of 0, 75, 150, or 300 mg/kg DAP (Shelby and Witt 1995). There was a significant increase in the percentage of cells with chromosomal aberrations at the highest dose group in one of two trials. The dose response trend in this study was weakly statistically significant in both trials. Overall, evidence for the clastogenicity of diallyl phthalate is equivocal.

Carcinogenicity

Groups of 50 B6C3F1 mice per sex per dose received gavage doses of 0, 150, and 300 mg/kg bw/day DAP 5 days/week for 103 weeks, followed by a three-week, post-exposure period (NTP 1983). Because survival, mean body weights, and pathological lesions unrelated to proliferative liver changes were not observed, the critical carcinogenic dose may not have been achieved in this study. A dose response for forestomach chronic inflammation and hyperplasia was observed in both sexes, but these were most likely portal-of-entry effects, rather than true treatment-related results. Similarly, the development of squamous cell papillomas in the forestomach may have been an effect of test substance administration, but data were insufficient to determine the true nature of this relationship. An increase in lymphomas was observed in treated male mice (control: 12%; low: 10%; high: 24%), but the incidence was not significantly greater than typical historical controls. There was a statistically significant positive trend in the incidence of male mice with hepatocellular adenomas (control: 0%; low: 0%; high: 6%), but no pairwise comparison between dosed-groups, and control was significantly different. Similarly, a negative trend (control: 8%; low: 4%; high: 0%), but no statistically different pairwise comparisons were observed in female mice with endometrial stromal polyps. Results of this two year assay do not provide evidence that DAP is carcinogenic to mice.

Fischer 344 rats (50/sex/dose) were gavaged with 0, 50, or 100 mg/kg bw/day DAP 5 days/week for 103 weeks, followed by a three-week, post-exposure period (NTP 1985). There was no statistically significant difference in body weights or survival in treated versus control rats throughout the study. High-dose rats of both sexes developed chronic liver disease, manifesting as periportal fibrosis, periportal accumulation of pigment, and severe bile duct hyperplasia. Pigment accumulation also occurred at the low dose in both sexes. DAP produced a statistically significant increase in the incidence of mononuclear cell leukemia by trend, and in female rats in the high-dose group compared to controls (control: 15/50; low dose: 15/43; high dose: 25/49). However, because the incidence of mononuclear cell leukemia in control-aged Fischer 344 rats is variable (10–72%; Caldwell 1999) and difficult to definitively diagnose, the authors considered the results to be equivocal evidence of carcinogenicity in female rats. No increase in mononuclear cell leukemia or other potential evidence of carcinogenicity was observed in male rats.

References

Bingham, E., B. Cohrssen, and C.H. Powell. 2001. Patty's Toxicology Volumes 1–9 5th ed. John Wiley & Sons. New York, N.Y. p. V6 865.

Caldwell, D.J. 1999. Review of mononuclear cell leukemia in F-344 rat bioassays and its significance to human cancer risk: A case study using alkyl phthalates**.** Regulatory toxicol pharmacol. 30(1): 45–53.

ChemIDPlus. 2010. Entry for diallyl phthalate. Accessed November, 2010. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM

Ekwall, B., C. Nordensten, and L. Albanus. 1982. Toxicity of 29 plasticizers to HeLa cells in the MIT-24 system. Toxicol. 24 (3–4).

Ethyl Corporation. 1979. Skin Corrosion Test in Rabbits Using Ethyl Corporation Compound Diallyl Phthalate (4-hr Exposure) (Final Report) with Attachments and Cover Letter Dated 060989. EPA/OTS Document No. 86-890000411, Fiche No. OTS0520458 [TSCATS].

FMC Corporation. 1977. DAP Monomer Mutagenicity Screening Test Salmonella Microsomal Assay & Activity of C-8013-3 in the Salmonella/Microsomal Assay for Bacterial Mutagenicty with Cover Letter 081789. EPA/OTS; Doc #86-890001479 [TSCATS].

FMC Corporation. 1978. Acute Oral and Dermal Toxicity and Eye and Skin Irritation of Diallyl Phthalate Monomer with Cover Letter Dated 061289. EPA Doc. No. 86-890000332 and 86-890001475 [TSCATS].

FMC Corporation. 1980. An acute inhalation toxicity study of compound "D" in the rat with attachments and cover letter dated 081789, EPA/OTS; Doc #86-890001477 [TSCATS].

FMC Corporation. 1982. One-hour acute aerosol inhalation toxicity study in rats of 180-439 with attachments and cover letter dated 081789. EPA/OTS; Doc #86-890001478 [TSCATS]

FMC Corporation. 1989a. Acute oral toxicity of diallyl phthalate monomer in dogs at a dose of 800 mg/kg (rat LD50 dose) with cover letter dated 081789. EPA/OTS; Doc #86-890001474 [TSCATS]

FMC Corporation. 1986. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with Cover Sheet and Letter Dated 081789. EPA Doc. No. 86-890001480, Fiche No. OTS0521094. [TSCATS].

Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986, p. 1021.

Gulati, D., Witt, K., Anderson, B., Zeiger, E., and Shelby, M. 1989. Chromosome aberration and sister chromatid exchange test in Chinese hamster ovary cells *in vitro* III: Results with 27 chemicals Environ. Molec. Mutagen. 13:133–193. Cited in NTP 2010.

Hazardous Substances Database (HSDB). 2010. Entry for diallyl phthalate. Accessed November, 2010. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Lagente, M., F. de la Farge, and P. Valdiguie. 1979. The influence of phthalate esters on human plasma. Lipids 14(6): 533–4.

Myhr, B. and Caspary, W. 1991. Chemical mutagenesis at the TK locus in L5178Y mouse lymphoma cells. I. Results for 31 coded compounds in the National Toxicology Program. Environ. Molec. Mutagen. 18: 51–83. Cited in NTP 2010.

Nakai M, Y. Tabira, D. Asai, Y. Yakabe, T. Shimyozu, M. Noguchi, M. Takatsuki, and Y. Shimohigashi. 1999. Binding characteristics of dialkyl phthalates for the estrogen receptor. Biochem Biophys Res Commun. 254(2): 311-4.

National Toxicology Program (NTP). 1983. Carcinogenesis bioassay of diallyl phthalate (CAS No.131-17-9) in B6C3F1 Mice (gavage study), National Toxicology Program Technical Report, TR 242.

National Toxicology Program (NTP). 1985. Toxicology and carcinogenesis studies of diallylphthalate (CAS No. 131-17-9) in F344/N rats (gavage studies), National Toxicology Program Technical Report, TR 284.

Organization for Economic Cooperation and Development (OECD). 2004. Screening Information Dataset (SIDS) initial assessment report for diallyl phthalate. Available: www.chem.unep.ch/irptc/sids/oecdsids/131179.pdf.

Saillenfait, A.M., F. Gallissot, and J.P. Sabaté. 2008. Evaluation of the developmental toxicity of diallyl phthalate administered orally to rats. Food Chem Toxicol. 46(6):2150-6.

Sato, M., H. Nagase, K. Sato, M. Niikawa, and H. Kito. 1994. Enhancement of the mutagenicity of amino acid pyrolysates by phthalate esters. Environmental and Molecular Mutagenesis. 24(4): 325–31.

Seed, J.L. 1982. Mutagenic Activity of Phthalate Esters in Bacterial Liquid Suspension Assays. Environ. Health Perspect. 45: 111–114.

Shelby, M.D. and K.L.Witt. 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests, Environ Mol Mutagen, **25** (4), 302–13.

Shelby, M.D., G.L. Erexson, G.J. Hook, and R.R. Tice. 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol, results with 49 chemicals. Environ Mol Mutagen, 21 (2), 160–79.

U.S. Environmental Protection Agency (U.S. EPA). 2006. Inventory Update Reporting (IUR) data for 2006. Available: http://www.epa.gov/iur/tools/data/index.html

Valencia, R., Mason, J.M., Woodruff, R.C., and Zimmering, S. 1985. Chemical mutagenesis testing in Drosophila: III. Results of 48 coded compounds tested for the National Toxicology Program. Environ. Mutagen. 7: 325–348.

Zeiger, E., Haworth, S., Mortelmans, K., and Speck, W. Mutagenicity testing of Di(2-ethylhexyl)phthalate and related chemicals in Salmonella Environ. Mutagen. Vol. 7 (1985) 213–232.

