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DRINKING WATER CRITERIA DOCUMENT FOR
PHTHALIC ACID ESTERS (PAES)

Prepared for

OFFICE OF WATER

Prepared by

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DRAFT: DO NOT CITE OR QUOTE

NOTICE

This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comments on its technical accuracy and policy implications.

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1986; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

ACAT	Cholesterol acryltransferase
ADP	Adenosine diphosphate
AHP	Phthalic anhydride
ATP	Adenosine tripnosphate
BBP	Butyl benzyl phthalate
BOP	Butyloctyl phthalate
BPBG	Butylphthalyl butylgylcolate
BSP	Bromosulfophthalein
bw	Body weight
CoA	Coenzyme A
CRAVE	Carcinogen Risk Assessment Verification Endeavor
DAP	Diallyl phthalate
DBOP	Dibutoxyethyl phthalate
DBP	Dibutyl phthalate
DCP	Dicyclohexyl phthalate
DDP	Diisodecyl phthalate
DEHA	Di-(2-ethylhexyl)adipate
DEHP	Di(2-ethylhexyl) phthalate
DEN	Diethylnitrosamine
DEP	Diethyl phthalate
DHP	Dihexyl phthalate
DIB	Diisobutyl phthalate
DID	Disodecyl phthalate
DIOP	Diisooctyl phthalate
DMEP	Dimethoxyethyl phthalate
DMP	Dimethyl phthalate
DnBP	Di-n-butyl phthalate
DnOP	Di-n-octyl phthalate
DNP	Dinonyl phthalate
n-DP	Decyl phthalate
DTP	Di-tridecyl phthalate
DWEL	Drinking Water Equivalent Level
FSH	Follicle stimulating hormone
GC/ECD	Gas chromatography/electron capture detector
GGT	Gamma glutamyl transpeptidase

LIST OF ABBREVIATIONS (cont.)

GI	Gastrointestinal
GLC	Gas-liquid chromatography
HA	Health Advisory
n-HP	Hexyl phthalate
HPLC	High pressure liquid chromatography
i.a.	Intra-arterial
i.m.	Intramuscular
i.p.	Intraperitoneal
IRIS	Integrated Risk Information System
i.v.	Intravenous
LH	Luteinizing hormone
LOAEL	Lowest-observed-adverse-effect level
MBP	Monobutyl phthalate
MEHP	Mono(2-ethylhexyl) phthalate
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NTP	National Toxicology Program
PA	Phthalic acid
PAEs	Phthalic acid esters
PAM	Phthalamide
PB	Phenobarbitol
p.o.	per oral
PVC	Polyvinyl chloride
RFD	Reference dose
s.c.	Subcutaneous
SDH	Succinate dehydrogenase
SER	Smooth endoplasmic reticulum
SGOT	Serum glutamic oxalacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TLC	Thin-layer chromatography

I. SUMMARY

Phthalic acid esters (PAEs) are primarily used as plasticizers in polyvinyl chloride resins. These compounds are environmentally ubiquitous due to their widespread use and ease of extraction: PAEs have been detected in soil, water, air, and food indicating widespread potential for human exposure. Their presence has also been detected in human tissues.

PAEs generally occur as colorless liquids characterized by low water solubility, high solubility in oils and organic solvents and, for the higher molecular weight compounds, low volatility. Although phthalate has three isomers (ortho, meta, and para positions), the term phthalic acid esters generally refers to esters formed from the ortho phthalic acid isomer. This document will be primarily concerned with the ortho isomer compounds.

PAEs are rapidly absorbed from the intestine, skin, peritoneum, blood, and lungs. A large percentage of the diesters are hydrolyzed to monoesters, although the intact compounds are found in excretory products. Distribution studies indicate that PAEs and their metabolites are found mainly in adipose tissue, liver, kidney and intestine. Accumulation and retention of these compounds is minimal. Most dialkyl phthalates are metabolized to their corresponding monoesters; however, short-chain alkyl phthalates such as dimethyl phthalate (DMP) may be metabolized to phthalic acid. In most species, glucuronide conjugates are formed with the monoester; however, rats appear to be unable to form glucuronide conjugates of mono(2-ethylhexyl) phthalate (MEHP) while forming glucuronide conjugates with monobutyl phthalate (MBP). PAEs and their metabolites are eliminated through the urine, feces, and bile.