Table 4: Summary of Toxicity Data for 1,2-Benzenedicarboxylic Acid, Di-2-propenyl Ester (Diallyl Phthalate; CAS #131-17-9)							
Organ/ System	**Model**	**Exposure Route**	**Dose**	**Dose Duration**	**Endpoint**	**Effect**	**Reference**
Systemic	F344 rats	Gavage	0. 50, 100, 200, 400, 600 mg/kg bw/day	14 days	NOAEL = 50 mg/kg/day (F); undetermined (M) LOAEL = 100 mg/kg/day (F); 50 mg/kg/day (M)	Liver, lung, stomach, cecum, spleen	NTP 1985
Systemic	F344 rats	Gavage	0, 25, 50, 100, 200, 400 mg/kg bw/day	5 days/week for 13 weeks	NOAEL = 50 mg/kg/day (F); undetermined (M) LOAEL = 100 mg/kg/day (F); 50 mg/kg/day (M)	Periportal lesions of hepatic lobules, necrosis, fibrosis, bile duct hyperplasia, hepatocellular hyperplasia, multifocal renal cortical tubular necrosis	NTP 1985
Systemic	Sprague Dawley rats	Gavage	0, 16.7, 50, 150 mg/kg bw/day	$\leq$54 days	NOAEL= 50 mg/kg/day (M,F)	Periportal hepatocyte necrosis, enlargement and basophilia, bile duct proliferation and periportal fibrosis, stomach ulceration	DAP Consortium, cited in OECD 2004
Systemic	B6C3F1 mice	Gavage	0. 50, 100, 200, 400, 600 mg/kg bw/day	14 days	NOAEL= 200 mg/kg/day (M,F) LOAEL= 400 mg/kg/day (M,F)	Death at 400, 600 mg/kg; absence of chemical induced lesions at necropsy	NTP 1983
Systemic	B6C3F1 mice	Gavage	0, 25, 50, 100, 200, 400mg/kg bw/day	5 days/week for 13 weeks	NOAEL= 400 mg/kg/day (M,F)	None	NTP 1983
Reproductive/ Developmental	Sprague-Dawley rats	Gavage	0, 16.7, 50, 150 mg/kg bw/day	14 days pre-mating through PND 4	$NOAEL_{reproductive}$ = 50 mg/kg/day $LOAEL_{reproductive}$ = 150 mg/kg/day $NOAEL_{developmental}$ = 150 mg/kg/day	Dystocia and death of 3/10 females at 150 mg/kg	DAP Consortium, cited in OECD 2004

Developmental	Sprague-Dawley rats	Gavage	0, 100, 150, 200, 250 mg/kg bw/day	GD 6-20	$NOAEL_{developmental}$ = 150 mg/kg/day $LOAEL_{developmental}$ = 200 mg/kg/day $NOAEL_{maternal}$ = 100 mg/kg/day	Developmental effects: At 200 mg/kg and higher, ↓ fetal body weight. At 250 mg/kg, ↑ percent of litters and fetuses with skeletal variations; delayed bone ossification (forelimb and hindlimb phalanges, metatarsals, caudal vertebrae). Maternal effects: At 150 mg/kg and higher, liver lesions and body weight changes	Saillenfait et al. 2008
Carcinogenicity	B6C3F1 mice	Gavage	0, 150 300 mg/kg bw/day	5 days/week for 103 weeks + 3 weeks recovery	No evidence of carcinogenicity in either sex	Significant positive trend in incidence of hepatocellular adenomas (M) and negative trend endometrial polyps (F)	NTP 1983
Carcinogenicity	Fisher 344 rats	Gavage	0, 50, 100 mg/kg bw/day	5 days/week for 103 weeks + 3 weeks recovery	At 100 mg/kg/day, equivocal evidence of carcinogenicity in females	↑ incidence mononuclear cell leukemia with dose; At 100 mg/kg, periportal fibrosis, periportal accumulation of pigment, severe bile duct hyperplasia (M,F); ↑ incidence mononuclear cell leukemia (F) compared to controls, but not compared to historical controls At 50 mg/kg, periportal pigment accumulation (M,F)	NTP 1985

BIS(METHOXYETHYL) PHTHALATE (CAS #117-82-8)

Bis(methoxyethyl) phthalate (CAS #117-82-8), commonly called dimethoxyethyl phthalate or DMEP, is manufactured in a closed reactor (Eastman Kodak 1991a) by esterification using ethylene glycol monomethyl ether and phthalic anhydride (HSDB 2010). DMEP is used as a plasticizer for cellulosic resins, some vinyl ester resins, PVC, and as a solvent, a molding component, and in adhesives, laminating cements, and flash bulb lacquers. In Italy, dimethoxyethyl phthalate is permitted for use with food.

U. S. production of DMEP was estimated to be greater than 5000 pounds in 1977 and 1979 (HSDB 2010). The U.S. EPA's Inventory Update Report (IUR) lists U.S. production/importation volume of DMEP to be between 500,000 and 1,000,000 pounds in 1986, and 10,000 to 500,000 pounds in the surveys conducted every four years from 1990–1998 (U.S. EPA 2002). After 1998, DMEP production was no longer tracked by IUR.

Exposure

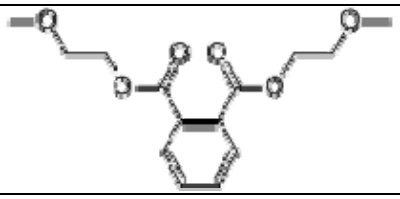
According to the main manufacturer of DMEP, the potential for occupation exposure is low because it is made in a closed reactor (Eastman Kodak 1991a). The airborne concentrations of DMEP in this facility were reported to be below the limit of detection of 0.001-0.005 ppm. Wipe sampling tests confirmed that employee exposure was low. As of 1991, DMEP was not sold commercially and was present in final articles only at very low concentrations.

Physicochemical Properties

Some physical and chemical properties of DMEP are summarized below in Table 4.

Table 5: Physicochemical Properties of Bis(methoxyethyl) Phthalate (DMEP)

Identification	Information
Chemical Name	Bis(methoxyethyl) phthalate
Synonyms	DMEP; Dimethoxyethyl phthalate; 1,2-Benzenedicarboxylic acid, bis(2-methoxyethyl) ester; 2-Methoxyethyl phthalate; Bis(2-methoxyethyl) phthalate; Bis(methoxyethyl) phthalate; Di(2-methoxyethyl) phthalate; Dimethoxyethyl phthalate; Dimethyl cellosolve phthalate; Dimethylglycol phthalate; Kesscoflex MCP; Kodaflex; Methyl glycol phthalate; Phthalic acid, bis(2-methoxyethyl) ester; Phthalic acid, di(methoxyethyl) ester
CAS Number	117-82-8

Structure	
Chemical Formula	$C_{14}H_{18}O_6$
Molecular Weight	282.29
Physical State	Oily liquid
Color	Colorless
Melting Point	-45°C
Boiling Point	340°C
Vapor Pressure	$2.28x10^{-4}$ mm Hg @25°C
Water Solubility	8500 mg/L (temperature not specified)
Log K_{ow}	1.11
Flashpoint	410°C
Source: HSDB 2010	

Toxicokinetics

Dimethoxyethyl phthalate (20 mL/kg bw) applied to the depilated abdomen of guinea pigs showed no evidence of percutaneous absorption after 24 hours (Eastman Kodak 1984). Based on an *in vitro* assay, DMEP is predicted to absorb very slowly into human skin, with a steady state absorption rate of 8 μg/cm^2/hour (Eastman Kodak 1991a).

The metabolic pathway for dimethoxyethyl phthalate begins with its hydrolysis to monomethoxyethyl phthalate (MMEP) and 2-methoxyethanol (2-ME). 2-ME is then oxidized to methoxyacetic acid (MAA) (Ritter et al. 1985). This pathway is depicted below in Figure 2.

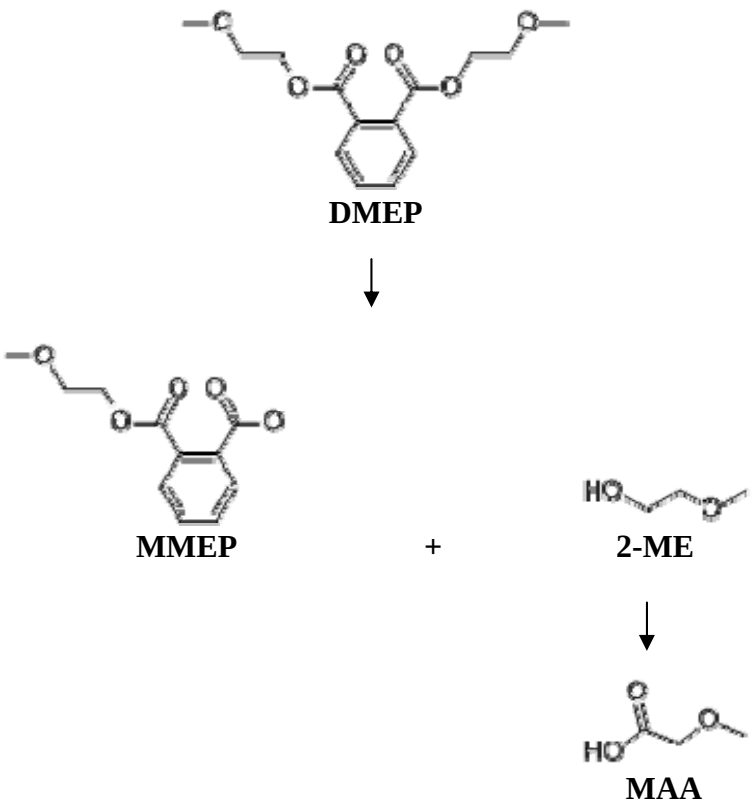


Figure 1: Metabolic pathway of DMEP

DMEP metabolism has been studied in pregnant rats. Following an intraperitoneal (ip) injection of 0.6 mL/kg bw ^{14}C-DMEP to pregnant Wistar rats on gestation day (GD) 13, there was a rapid transfer of the unmetabolized DMEP across the placenta to the fetus (Parkhie et al. 1982). In the rat fetus, the initial hydrolysis to MMEP does not take place to any significant extent, although MMEP is present in the fetus shortly after the dam is dosed with DMEP, presumably a result of transfer from the placenta (Campbell et al. 1984). No other phthalate metabolites were formed in, or transported to, the fetus.