Acute animal toxicity of PAEs is low and generally tends to be inversely related to the molecular weight of the compound. Subchronic and chronic toxicity includes decreased body weight and increased liver and kidney weights. Target organs of PAEs include the testes and the liver, although these effects have not been observed with all PAEs. Testicular atrophy has been associated with exposure to di(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBP), and dibutyl phthalate (DBP). Hepatic effects commonly reported include enlargement of the liver, effects on the mitochondria, and decreased succinate dehydrogenase activity. Reproductive effects have been reported following exposure to PAEs during mating and gestation. DEHP has been shown to decrease fertility and reproductive performance in mice. Decreased fertility was attributable to effects in both males and females. PAEs are generally regarded as nonmutagenic. Two PAEs have been tested in 2-year carcinogenesis bioassays performed by the National Toxicology Program. DEHP was found to cause increased incidence of hepatocellular carcinomas in both rats and mice. There was limited evidence that BBP induces leukemia in female rats. The rat portion of the NTP bioassay on BBP is currently being repeated.

Information on the effects of PAEs in humans, particularly for oral exposures, is limited. A single dose of 5 or 10 g of DEHP caused mild GI effects in one individual. Accidental ingestion of 10 g of DBP caused nausea, vertigo, keratitis, and toxic nephritis. Dermal exposure to most PAEs does not cause irritation or sensitization. Studies of human tissues and cell cultures demonstrated inhibition of cellular growth and decreases in platelet function but did not induce chromosomal damage. In epidemiologic studies the results have been largely confounded by exposure to multiple chemicals and lack of quantitative information on levels and

duration of exposure. Only two studies reported to date identify specific phthalate exposure. However, lack of exposure data and limited chemical details result in a relatively weak data base. The highest risk group in humans appears to be among patients receiving blood transfusions or hemodialysis due to leaching of PAEs from plastic blood bags or plastic tubing. Hepatitis in hemodialysis patients and necrotizing enterocolitis in infants given blood tranfusions or umbilical catheters were related to PAE exposures, but a causal relationship could not be conclusively demonstrated.

Researchers have investigated several possible mechanisms of PAE toxicity; however, there is no conclusive evidence on any one mechanism. Mechanistic studies have indicated that PAEs may be interfering with the normal enzymatic or metabolic processes that occur at the cellular level. However, the exact processes involved in these alterations have not been clearly delineated. It has been suggested that PAEs exert their toxic effects by altering the physical state of membrane lipids, thereby changing membrane fluidity. In the liver, PAEs act to increase fatty acid metabolism by inducing peroxisomes, mitochondria and enzymatic activities. PAEs may become associated with hepatic DNA as a result of biosynthetic incorporation of PAE metabolites into the genetic material. Gonadal toxicity of PAEs in males has been related to the antagonistic effect of PAEs upon endogenous testicular zinc levels. Testicular lesions may result from morphologic changes of Sertoli cells induced by PAE exposure. It would appear that different mechanistic processes are operating on the various target organs.

The 1-day and 10-day HAs for DEHP were derived based on the dose producing noncarcinogenic effects in animals after oral administration. The

1-day and 10-day HAs for DEHP for a 10 kg child are 1 mg/ℓ and 0.5 mg/ℓ, respectively. The recommended longer-term HAs are 0.5 mg/ℓ and 2 mg/ℓ for a 10 kg child and 70 kg adult, respectively. A lifetime DWEL based upon a LOAEL for guinea pigs administered DEHP in the diet was determined to be 0.7 mg/ℓ.

Lack of sufficient data preclude the derivation of a 1-day HA for BBP. It is recommended that the 10-day HA (20 mg/ℓ) be adopted as the 1-day HA. The 1-day and 10-day HA for BBP for a 10 kg child are 20 mg/ℓ. The longer-term HAs were based on a NOAEL derived from orally exposed rats. The longer-term HAs are 20 mg/ℓ for a child and 60 mg/ℓ for an adult. A lifetime DWEL based on the same study as the longer-term HAs was determined to be 7 mg/ℓ.