Following an ip dose of 2.49 mmol/kg (702 mg/kg bw) of ^{14}C-labeled DMEP or MMEP to pregnant Wistar-Porton rats on GD 14, total phthalate concentration in placenta and fetus was maximal after 30 to 60 minutes (Campbell et al. 1984). Clearance was rapid; DMEP and MMEP concentrations decreased by 96 percent in the fetus between 45 minutes and four hours of administration to the dam. Likewise, the *in vitro* hydrolysis of DMEP to MMEP was complete within two minutes in maternal liver homogenates and four hours in maternal placenta homogenates.

Irritation and Sensitization

Dermal exposure of undiluted DMEP to rabbit and guinea pig skin caused slight skin irritation (HSDB 2010). DMEP (purity 78%) caused slight skin irritation when applied for 24 hours to

depilated guinea pig abdomen under an occlusive wrap at doses up to 20 mL/kg bw (Eastman Kodak 1984). Irritation was not exacerbated after nine repetitions of a 0.5 mL exposure to the clipped backs of guinea pigs. A 0.2 mL intradermal injection of 100 mg/mL DMEP into the clean-shaven back of rabbits produced a moderate inflammatory response over 26 minutes (Calley et al. 1966).

Undiluted DMEP produced a significant degree of irritation to mice when injected intradermally, but produced mild or no ophthalmic irritation in the rabbit eye test (Lawrence et al. 1975; HSDB 2010). Another ocular irritation test gave a very slight irritation response in unwashed rabbit eyes only (Eastman Kodak 1984). Because compounds inducing skin irritation intradermally are usually also associated with ophthalmic irritation, the results of the intradermal mouse test are ambiguous.

No sensitization was observed after a standard sensitization procedure in 10 guinea pigs (Eastman Kodak 1984). No further details of this study were available.

Acute and Subacute Toxicity

Acute toxicity data are summarized below in Table 6. A single oral dose toxicity study of DMEP was conducted with male and female rats (Eastman Kodak 1984). The oral LD_{50} was reported to be greater than 3200 mg/kg bw in both male and female rats with clinical effects limited to slight to moderate weakness in both sexes. In another acute study, none of six adult Wistar rats, ingesting 2000 mg/kg bw died, but at 2500 and 2750 mg/kg bw DMEP, 3 out of 12 and 5 out of 10 rats died, respectively, within 36 hours of ingestion (HSDB 2010).

In an acute dermal toxicity study, guinea pigs received dermally administered DMEP (purity 78%) using the cuff technique. There was no evidence of percutaneous absorption, only slight, transient erythema and edema. The dermal LD_{50} was reported to be greater than the highest dose of 20 mL/kg (equivalent to 23,460 mg/kg bw) (Eastman Kodak 1984).

Intraperitoneal LD_{50} values in mice range from 2510 to 4180 mg/kg bw (Lawrence et al. 1975; Calley et al. 1966). Singh et al. (1972) derived an ip LD_{50} of 3.7355 mL/kg bw (4382 mg/kg bw) in Sprague-Dawley rats.

Table 6: Acute Toxicity of Bis(methoxyethyl) Phthalate (DMEP)

Route	Species (Strain)	Endpoint	Dose	Reference
Oral	Rat (unspecified)	LD_{50}	>3200 mg/kg (M,F)	Eastman Kodak 1984
	Rat (Wistar)	LD_{50}	2750 mg/kg	HSDB 2010
Intraperitoneal	Rat (Sprague Dawley)	LD_{50}	4382 mg/kg	Singh et al. 1972
	Mice (ICR)	LD_{50}	2510 mg/kg	Lawrence et al. 1975
	Mice (Swiss Webster)	LD_{50}	4180 mg/kg	Calley et al. 1966
Dermal	Guinea pigs	LD_{50}	>23,460 mg/kg	Eastman Kodak 1984

Calley et al. (1966) ran a battery of acute and subacute tests on various phthalates, including DMEP exploring a number of endpoints. A single ip dose of 500 mg/kg bw DMEP produced no effect on central nervous system activity in Swiss Webster mice (n=10), as measured by hexobarbital-induced sleeping time. However, when repeated doses of 50 mg/kg bw DMEP (emulsified in a 3% acacia solution) were administered intravenously into the heart of anesthetized rabbits, there was no apparent effect on blood pressure, but a temporary increase in respiration rate was noted. The electroencephalogram pattern indicated minor CNS depression (Calley et al. 1966). Similarly, male ICR mice (10/dose) receiving ip injections of 418, 836, or 2090 mg/kg bw (equivalent to 0.1, 0.2, or 0.5 of the 3.57 mL/kg bw LD_{50}, respectively) for three consecutive days, followed by an ip injection of pentobarbital, experienced a dose-dependent reduction in sleep induction time significant at all three doses, and a dose-dependent increase in sleeping duration significant at the highest dose (Lawrence et al. 1975). Study authors posited that the phthalate-induced increase in sleeping time was related to DMEP's ability to impede metabolism or excretion of pentobarbital, or to increase the central nervous system sensitivity to the sleeping agent.

In Vitro Toxicity

A 50 mg/mL emulsion of DMEP produced no toxic effect on chick embryos (Calley et al. 1966). However, some cell death was noted in strain L929 mouse fibroblast cells after 48 hours of exposure. No further information was given on these two cellular toxicity studies.

Repeat Dose Toxicity

DMEP was found to reduce significantly relative testes weight in mice at ip doses of 250 mg/kg/day given for six weeks (Calley et al. 1966). No other relative organ weights (liver, heart, lungs, kidney, or spleen) were affected, nor was hematology. Acute peritonitis, possibly an effect of chemical administration, periportal hepatitis, and extramedullary hematopoiesis in the liver and spleen were also observed.

Five male rats per dose (strain not reported) were exposed by gavage to DMEP (purity 78%) at dose levels of 0, 100, or 1000 mg/kg/day for 12 treatments over the course of 16 days (Eastman Kodak 1984). Compared to controls, rats receiving 1000 mg/kg/day showed statistically significant decreases in absolute liver and kidney weights, absolute and relative thymus and testes weights, and absolute red and white blood cell counts. Levels of hemoglobin and hematocrit were reduced at both dose levels. High-dose rats also had statistically higher relative kidney weights, minor to severe thymic atrophy, moderate to severe testicular atrophy, and accessory sex organ and adipose tissue atrophy. Four of five low-dose rats had minimal to minor thymic medulla hemorrhage. The histopathology exam showed atrophied seminiferous tubules, degeneration of the sperm in the seminiferous tubules and epididymides, and the presence of giant spermatids in high-dose rats. The systemic LOAEL was determined to be 100 mg/kg/day based on hematological changes and thymus damage. A NOAEL could not be established for systemic changes.

Reproductive/Developmental Toxicity

Reproductive Toxicity
As described under the Repeat Dose Toxicity section, male rats receiving 12 gavage doses of 1000 mg/kg/day had reduced absolute and relative testes weights, moderate to severe testicular atrophy, accessory sex organ atrophy, atrophied seminiferous tubules, degeneration of the sperm in the seminiferous tubules and epididymides, and giant spermatids (Eastman Kodak 1984). Based on these effects, the reproductive NOAEL was determined to be 100 mg/kg bw/day.

Ten-week-old male Wistar rats (5/treated group, 20/control) received single gavage doses of 1000, 1500, or 2000 mg/kg bw DMEP (Cassidy et al. 1983). Rats were necropsied on the eleventh day following dosing, and testes were removed for analysis. There was a statistically significant dose-related decrease in mean testes weights and an increase in the percentage of abnormal sperm heads (straight, excessively curved, folded, coiled, thin, or amorphous) at the top two doses. Rats receiving 2000 mg/kg/day had significantly more sperm heads per gram of testis than control rats. No treatment-related changes were observed with respect to clinical signs or gross appearance of the testes. A NOAEL of 1000 mg/kg/day for reproductive effects can be established based on the decreased testes weight and abnormal sperm at the next highest dose.

Developmental Toxicity of DMEP and its Metabolites
The metabolic basis of DMEP teratogenicity was examined in several *in vitro* and *in vivo* studies. The metabolic pathway for dimethoxyethyl phthalate begins with its hydrolysis to monomethoxyethyl phthalate (MMEP) and 2-methoxyethanol (2-ME). 2-ME is then oxidized to methoxyacetic acid (MAA) (Ritter et al.1985). Yonemoto et al. (1984) examined the *in vitro* toxicity of DMEP, MMEP, 2-ME, and MAA. Cultured rat embryos at the somite stage (embryonic age 9.5 days) showed no signs of embryotoxicity following 46-hour exposure to 5 mM DMEP, MMEP, or 2-ME. On the other hand, MAA concentrations of 2–5 mM significantly decreased head length and morphological scores, and 3–5 mM MAA decreased somite number and crown-rump length. At 5mM, a reduction in the yolk sac diameter was seen, and its vasculature appeared disorganized. The fusion of the neural tube was also irregular, and open neural tubes and stunted telencephalic hemispheres were noted in these embryos. No significant difference in developmental parameters was observed at an exposure level of 1 mM MAA compared to controls.

Campbell et al. (1984) administered a single ip dose of 2.49 mmol/kg bw DMEP, MMEP, or 2-ME to pregnant Wistar rats on GD 8, 10, 12, or 14. Doses were equivalent to approximately 702 mg/kg bw DMEP, 558 mg/kg bw MMEP, and 189 mg/kg bw 2-ME. Rats were sacrificed on GD 20. DMEP and 2-ME induced similar teratogenic and fetotoxic responses, most notably when given on GD 8, when they caused three-to fourfold increases in the number of dead or resorbed fetuses as compared to controls. Survival was higher following treatment on GD 10 or later, but most survivors were malformed, especially in the kidneys and bladders. MMEP was not teratogenic under the conditions of this study.

Ritter et al. (1985) further explored the *in vivo* teratogenicity of DMEP and its metabolites. On GD 12, pregnant Wistar rats received an intraperitoneal (i.p.) dose of 1.03, 2.07, or 4.14 mmol/kg DMEP; an ip or gavage dose of 2.07 or 4.14 mmol/kg 2-ME; or a gavage dose of 2.07

or 4.14 mmol/kg MAA. In some rats receiving 2-ME, a concurrent ip dose of the alcohol dehydrogenase inhibitor 4-methylpyrazole (4-MP) was given. Pregnancy was terminated on GD 20. The number of implants and litters, the percent dead/resorbed embryos, and the percent of malformed fetuses were evaluated and translated into an overall total embryotoxicity, equivalent to the sum of dead, resorbed, and malformed fetuses as a percent of total implantations. The percent total embryotoxicity was statistically greater in all treatment groups than in the control group. DMEP, 2-ME, and MAA all caused a statistically significant, dose-related increase in total embryotoxicity. There was no effect of dosing route on embryotoxicity of 2-ME. Molar equivalent doses of DMEP, 2-ME, and MAA each gave rise to statistically similar degrees of embryotoxicity, suggesting a common mechanism of action or metabolite in all three compounds. In addition, fetotoxicity associated with the three compounds manifested in similar and unusual abnormalities: hydronephrosis, unusual heart defects, short limbs and tails, and ventral polydactyly. However, rats receiving 4-MP in addition to 2-ME showed significantly lower embryotoxicity (16.8 versus 100 percent) than when 2-ME was given by itself. Because 4-MP interferes with the oxidation of 2-ME to MAA, and because MAA itself is a known potent teratogen, it is reasonable to conclude that MAA, or one of its metabolic products, is the proximate teratogen in DMEP toxicity (Ritter et al. 1985).

Developmental Toxicity of DMEP

Pregnant Wistar rats received single ip injections of 0.6 mL/kg bw (702 mg/kg bw) ^{14}C-DMEP on GD 10, 11, 12, 13, or 14, the period of organogenesis, and were sacrificed on GD 20 (Parkhie et al. 1982). ^{14}C-DMEP exposure was associated with a high incidence of fetal deaths and resorptions, most markedly when exposure took place at earlier gestational times. The mean weight of living fetuses was significantly lower in treated animals than controls, regardless of exposure day. Fetotoxicity was pronounced, with greater effects associated with exposure on earlier gestational days (10 or 11) versus later. Specific toxic effects included a significant reduction in fetal weights, and an increase in hydrocephalus interna, a congenital brain deformity, congenital skeletal malformations, and skeletal and appendicular brain malformations.

Pregnant female Sprague-Dawley rats (5/dose) received ip doses approximately equivalent to 418, 1392, or 2089 mg/kg bw DMEP (corresponding to 1/10, 1/3, or 1/2 of the LD_{50}, respectively) on GD 5, 10, and 15 (Singh et al. 1972). An untreated control group was included. On GD 20, rats were sacrificed and fetuses examined for toxicity. All three doses of DMEP produced significant resorptions: 96.5, 89.7, and 27.6 percent in the high, medium, and low doses, respectively. The combined percentage of fetal deaths, plus resorptions was 98.2, 96.6, and 56.9 percent for the three doses in descending order. Average fetal weights also were reduced significantly in all dose groups compared to controls ($p \leq 0.01$). Gross abnormalities were observed in 100, 83.3, and 2.4 percent of the fetuses in the high-, medium-, and low-dose groups, respectively, and included absence of tail or eyes, anophthalmia, twisted hind legs, and hematomas. Skeletal malformations were seen in all high- and medium-dose fetuses and 92.9 percent of low-dose fetuses. The most common skeletal malformations were elongated and fused ribs (bilateral and unilateral), absence of tail bone, abnormal or incomplete skull bones, and incomplete or missing leg bones. Of the eight phthalate compounds tested in this study (including dimethyl, diethyl, dibutyl, diisobutyl, butyl carbobutoxymethyl, dioctyl and di-(2-ethylhexyl) phthalates), DMEP was the most toxic to embryos and fetuses (Singh et al. 1972).

Because there were reproductive and developmental effects at all dose levels, a NOAEL could not be established for these endpoints.

Pregnant Sprague-Dawley rats (10/dose) received gavage doses of 60, 180, or 600 mg/kg bw/day DMEP on GD 6–15 (Eastman Kodak 1991b). Pup survival and weight gain were monitored through postnatal day (PND) 5. Maternal body weight gain was reduced significantly at the high dose, as was feed consumption on certain days. Clinical signs of maternal toxicity were evident at this dose. Although the number of implantation sites in high- dose animals compared to control animals was not significantly different, none of the high-dose dams delivered a litter. At the middle dose, all dams but one delivered viable litters. Four pups from three of the nine litters contained pups with external malformations, including shortened lumbosacral region, absent or filamentous tail. At birth and from PND 1–5, pup survival was reduced significantly, and body weight gain for surviving pups was less than that of controls. In the low-dose group, pup survival was slightly but significantly reduced during PND 1–5, as was mean body weight gain. The NOAEL for maternal effects was 180 mg/kg bw. A developmental NOAEL could not be established because there were effects at the lowest dose.

Genotoxicity

In vitro

DMEP was tested for mutagenicity in the Ames reverse mutation assay in *Salmonella typhimurium* tester strains TA98 and TA100 at concentrations up to 10,000 μg/plate with and without metabolic activation (NTP 1993). The test compound did not cause a significant increase in reverse histidine mutations in the presence of metabolic activation. With no activation, positive results were obtained in strain TA98.

In vivo

The genotoxicity of DMEP was assessed in the dominant lethal assay (Singh et al. 1974). The calculation of the dominant lethal effect is based on a comparison of the live implants per female in the treated group to the live implants per female in the control group, reflecting the post-implantation loss. Ten male Harlan/ICR Swiss mice per dose received 1392, 2089, or 2785 mg/kg bw DMEP (approximately equivalent to one-third, one-half, and two-thirds, respectively, of the 3.57 mL/kg bw LD_{50}) via ip injection. Males were then mated with untreated females in sequential mating intervals. Between gestational days (GD) 13 and 17, pregnant mice were sacrificed and necropsied. There was no relationship between the dose of DMEP received by males and late fetal deaths. The percent of pregnant dams, implants per pregnancy, early fetal deaths per pregnancy, and litter size per pregnancy showed an overall dose-related trend. The high dose of DMEP statistically reduced the incidence of pregnancies and the number of implants per pregnancy compared to the control group, indicating a dominant lethal effect at this dose.

Carcinogenicity

No studies of DMEP's carcinogenicity were identified in a search of the literature.

References

Calley D., J. Autian, and W.L. Guess. 1966. Toxicology of a series of phthalate esters. J Pharmaceutical Sci 55(2): 158–162.

Campbell, J., D. Holt, and M. Webb. 1984. Dimethoxyethylphthalate metabolism: teratogenicity of the diester and its metabolites in the pregnant rat. J Appl Toxicol. 4(1):35–41.

Cassidy, S.L., Dix, K.M., and T. Jenkins. 1983. Evaluation of a testicular sperm head counting technique using rats exposed to dimethoxyethyl phthalate (DMEP), glycerol alpha-monochlorohydrin (GMCH), epichlorohydrin (ECH), formaldehyde (FA), or methyl methanesulphonate (MMS). Arch Toxicol. 53(1):71–8.

Eastman Kodak Company. 1984. Basic Toxicity of bis(2-methoxyethyl)phthalate. Acc No 902511; HAEL No 840080; with cover letter dated 031385. EPA Document No. FYI-OTS-0385-0329, Fiche No. OTS0000329-1 [TSCATS].

Eastman Kodak Company. 1991a. U.S. EPA Submission summary: dimethyoxyethyl phthalate with cover letter. EPA/OTS; Doc #8EHQ-0191-1170 [TSCATS].

Eastman Kodak Company. 1991b. Letter from Eastman Kodak Co to U.S. EPA submitting information on developmental toxicity screening study using dimethyoxyethyl phthalate. EPA/OTS; Doc #89-910000086 [TSCATS].

Hazardous Substances Database (HSDB). 2010. Entry for dimethoxyethyl phthalate. Accessed November, 2010. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Lawrence WH, Malik M, Turner JE, Singh AR, & Autian J (1975) Toxicology investigation of some acute short term and chronic effects of administering di-2-ethylhexyl phthalate (DEHP) and other phthalate esters. Environmental Research, 9(1): 1–11.

National Toxicology Program (NTP). 1993. Salmonella Study Overview A59190, Di(2-methoxyethyl)phthalate.

Parkhie, MR, Webb, M., Norcross, M.A. 1982. Dimethoxyethyl phthalate: embryopathy, teratogenicity, fetal metabolism and the role of zinc in the rat. Environ Health Perspect. 45:89–97.

Ritter, E.J., W.J. Scott Jr., J.L. Randall, and J.M. Ritter. 1985. Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxyethanol and methoxyacetic acid in the rat. Teratol. 32(1):25–31.