The 1-day HA of 50 mg/ℓ for DBP is based on a NOAEL for testicular effects in rats. The 10-day HA and the longer-term HAs were based on a NOAEL derived from orally exposed rats. The corresponding 10-day HA and longer-term HA for a child is 10 mg/ℓ. The longer-term HA for an adult is 40 mg/ℓ. A lifetime DWEL based on the same study as the longer-term HAs was determined to be 4 mg/ℓ.

Lack of sufficient data precludes the derivation of a 1-day and 10-day HA for DEP. The recommended longer-term HAs are 75 mg/ℓ for children and 300 mg/ℓ for adults, based on a NOAEL in rats after oral exposure. A lifetime DWEL based on the same oral rat data was determined to be 30 mg/ℓ.

Lack of sufficient data precludes the derivation of 1-day and 10-day HAs, longer-term HAs or DWEL for DMP.

There is sufficient evidence to classify DEHP as a B2, probable human carcinogen (i.e., inadequate evidence from human studies and sufficient evidence from animal studies). Questions have been raised concerning the mechanisms of DEHP cancer induction and dosimetry. A re-evaluation of DEHP may be performed when more information becomes available. The drinking water risk levels of 10^{-4}, 10^{-5} and 10^{-6} for DEHP are 300, 30 and 3 µg/ℓ, respectively.

There is limited evidence to classify BBP as a Group C possible human carcinogen. Pertinent data regarding the carcinogenicity of DBP, DEP and DMP are nonexistent. Under the U.S. EPA guidelines DBP, DEP and DMP should be placed in Group D, not classified as to human carcinogenicity.

II. PHYSICAL AND CHEMICAL PROPERTIES

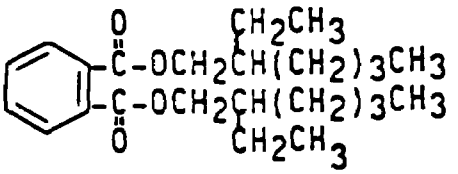
Introduction

Phthalate acid esters, commonly referred to as PAEs, are colorless liquids characterized by low volatility, low solubility in water, and high solubility in oils and organic solvents. Structures for the compounds considered in this document are listed in Figure II-1. Table II-1 summarizes the pertinent chemical and physical properties for various phthalic acid alkyl and aryl esters including the PAEs of particular interest reviewed in this document.

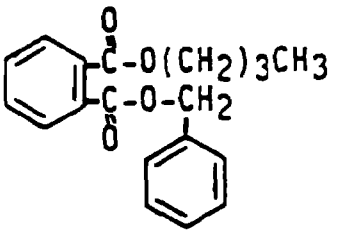
Preparation

The reaction of phthalic acid (benzene dicarboxylic acid) with a specific alcohol to produce the desired phthalic acid ester is a common method of preparation. PAEs are often manufactured industrially from phthalic anhydride rather than the acid. Figure II-2 illustrates the preparation of phthalic acid esters. Manufactured esters frequently contain mixtures of various isomers and impurities (U.S. EPA, 1980). Commercially produced PAEs are usually $\geq$99% pure with a residual maximum acidity of 0.01%. The remaining impurities may be mixtures of terephthalic acid, maleic anhydride or diesters of isophthalic acid (U.S. EPA, 1978). The term phthalate ester in this document refers to an ester formed from the ortho phthalic acid isomer. Other PAEs formed from the meta and para phthalic acid isomers are generally referred to as isophthalates and terephthalates, respectively. Since the ortho phthalic acid isomers are the most prevalent and extensively studied PAEs, this document will address those isomers.