Singh, A.R., W.H. Lawrence and J. Autian. 1972. Teratogenicity of phthalate esters in rats. J. Pharmac Sci. 61(1): 51–55.

Singh, AR, WH Lawrence and J Autian. 1974. Mutagenic and antifertility sensitivities of mice to DEHP and dimethoxyethyl (DMEP) phthalates. Toxicol Appl Pharacol. 29: 35–46.

U.S. Environmental Protection Agency (U.S. EPA). 2002. Non-confidential production volume information submitted by companies for chemicals under the 1986-2002. Inventory Update Reporting (IUR). 1,2-Benzenedicarboxylic acid, bis(2-methoxyethyl) ester (117-82-8). Available: http://www.epa.gov/oppt/iur/tools/data/2002-vol.html.

Yonemoto, J., N.A. Brown, and M. Webb. 1984. Effects of dimethoxyethyl phthalate, monomethoxyethyl phthalate, 2-methoxyethanol and methoxyacetic acid on post implantation rat embryos in culture. Toxicol Lett. 21(1):97–102.

Table 7: Summary of Toxicity Data for Bis(methoxyethyl)phthalate (CAS #117-82-8)							
Organ/ System	**Model**	**Exposure Route**	**Dose**	**Dose Duration**	**Endpoint**	**Effect**	**Reference**
Systemic	Swiss Webster mice	Intraperitoneal	250 mg/kg/day emulsion	6 weeks	LOAEL = 250 mg/kg/day (only dose)	Increased relative testes weight, acute peritonitis, periportal hepatitis, extramedullary hematopoiesis in liver and spleen.	Calley et al. 1966
Systemic	Rats (male)	Gavage	0, 100, 1000 mg/kg/day 78% pure DMEP	12 doses in 16 days	No systemic NOAEL $LOAEL_{systemic}$ =100 mg/kg/day	At 1000 mg/kg/day: ↓ absolute liver, kidney weights, absolute and relative thymus and testes weights, ↑ relative kidney weights; ↓ red and white blood cell counts, hemoglobin and hematocrit levels; atrophy of thymic and adipose tissue At 100 mg/kg/day: ↓ hemoglobin and hematocrit levels; minor thymic medulla hemorrhage	Eastman Kodak 1984
Reproductive	Rats (male)	Gavage	0, 100, 1000 mg/kg/day 78% pure DMEP	12 doses in 16 days	NOAEL =100 mg/kg/day LOAEL =1000 mg/kg/day	At 1000 mg/kg/day: Atrophy of testes, accessory sex organs, and seminiferous tubules; degeneration of the sperm in seminiferous tubules and epididymides; giant spermatids	Eastman Kodak 1984
Reproductive	Wistar rats (10 week old males)	Gavage	0, 1000, 1500, 2000 mg/kg bw	Single dose + 11 days	NOAEL = 1000 mg/kg bw LOAEL = 1500 mg/kg bw	At 2000 mg/kg/day: ↑ #sperm heads/gram testis. At 1500 and 2000 mg/kg/day: dose-dependent ↓ in testes weight and ↑ abnormal sperm heads	Cassidy et al. 1983

Developmental (DMEP and metabolites)	9.5-day old rat embryos	*In vitro*	2-5 mM DMEP, MMEP, 2-ME, MAA	Cultured on GD 9, exposed GD9-11 (somite stage)	Implication of MAA as proximate teratogen *in vitro*	DMEP, MMEP, 2-ME: No effects on embryonic growth or development MAA: ↓ Head length, morphological scores, somite number and crown-rump length, yolk sac diameter; irregular neural tube development	Yonemoto et al. 1984
Developmental (DMEP and metabolites)	Wistar-Porton rats (pregnant)	Intraperitoneal	2.49 mmol/kg DMEP, MMEP, or 2-ME (= 702 mg/kg bw DMEP, 558 mg/kg bw MMEP, 189 mg/kg bw 2-ME)	Single dose on GD 8, 10, 12 or 14	Implication of 2-ME or one of its metabolites as proximate teratogen *in vivo*	DMEP and 2-ME: ↑ number of dead or resorbed fetuses, particularly when dosed on GD 8; ↑ malformations on all dosing days MMEP: not teratogenic	Campbell et al. 1984
Developmental (DMEP and metabolites)	Wistar rats (pregnant)	Gavage and/or intraperitoneal	1.03, 2.07, 4.14 mmol/kg DMEP; 2.07, 4.14 mmol/kg 2-ME +/- 4-MP; 2.07, 4.14 mmol/kg MAA	Single dose on GD 12	Implication of MAA or one of its metabolites as proximate teratogen *in vivo*	All treatment groups unless 4-MP present: Dose-related ↑ total embryotoxicity (e.g. hydronephrosis, unusual heart defects, short limbs and tails, and ventral polydactyly); no effect of dosing route; presence of 4-MP ameliorated embryotoxic effects of 2-ME	Ritter et al. 1985
Developmental	Wistar rats (pregnant)	Intraperitoneal	702 mg/kg bw	Single dose on GD 10, 11, 12, 13 or 14	LOAEL = 702 mg/kg bw (only dose)	High incidence of fetal deaths and resorptions; ↓ fetal weight; ↑ hydrocephalus interna, congenital skeletal malformations, skeletal and appendicular brain malformations; teratogenicity more pronounced at earlier exposure times	Parkhie et al. 1982

Developmental	Sprague-Dawley rats (pregnant)	Intraperitoneal	0, 418, 1392, or 2089 mg/kg bw	GD 5, 10, and 15	No developmental NOAEL could be established LOAEL = 418 mg/kg bw	All doses: significant resorptions, fetal deaths ; ↓ average fetal weights; ↑ gross abnormalities (e.g., absence of tail or eyes, anophthalmia, twisted hind legs, hematomas); ↑ skeletal malformations (e.g. elongated and fused ribs (bilateral and unilateral), absence of tail bone, abnormal or incomplete skull bones, incomplete or missing leg bones)	Singh et al. 1972
Developmental	Sprague-Dawley rats (pregnant)	Gavage	0, 60, 180, 600 mg/kg bw/day	GD 6-15	$NOAEL_{maternal}$ =180 mg/kg bw No developmental NOAEL could be established $LOAEL_{develop}$ = 60 mg/kg bw	Maternal: At 600 mg/kg, ↓ body weight gain and food consumption; clinical signs of toxicity. Developmental: At 600 mg/kg, 0% parturition. At180 mg/kg, ↓ survival at birth and PND 1-5; ↓ weight gain; 4 pups with external abnormalities. At 60 mg/kg ↓ survival PND 1-5; ↓ weight gain PND 1-5	Eastman Kodak 1991b

2,2,4-TRIMETHYL-1,3-PENTANEDIOL DIISOBUTYRATE (CAS #6846-50-0)

2,2,4-Trimethyl-1,3-pentanediol-diisobutyrate, commonly known as TXIB, is a non-phthalate, low-viscosity plasticizer, intended for use as a secondary plasticizer (DEPA 2010). TXIB has higher hardness (lower efficiency) than phthalates, higher volatility (though lower than dibutyl phthalate), and higher water extractability. According to Eastman Chemical Company (2006a, 2009), the primary manufacturer of TXIB, this additive is compatible with polyvinyl chloride (PVC) and with common primary and secondary plasticizers like dioctyl phthalate and dioctyl terephthalate. TXIB is commonly used in polyurethane elastomers to lower viscosity (Eastman Chemical 2006b). A potential financial advantage to using TXIB as a plasticizer is it can be used with fillers without compromising the viscosity of the plastisol.

TXIB can be found in apparel, weather stripper, furniture, wallpaper, nail care, plastisols, sheet vinyl flooring, toys/sporting goods, traffic cones, vinyl compounding, vinyl gloves, and as a diluent for methyl ethyl ketone peroxide formulations (Eastman Chemical 2002, 2010). The use of TXIB in vinyl flooring declined in the 1990s, due to high emissions from end products (DEPA 2010). TXIB is also used in inks, coatings, urethane elastomers, and water-based paints (DEPA 2010, Kim et al. 2007). In the Netherlands, 14 percent of toys and childcare articles surveyed in 2007, contained TXIB; and in Germany, Austria, and Switzerland, 11 percent of such products contained TXIB (DEPA 2010).

The production volume of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate in Japan was estimated at 1,200 tonnes/year in 1990–1993 (OECD 1995). No such estimates were located for production in the United States; however, TXIB is not considered a high production volume chemical by the U.S. EPA.

Exposure

The general population may be exposed to TXIB via inhalation in the presence of building materials containing this compound. Levels of TXIB and other volatile organic compounds were significantly higher in freshly painted or newly constructed buildings compared with unpainted or existing buildings (Wieslander et al. 1997; Kim et al. 2007). Emissions of TXIB also come from PVC materials and adhesives associated with flooring; the specific emission rate was found to be higher in the finished products than in the individual materials themselves (Järnström et al. 2008). TXIB-specific emission rates from PVC materials measured in the laboratory ranged from <1 to 13 $\mu g/m^2$/hour, while emission rates from PVC-covered floor structures ranged from <1 to 53 $\mu g/m^2$/hour.

Occupational exposure to the monoisobutyrate of 2,2,4-trimethyl-1,3-pentanediol has been noted in painters using water-based paints and was associated with self-reported asthma and other respiratory issues (Wieslander et al. 1994).