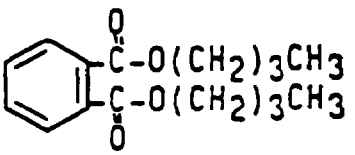
DEHP



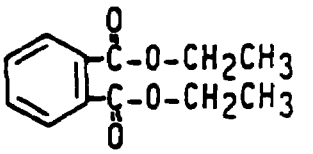
BBP



DBP



DEP



DMP

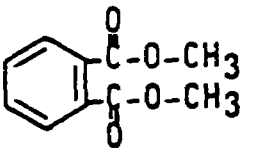


FIGURE II-1

Structures of Various PAEs

TABLE II-1

Chemical and Physical Properties of Various PAEs

Phthalate Acid Ester	Molecular Formula[a]	Molecular Weight[a]	CAS Number[a]	RTECS Number[a]	Boiling Point[b] (°C)	Melting Point (°C)	Density[c]	Specific Gravity[b]	Vapor Pressure[d] (mm) (25°C)	Water Solubility (mg/ℓ)	Log Octanol/ Water Partition Coefficient[e]
Butyl benzyl[1] (BBP)	$C_{19}H_{20}O_4$	312.39	85-68-7	TH99900	370[f]	-35	ND	1.111[f]	1.1×10^{-8}	2.69[d]	5.56
Butylloctyl (BOP)	ND	334.0[b]	ND	ND	225[f-2]	<-50[f]	ND	0.991[f]	ND	ND	ND
Butyl phthalylbutyl gylcolate (BPBG)	$C_{18}H_{24}O_6$	336.42	85-70-1	TI05350	219	<-35[g]	ND	1.097	ND	120[b]	ND
Diallyl (DAP)	$C_{14}H_{14}O_4$	246.28	131-17-9	CZ42000	156-175[f-1]	65[f]	ND	1.120	ND	100[b]	ND
Dibutoxyethyl (DBOP)	$C_{20}H_{30}O_6$	366.50	117-83-9	TI01750	270[g]	ND	ND	1.063	ND	300[b]	ND
Di-n-butyl[1] (DBP)	$C_{16}H_{22}O_4$	278.38	84-74-2	TI08750	340	-35[g]	1.0426[h]	1.0465	9.7×10^{-8}	11.2[d]	4.79[d]
Di-n-octyl (DnOP)	$C_{24}H_{38}O_4$	390.62	117-84-0	TI19250	220-248[f-1]	-25	ND	0.978	1.44×10^{-4}[j]	3.0[j]	9.87
Dicyclohexyl (DCP)	$C_{20}H_{26}O_4$	330.46	84-61-7	TI08890	212-218[f-2]	58-65[f]	1.383	1.148[f]	ND	Insoluble	ND
Diethyl (DEP)[1]	$C_{12}H_{14}O_4$	222.26	84-66-2	TI10500	296	-4[f]	1.1175	1.118[f]	2.2×10^{-1}	1080[d]	2.24[d]
Di-2-ethylhexyl[1] (DEHP)	$C_{24}H_{38}O_4$	390.62	117-81-7	TI03500	231[f-2]	-46[f]	0.9843[h]	0.985	8.6×10^{-4}	0.34[d]	9.61
Dihexyl (DHP)	$C_{20}H_{30}O_4$	334.50	84-75-3	TI11000	350[g]	-50[g]	ND	1.010-1.016[g]	1.9×10^{-8}	0.24[d]	ND
Diisobutyl (DIB)	$C_{16}H_{22}O_4$	278.38	84-69-5	TI12250	295-298[c]	ND	1.0490	1.040	ND	Insoluble	ND
Diisooctyl (DIOP)	$C_{24}H_{38}O_4$	385.57	27554-26-3	TI13000	370[g]	ND	ND	0.981	7.4×10^{-4}	0.09[d]	ND

TABLE II-1 (cont.)

Phthalate Acid Ester	Molecular Formula[a]	Molecular Weight[a]	CAS Number[a]	RTECS Number[a]	Boiling Point[b] (°C)	Melting Point (°C)	Density[c]	Specific Gravity[b]	Vapor Pressure[d] (mm) (25°C)	Water Solubility (mg/l)	Log Octanol/ Water Partition Coefficient[e]
Dimethoxyethyl (DMEP)	$C_{14}H_{18}O_6$	282.32	117-82-8	TI14000	340[g]	-45[g]	1.047	1.171	ND	8500[b]	ND
Dimethyl (DMP)[i]	$C_{10}H_{10}O_4$	194.20	131-11-3	TI15750	282	0-2[f]	1.1905	1.192[f]	2.2×10^{-3}	4000[d]	1.47[d]
Dinonyl (DNP)	$C_{26}H_{42}O_4$	418.68	84-76-4	TI18000	205-220[g]	ND	ND	0.9799	ND	Insoluble	ND