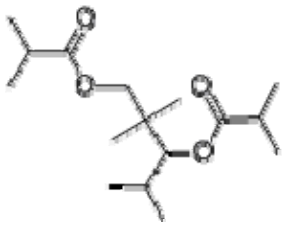
TXIB has been implicated in "sick building syndrome" based on sampling levels exceeding 10-100 $\mu g/m^3$ in the air of office buildings, other public buildings, and temporary housing (Rosell 1990; Norback et al. 1995; Maddalena et al. 2009). An average indoor air concentration of 1.64

$\mu g/m^3$ TXIB was reported in schools in Sweden and, along with other plasticizer compounds and microbial volatile organic compounds, was associated significantly with incidence of asthma, respiratory symptoms, and allergies in children ($p < 0.05$)(Kim et al. 2007). However, a study finding the threshold for sensory irritation to be about 500 ppb (v/v) (Cain et al. 2005), suggests that reports of irritation at low levels may be better attributed to simultaneous exposure to multiple chemicals in indoor air.

Physicochemical Properties

Some physical and chemical properties of 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate are summarized below in Table 8.

Table 8: Physicochemical Properties of 2,2,4-Trimethyl-1,3-pentanediol-Diisobutyrate

Identification	**Information**
Chemical Name	2,2,4-Trimethyl-1,3-pentanediol-diisobutyrate
Synonyms	2,2,4-Trimethylpentanediol-1,3-diisobutyrate; TMPD-DIB; Isobutyric acid, 1-isopropyl-2,2-dimethyltrimethylene ester; Kodaflex TXIB; TXIB; 1,3-Pentanediol, 2,2,4-trimethyl-, diisobutyrate (ester); 1-Isopropyl-2,2-dimethyltrimethylene diisobutyrate; Propanoic acid, 2-methyl-, 1,1'-(2,2-dimethyl-1-(1-methylethyl)-1,3-propanediyl) ester; Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(1-methylethyl)-1,3-propanediyl ester
CAS Number	6846-50-0
Structure	
Chemical Formula	$C_{16}H_{30}O_4$
Molecular Weight	286.42
Physical State	Liquid
Color	Colorless
Melting Point	-70°C
Boiling Point	280°C
Vapor Pressure	0.0009 @ 20°C
Water Solubility	1-2 mg/L @ 20.5°C
Log K_{ow}	>4.1 (DEPA 2010)
Flashpoint	128°C
Source: IUCLID 2000 (unless otherwise stated)	

Toxicokinetics

Absorption
The majority of an oral dose was absorbed quickly in Holtzman albino rats (Astill et al. 1972). An acetone extract of the feces 24 hours after a single oral dose of 475 mg/kg bw TXIB indicated that one-half to two-thirds of TXIB indeed had been absorbed by the animal. The percent of total oral doses of 236, 250, and 283 mg/kg bw ^{14}C-TXIB accounted for in urine and feces of rats was 98.9, 99.2, and 95.3 percent, respectively, after 7–10 days.

Distribution
Male Holtzman albino rats receiving single oral doses of radiolabeled TXIB (236–895 mg/kg bw) were sacrificed one-by-one 8, 15, and 22 days later (Astill et al. 1972, Eastman Chemical 2007). Liver, kidney, perirenal and omental fat, brain, lung, and carcass tissue were analyzed for radioactivity after sacrifice. After eight days of exposure, the carcass and organs combined accounted for 2.9 percent of the original dose, and at days 15 and 22, essentially no radioactivity remained (<1%).

Metabolism
After an oral dose of radiolabeled TXIB, the first metabolic step was hydrolysis to the monoisobutyrate of the parent glycol 2,2,4-trimethyl-1,2-pentanediol (TMPD), presumably in the gastrointestinal tract since a large proportion of the total dose detected in feces was this compound (Astill et al. 1972). A second hydrolysis takes place, transforming some of the dose into TMPD. Major urinary metabolites of TXIB detected three days after an oral dose of 255 mg/kg bw were free 2,2,4-trimethyl-3-hydroxyvaleric acid (11.6% of dose), as well as its glucuronide (3.1%) and its sulfate form (4.3%), and also TMPD in its glucuronide (6.5%), sulfate (1.8%), and free form (0.5%).

Forty-eight hours following an oral dose of TMPD (196 or 208 mg/kg bw), the major urinary metabolite in rats was the O-glucuronide of TMPD (72–73% of the dose); other compounds found in the urine were the sulfate (6.4–6.5%) and free (1–1.7%) forms of TMPD, and free 2,2,4-trimethyl-3-hydroxyvaleric acid (3%) and its glucuronide (4.3–4.4%).

Excretion
Three rats received single oral doses of radiolabeled TXIB (236, 250, or 283 mg/kg bw) and urine, feces and $^{14}CO_2$ were collected daily. The percentages of the total oral doses of 236, 250, and 283 mg/kg bw accounted for were 98.9 percent (after 10 days), 99.2 percent (after 10 days), and 95.3 percent (after seven days), respectively. Two-thirds of the total dose was excreted in 48hours, 90 percent in five days, and nearly 100 percent in 10 days. The major route (50–67% of the total dose) of elimination was urine. Fecal excretion accounted for 14–31 percent of the dose with the majority excreted within the first 48 hours; elimination was essentially complete in seven days. Radiolabeled CO_2 was not detected.

In rats administered 475 mg/kg unlabeled TXIB, fecal acetone extracts taken after 24 hours indicated 8–36 percent of the dose remained as TXIB, 18–27 percent was the monoester, and there were trace amounts of TMPD. In urine, concentrations were not quantified, but TXIB, TMPD, the monoester of TMPD, and conjugates of TMPD and 2,2,4-trimethyl-3-hydroxyvaleric acid were detected.

Oral doses of TMPD (196 and 208 mg/kg bw) were also eliminated rapidly and almost entirely in the urine of rats.

Irritation and Sensitization

TXIB was slightly irritating to the skin of guinea pigs when the skin was exposed uncovered, and more irritating when covered (Eastman Chemical 1962). The EC classification for skin response in this test was "not irritating." There was no evidence that TXIB was absorbed into the skin, but small skin flakes, desquamation, and little hair were visible after one week. After two weeks, desquamation and sparse hair persisted. No evidence of irritation was observed after a 24-hour occlusive patch exposure in rabbits performed under OECD test guideline 404 (Eastman Chemical 2007).

Human volunteers received dermal applications of 1 percent (v/v) TXIB under a semi-occlusive patch three times per week for three weeks (David et al. 2003). Following a two-week rest period, reactions to a challenge dermal exposure were observed. Slight erythema was noted in three subjects exposed to TXIB, one of which resolved after four days, and one that occurred only after four days. Researchers concluded that TXIB was not a sensitizer or skin irritant in humans. Likewise, guinea pigs receiving TXIB injections in the footpad, followed by a topical challenge, showed no signs of sensitization after 24 or 48 hours (Eastman Chemical 1961).

In ocular irritation testing performed under OECD Guideline 405, TXIB classified as not irritating. Each of six rabbits received a 0.1 mL dose of TXIB into one eye, while the other eye was washed with water, serving as control for each animal. Animal eyes were determined to be clinically normal after 24 hours. Therefore, TXIB was not considered an eye irritant (Eastman Chemical 1990).

Acute Toxicity

The acute oral LD_{50} of TXIB was reported to be >3200 mg/kg bw in rats and >6400 mg/kg bw in mice (Astill et al. 1972). LD_{50} values for rats and mice following intraperitoneal (ip) injection were 3200 and 6400 mg/kg bw, respectively. No mortality was observed in three rats after inhalation exposure of 0.12-5.3 mg/L TXIB for six hours and a two-week rest period (Eastman Chemical 1962). The LC_{50} value for TXIB, therefore, was determined to be greater than 5.3 mg/L. The dermal LD_{50} for TXIB in guinea pigs (Eastman Chemical 1962) and rabbits (Eastman Chemical 2007) was greater than 2000 mg/kg bw.

Repeat Dose Toxicity

Astill et al. (1972) reported on two repeat-dose studies on TXIB performed by Eastman Kodak Company. In the first, four beagle dogs/sex/group received dietary doses of 0.1, 0.35, or 1.0 percent TXIB by weight, six days per week for 13 weeks (approximately equivalent to 22, 77,

and 221 mg/kg bw/day for males and 26, 92, and 264 mg/kg/day for females)[2]. At the end of the study, animals were sacrificed and extensive gross, microscopic, and histopathological analyses were conducted. There was no mortality or evidence of neurological stimulation, depression, or reflex abnormality. Nor were there effects on growth or food consumption amongst treated dogs, and no changes were observed in the hematology, clinical chemistry, histopathology, or urine analyses. Relative organ weights were similar to control animals, except for the liver and pituitary gland in the two higher dose groups, which were increased slightly compared to controls. However, elevated pituitary gland weights were still within normal ranges, and the absence of microscopic pathological findings in both of these organs indicates that the observed weight change was not adverse. Therefore, Astill et al. (1972) interpreted these findings as having no toxicological significance. The NOEL for this studied was 0.1 percent, or 22–26 mg/kg/day, and the NOAEL was greater than 1 percent, or 221 and 264 mg/kg/day for male and female dogs, respectively.

The second study reported by Astill et al. (1972) was a feeding study in rats. Ten albino Holtzman rats per sex, per dose, received TXIB for 103 days in the diet at levels of 0.1 or 1.0 percent by weight (average doses equivalent to 75.5 and 772 mg/kg/day for males and 83.5 and 858.5 mg/kg/day for females).[3] Appropriate vehicle control groups were also run. At the end of the dosing period, rats were necropsied and tissues (esophagus, small and large intestine, liver, trachea, lung, thyroid, parathyroid, spleen, brain, heart, kidney, bladder, adrenal, gonad, and bone) were removed for histological examination. There were no significant differences between feed consumption and weight gain or growth between controls and treated rats. Relative liver weights in both sexes[4] and absolute liver weights in male rats were slightly significantly higher in high-dose rats compared with controls. These weights were still within the normal range of values, however. No other effects in organ weights or histology were noted. As with the dog study, Astill et al. (1972) considered the liver weight effects seen in this study to be without toxicological significance because they were not abnormally high. Study authors derived a NOAEL of 1.0 percent, or 772–858.5 mg/kg bw/day.

Krasavage et al. (1972) carried out three concurrent experiments that support the conclusions about liver weights drawn by Astill et al. (1972). Sprague-Dawley rats (10/sex/group) were fed 0, 0.1, or 1.0 percent TXIB by weight (approximately equivalent to 0, 147.5, and 1475 mg/kg/day).[5] Rats were fed this diet continuously for 52 days in experiment I, 99 days in experiment II, and for experiment III, rats either received the TXIB diet for 52 day followed by the control diet for 47 days, or they received control diet for 52 days followed by TXIB diet for 47 days. Following sacrifice, livers were collected for the analysis of activity of four microsomal enzymes: glucose-6-phosphatase (G-6-Pase), *p*-nitroanisole demethylase (*p*-NDase), UDP-*p*-aminophenol-beta-D-glucuronyl transferase (*p*-AG-Tase), and UDP-bilirubin-beta-D-

[2] Assuming a food intake of 0.4 kg food/day and a body weight of 15.5 kg (males) and 13 kg (females) for beagle dogs (U.S. EPA 1988). Example calculation: 0.4 kg food/15.5 kg bw/day * 6 days/7days * 1% TXIB = 221 mg TXIB/kg bw/day.

[3] Reported in Eastman Chemical 2007.

[4] Astill et al. (1972) reported that relative liver weights in females were significantly higher in the high-dose group. In Eastman Chemical's 2007 summary of this study, they note that the laboratory report did not report this result as significant and that the published manuscript contained this finding in error.

[5] Assuming an average food factor of 0.1475 kg food/ kg bw/day for Sprague-Dawley rats weighing 64–77 grams (U.S. EPA 1988).

glucuronyl transferase (BG-Tase). In addition, groups of six male rats received seven daily ip doses of 25 or 100 mg/kg bw TXIB or 2,2,4-trimethyl-1,3-pentanediol (TMPD), the parent glycol and a metabolite of TXIB in rats, followed by sacrifice one day after the last injection. Livers from these animals were collected for analysis of *p*-NDase and BG-Tase activity.

There was no significant treatment-related effect on mean body weight gain, group feed consumption, hematological parameters, alkaline phosphatase activity, tissue histology, or absolute organ weight in any group compared to controls (Krasavage et al. 1972). Serum glutamic oxaloacetic transaminase levels were elevated in all high-dose animals relative to controls, except for females in experiment I. However, elevated levels were still within normal ranges. The relative liver weights of male and female rats fed 1 percent TXIB were significantly greater than controls in all three experiments, except for experiment III rats fed TXIB first and control diet second. Differences in other relative organ weights were not determined to be treatment-related. Likewise, the only consistent finding with respect to microsomal enzymes was an increase in activity at the high-dose level, but only when the animal was consuming TXIB at the time of sacrifice (i.e., not in the experiment III rats that ate a control diet in the second part of the experiment). Repeat ip injections of TXIB and TMPD significantly increased P-NDase levels in male rats receiving the higher dose of either compound; BG-Tase levels were unaffected. A lower level of enzyme induction by TMPD suggests that TXIB is the active inducer, and not its metabolic product. Temporary liver weight increase and microsomal enzyme activity induction are responses frequently associated with stress. In the absence of hepatic damage, they can be interpreted as physiological adaptations, rather than a true chemical-induced toxic response.

OECD test guideline 422, the combined repeated dose and reproductive/developmental toxicity screening test, was performed on TXIB using male and female Sprague-Dawley rats (JMHLW 1993; OECD 1995; Eastman Chemical 2007). Rats (12/sex/dose) were administered gavage doses of 0, 30, 150 or 750 mg/kg/day TXIB (purity: 99.7%) starting 14 days before mating. Males continued receiving the test substance for 30 days thereafter, and females, through day three of lactation. At the high-dose level, depressed body weight gain (males) and increased food consumption (females) were observed. Rats receiving 150 or 750 mg/kg/day had higher levels of creatinine and total bilirubin, and high-dose males had higher total protein content in the blood, suggesting liver and kidney effects. Indeed, relative liver weights were higher for male rats receiving the two higher doses of TXIB, with discoloration and hepatocellular swelling and decreased fatty change at the highest dose. Absolute and relative kidney weights were elevated in high-dose males and basophilic changes in the renal tubular epithelium and degeneration of hyaline droplet were observed in male rats receiving 150 mg/kg/day or more. Additionally, necrosis and fibrosis of the proximal tubule and dilatation of the distal tubule were observed in male rats receiving 750 mg/kg/day. At the lowest dose only, there was a decrease in absolute but not relative thymus weight, which was not considered treatment-related. Eastman Chemical (2007) determined a NOEL for systemic toxicity of 30 mg/kg/day for males and 150 mg/kg/day for females. The NOAEL was determined to be 150 mg/kg/day based on the assertion that effects seen at 150 mg/kg/day were adaptive in nature.

Developmental and Reproductive Toxicity

In the combined repeated dose and reproductive/developmental toxicity screening test described in the repeat-dose section above, male and female Sprague-Dawley rats were administered gavage doses of 0, 30, 150, or 750 mg/kg/day TXIB from 14 days before mating until 30 days after (males) or day three of lactation (females) (JMHLW 1993; OECD 1995; Eastman Chemical 2007). TXIB had no significant effect on mating, fertility, the estrous cycle, delivery, or lactation period. Parameters evaluating developmental toxicity were limited to body weights at postnatal days (PND) 0 and 4, and autopsy findings at PND 4; these examinations revealed no TXIB-related effects at any dose. The reproductive and developmental NOAEL, therefore, is 750 mg/kg/day.

A reproductive/developmental toxicity screening test was performed by Eastman Chemical Company under OECD test guideline 421 (Eastman Chemical 2001). Sprague-Dawley rats (12/sex/dose) received dietary doses of 0, 120, 359, or 1135 mg/kg/day (females) or 0, 91, 276, or 905 mg/kg/day (males) for 14 days before mating, during mating (1–8 day), throughout gestation (21–23 days), and through PND 4–5. Significant reductions in mean body weight, body weight gain, and feed consumption/utilization were observed in both sexes of the parent generation at the high-dose level, but were transient in nature. Reductions in mean number of implantation sites were observed in the high-dose group and correlated to the number of corpora lutea. However, there was no corresponding effect on pre- or post-implantation loss, or litter size on PND 0. Mean litter weights in the high-dose group were statistically lower than those of the control group on PND 0 and 4, an effect attributed to the smaller litter sizes rather than a difference in individual pup size. The mean number of live pups at PND 4 was lower in high-dose litters compared to control litters. Mean absolute epididymal sperm counts were statistically lower in all treated groups compared to the control group; however, when counts were normalized for organ weight, values were not statistically different. Males in the high- and low-dose groups had lower mean absolute and/or relative testicular sperm counts. The significance of this was unclear, as there was no effect on relative epididymal sperm counts, fertility, or microscopic lesions in the testes. Authors considered both sperm type changes to be nonadverse. Other reproductive parameters, including reproductive organ weights, gross or microscopic lesions, and mean sperm motility were not affected. Study authors concluded that the NOAEL for reproductive or developmental toxicity was 276 mg/kg bw/day for males and 359 mg/kg bw/day for females, based on decreased total litter weight and litter size on PND4, decreased number of implants and number of corpora lutea (Eastman Chemical 2001).

Genotoxicity

Eastman Chemical (2007) reported briefly on three genotoxicity assays performed on TXIB under GLP conditions. TXIB did not induce reverse mutations in *Salmonella typhimurium* or *Escherichia coli* tester strains in the presence or absence of exogenous metabolic activation. Similarly, no significant increase in forward mutations at the hypoxanthine-guanine phosphoribosyltransferase locus was observed in Chinese Hamster Ovary (CHO) cells in the presence or absence of metabolic activation. TXIB also did not induce chromosomal aberrations in CHO cells with or without metabolic activation. No further details of these studies were available.

The genotoxicity of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate was examined in another reverse mutation assay and in the chromosomal aberration test in cultured Chinese Hamster Lung (CHL/IU) cells (JMHLW 1993). TXIB (purity 99.7%) was not mutagenic in *S. typhimurium* tester strains TA100, TA98, TA1535, TA1537 or *E. coli* WP2 uvrA at concentrations of 0, 312.5, 625, 1250, 2500, or 5000 µg/plate with or without metabolic activation. Neither structural nor numerical chromosomal aberrations were induced in CHL/IU cells under short-term treatment with metabolic activation at 0–1.3 mg/mL and without activation at 0–0.018 mg/mL. Cells under continuous treatment without metabolic activation also showed no increase in chromosomal aberrations at concentrations of 0–0.04 mg/mL.