[a]NIOSH, 1985

[b]U.S. EPA, 1980

[c]Weast, 1983

[d]Howard et al., 1985

[e]U.S. EPA, 1982

[f]Mark et al., 1982
(1) At 533 Pa (4.0 mm Hg)
(2) At 666 Pa (5.0 mm Hg)

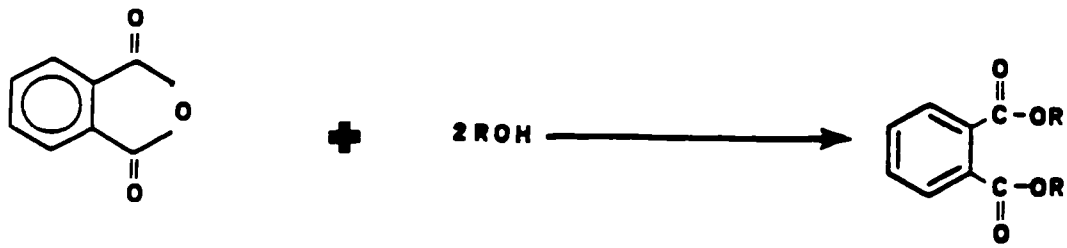
[g]Hawley, 1981

[h]Dean, 1979

[i]Phthalates of primary concern in this document

[j]U.S. EPA, 1987a

ND = No data



ortho phthalic acid + alcohol ⟶ phthtalic acid ester

FIGURE II-2

Preparation of Phthalic Acid Esters

Source: U.S. EPA (1980)

Analytical Methods

Analysis for specific PAEs is complicated due to matrix interferences and limited analytical methods and analysis procedures. To preserve samples for analysis, containers must be refrigerated at 4°C and protected from light (40 CFR Part 136). This will reduce matrix interferences caused by evaporation and photosensitivity.

PAEs are readily soluble only in organic solvents. Ease of solvent extraction increases with increasing molecular weight of the organic solvent (Leah, 1977). Since phthalates are a component of many plastic and rubber products, contamination of laboratory apparatus and solvents may occur requiring a sample clean-up procedure. Clean-up procedures require sample extraction by either a florisil or alumina column (40 CFR Part 136). Extracted PAEs are separated and quantified by gas chromatography with an electron capture detector (40 CFR Part 136).

Uses and Industrial Sources

PAEs are used primarily to impart flexibility to plastics. The final products may contain as much as 50% PAEs by weight (Kluwe, 1982b). DEHP is the most commonly used plasticizer in polyvinyl chloride (PVC) products, which include syringes, dialysis tubing and other medical devices (Kluwe, 1982b). DEHP may constitute as much as 40% of the plastic material in blood storage bags and medical tubing (Sjöberg et al., 1985b). PVC resins are also used in the production of high temperature electric wire, cable insulation, flooring material, swimming pool liners, furniture upholstery, wall coverings, seat covers for cars, footwear and packaging materials (Graham, 1973). Nonplasticizer uses include pesticide carriers, cosmetics, fragrances, munitions, industrial oils and insect repellents (USITC, 1983).

In 1984, the United States produced 1179 million pounds of PAEs (USITC, 1985). Table II-2 indicates various PAEs and their corresponding production figures. Annual production on a worldwide scale is estimated to be between 3 and 4 billion pounds (U.S. EPA, 1980).

Distribution

PAEs enter the environment as a result of release during production, use and disposal. Due to their widespread use PAEs are available as potential contaminants to the environment. The U.S. EPA (1980) Ambient Water Quality Criteria for Phthalate Esters cites publications detailing numerous detections of PAEs in the soil (Ogner and Schnitzer, 1970), water (Ewing and Chian, 1977; Corcoran, 1973; Hites and Bieman, 1972), air (Mathur, 1974), fish (Mayer, 1976; Stalling et al., 1973) and biologic tissues (Nazir et al., 1971; Rubin and Shiffer, 1976; Jaeger and Rubin, 1970). In a U.S. EPA monitoring survey of U.S. surface waters DEHP and DEP were found with the greatest frequency (levels not provided in the report) out of 89 base extractable compounds (Ewing and Chian, 1977). However, because of their low water solubility, PAEs tend to partition to soil, sediment and biota in an aqueous environment (Gledhill et al., 1980).