Carcinogenicity

No data on the carcinogenicity of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate were identified in a search of the literature.

References

Astill, B.D., C.J. Terhaar, and D.W. Fassett. 1972. Toxicology and fate of 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate in the rat. Toxicol Appl Pharmacol 22: 387–399.

Cain, W.S., R. A. de Wijk, A. A. Jalowayski, G. Pilla Caminha, and R. Schmidt. 2005. Odor and chemesthesis from brief exposures to TXIB. Indoor Air 15(6):445–457.

David, R.M., L.K. Lockhart, and K.M. Ruble. 2003. Lack of sensitization for trimellitate, phthalate, terephthalate and isobutyrate plasticizers in a human repeated insult patch test. Food Chem Toxicol.41(4):589–93.

Danish Environmental Protection Agency (DEPA). 2010. Identification and assessment of alternatives to selected phthalates. Environmental Project No. 1341 2010. Available: http://www2.mst.dk/udgiv/publications/2010/978-87-92708-00-7/pdf/978-87-92708-01-4.pdf .

Eastman Chemical. 2001. Reproduction/developmental toxicity screening test in the rat with 2,2,4-trimethyl-1,3-pentanediol diiosbutyrate - final report w/cover letter dated 082401. U.S. EPA/OPTS Public Files; Fiche #: OTS0560045-1; Doc#: 89010000299. TSCATS.

Eastman Chemical. 2002. Eastman Plasticizers Selector Chart. Publication L-174L, June 2002. Available: http://www.eastman.com/Literature_Center/L/L174.pdf .

Eastman Chemical. 2006a. Eastman TXIB formulation additive for vinyl plastisols. Available: http://www.eastman.com/Literature_Center/L/L231.pdf .

Eastman Chemical. 2006b. Eastman TXIB Formulation Additive technical tip. TT-78A. http://www.eastman.com/Literature_Center/T/TT78.pdf .

Eastman Chemical. 2007. Toxicity summary for Eastman ® TXIB Formulation Additive. Available: http://www.cpsc.gov/about/cpsia/docs/EastmanTXIB11282007.pdf.

Eastman Chemical. 2009. Eastman product data sheet for TXIB. Available: http://www.eastman.com/ProductCatalogApps/PageControllers/ProdDatasheet_PC.aspx?Product=71066420&sCategoryName=Generic.

Eastman Chemical. 2010. Eastman TXIB Formulation Additive. Product overview. Available: http://www.eastman.com/Brands/Eastman_TXIB/Pages/Overview.aspx.

Hazardous Substances Database (HSDB). 2010. Entry for 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate. Accessed November, 2010. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

International Uniform Chemical Information Database (IUCLID). 2000. IUCLID Dataset for 2,2,4-Trimethyl-1,3-pentanediol-diisobutyrate. Available: http://ecb.jrc.ec.europa.eu/iuclid-datasheet/6846500.pdf .

Japanese Ministry of Health, Labor, and Welfare (JMHLW). 1993. Japan Existing Chemical Data Base(JECDB). Test report on 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (6846-50-0). Abstract only. Available: http://dra4.nihs.go.jp/mhlw_data/home/file/file6846-50-0.html.

Järnström, H., K. Saarela, P. Kalliokoski, and A.L. Pasanen. 2008. Comparison of VOC and ammonia emissions from individual PVC materials, adhesives and from complete structures. Environ Int. 2008, Apr; 34(3):420–7.

Kim, J.L., L. Elfman, Y. Mi, G.Wieslander, G. Smedje, and D. Norbäck. Indoor molds, bacteria, microbial volatile organic compounds and plasticizers in schools--associations with asthma and respiratory symptoms in pupils. Indoor Air. 2007, Apr; 17(2):153–63.

Krasavage, W.J, K.S. Tischer, and R Roudabush. 1972. The reversibility of increased rat liver weights and microsomal processing enzymes after feeding high levels of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. Toxicol Appl Pharmacol 22 (3): 400–408.

Maddalena R, Russell M, Sullivan DP, and Apte MG. 2009. Formaldehyde and other volatile organic chemical emissions in four FEMA temporary housing units. Environ Sci Technol 43(15): 5626–32.

Norback, D., G.Wieslander, and C. Edling. 1995. Occupational exposure to volatile organic compounds (VOCs), and other air pollutants from the indoor application of water-based paints. Annals of Occupational Hygiene 39(6): 783–794.

Organization for Economic Cooperation and Development (OECD). 1995. Screening Information Dataset (SIDS) initial assessment report for 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate. Available: http://www.inchem.org/documents/sids/sids/6846500.pdf

Rosell, L. 1990. High Levels of a Semi-VOC in Indoor Air due to Emission from Vinyl Floorings. Indoor Air '90, The 5th International Conference on Indoor Air Quality and Climate, Toronto, July 29 August 3, 1990, Volume 3, pages 707–712. Abstract only.

U.S. EPA 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. EPA/600/6-87/008.

Wieslander, G., D. Norback, E. Bjornsson, C. Janson, and G. Boman. 1997. Asthma and the Indoor Environment: The Significance of Emission of Formaldehyde and Volatile Organic Compounds from Newly Painted Indoor Surfaces. International Archives of Occupational and Environmental Health, Vol. 69, No. 2, pages 115–124. Abstract only.

Wieslander G, Janson C , Norback D, Bjornsson E, Stalenheim G, Edling C. 1994. Occupational exposure to water-based paints and self-reported asthma, lower airway symptoms, bronchial hyperresponsiveness, and lung function. International Archives Of Occupational And Environmental Health; 66 (4). 261–267.

Table 9: Summary of Toxicity Data for 2,2,4-Trimethyl-1,3-pentanediol-diisobutyrate (CAS #6846-50-0)							
Organ/ System	**Model**	**Exposure Route**	**Dose**	**Dose Duration**	**Endpoint**	**Effect**	**Reference**
Systemic	Beagle dogs	Dietary	~22, 77, 221 mg/kg bw/day (M); 26, 92, 264 mg/kg/day (F)	6 day/week for 13 weeks	NOEL= 22-26 mg/kg/day NOAEL = 221-264 mg/kg/day	Relative liver and pituitary gland weights elevated but still within historically normal range	Astill et al. 1972
Systemic	Holtzman rats	Dietary	75.5, 772 mg/kg/day (M); 83.5, 858.5 mg/kg/day (F)	103 days	NOAEL = 772-858.5 mg/kg/day	Relative liver weight elevated but still within historically normal range	Astill et al. 1972
Systemic	Sprague-Dawley rats	Dietary	0, 147.5, 1475 mg/kg bw/day	52 days (exper. I), 99d (exper. II), 52d + 47d control or 52d control + 47d (exper. III)	NOAEL = 1475 mg/kg/day	At 1475 mg/kg, ↑ relative liver weights (M,F) and ↑ microsomal enzyme activity except for experiment III rats fed TXIB first and control diet second; no accompanying hepatic damage	Krasavage et al. 1972

Systemic	Sprague-Dawley rats	Gavage	0, 30, 150, 750 mg/kg bw/day	14 days pre-mating + 30 days (M) or through PND3	NOEL = 30 mg/kg/day (M); 150 mg/kg/day (F); NOAEL = 150 mg/kg/day	At 750 mg/kg, ↓ body weight gain (M); ↑ food consumption (F); ↑creatinine, total bilirubin and blood protein (M); ↑ liver weights (F, M) with swelling and abnormal color (M); changes in renal tubular epithelium (M); ↑ kidney weight (M); proximal tubule necrosis, fibrosis (M); distal tubule dilatation (M). At 150 mg/kg, ↑ creatinine and total bilirubin (M); ↑ liver weights (M); basophilic changes in renal tubular epithelium (M).	JMHLW 1993; Eastman Chemical 2007
Systemic	Sprague-Dawley rats	Dietary	0, 120, 359, 1135 mg/kg bw/day (F); 0, 91, 276, 905 mg/kg bw/day	14 days pre-mating through PND4 or 5	NOAEL = 1135 mg/kg/day (F) and 905 mg/kg/day (M)	At high dose, transient ↓ body weight, body weight gain, and feed consumption/ utilization (M,F).	Eastman Chemical 2001
Reproductive/ developmental	Sprague-Dawley rats	Gavage	0, 30, 150, 750 mg/kg bw/day	14 days pre-mating + 30 days (M) or through PND3 (F)	$NOAEL_{reproductive}$ = $NOAEL_{developmental}$= 750 mg/kg bw/day	No effects on reproductive or developmental parameters observed	JMHLW 1993; Eastman Chemical 2007

Reproductive/ developmental	Sprague-Dawley rats	Dietary	0, 120, 359, 1135 mg/kg bw/day (F); 0, 91, 276, 905 mg/kg bw/day	14 days pre-mating through PND4 or 5	$NOAEL_{reproductive}$ = $NOAEL_{developmental}$= 276 mg/kg bw/day (M) and 359 mg/kg bw/day (F)	At high dose, ↓ # implantation sites and corpora lutea; ↓ litter size and weight PND 0 and 4; ↓ mean number of live pups/litter PND 4. High and low dose, ↓ absolute and/or relative testicular sperm counts. All doses, ↓ absolute but not relative epididymal sperm count.	Eastman Chemical 2001