Fate and Transport

PAEs are removed from the environment mainly by biologic degradation. Biodegradation of PAEs is the primary method of removal from soil, water and sediment. Phthalate esters have been reported to be metabolized in water by mixed and pure cultures of microorganisms. The rates of degradation vary widely depending upon environmental conditions, such as temperature, pH, amount of dissolved oxygen and the structure of the phthalate

TABLE II-2

Production of Individual Phthalic Acid Esters in the United States from 1977-1984

Ester	Volume Produced (million pounds)	Year	Reference
BBP	101-510	1977	U.S. EPA, 1985
DBP	22.21	1984	USITC, 1983
DEP	17.75	1984	USITC, 1985
DDP	1-10	1977	U.S. EPA, 1985
DMP	8.64	1984	USITC, 1985
Dioctyl			
DEHP	251.1	1982	USITC, 1983
Other dioctyl phthalates	301.12	1984	USITC, 1985
DIOP	1-10	1977	U.S. EPA, 1985
DTP	21.79	1984	USITC, 1985
DID	145.82	1984	USITIC, 1985

(Hattori et al., 1975). The degradation of phthalate esters by pure culture isolated from natural water, activated sludge and soil have been studied by several investigators (Taylor et al., 1981; Kurane et al., 1979a,b; Engelhardt et al., 1975, 1977; Engelhardt and Wallnofer, 1978; Klausmeier and Jones, 1960; Perez et al., 1977; Ohta and Nakamoto, 1979). Several authors have studied the biodegradation of phthalate esters by mixed microorganisms. Thus, activated sludge, domestic wastewater and natural river water have been used as microbial inoculum to study the biodegradation of phthalate esters (O'Grady et al., 1985; Saeger and Tucker, 1973b, 1976; Sasaki, 1978; Sugatt et al., 1984). Tabak et al. (1981) observed 100% degradation of DMP, DEP, DBP and BBP in 7 days with unacclimated microorganisms from domestic wastewater. On the other hand, DEHP and DOP required 21 days of acclimatization with microorganisms before 90% biodegradation in 7 days occurred (Tabak et al., 1981). Similarly, the mineralization of >85% occurred with various phthalates in 28 days with both activated sludge and river water (Saeger and Tucker, 1976; Sugatt et al., 1984). The metabolic pathway data indicate that phthalate esters first undergo enzymatic hydrolysis to form the monoester, followed by further hydrolysis to phthalic acid. The phthalic acid is further degraded to carbon dioxide and water (U.S. EPA, 1978; Saeger and Tucker, 1976).

Saeger and Tucker (1973a,b, 1976) and Gledhill et al. (1980) concluded from their river die-away and activated sludge studies that phthalate plasticizers, as a class, undergo rapid primary degradation and mineralization by bacteria commonly found in the environment. In a simulated lake microcosm, Gledhill et al. (1980) observed >95% primary degradation of BBP in 7 days (C_0=1 mg/ℓ). The biodegradation half-life for BBP in this natural water system was <4 days. The length and

configuration of the alkyl ester chains significantly influences the biodegradation rate of phthalates in freshwater ecosystems, whereas acclimation of microbes appears to have little effect (Hattori et al., 1975, Johnson et al., 1984). In freshwater systems, phthalates such as DMP and DEP are expected to degrade faster than the larger and more complex phthalate esters (Johnson et al., 1984; Hattori et al., 1975). However, in relatively clean ocean water, ~14-20% degradation of DEP and DMP phthalate was measured after 14 days, while the larger phthalates were decomposed >30% during the same period. The degradation of all the phthalate esters were much higher with impure ocean water. For example, while 33% of DBP and 14% of DEP degraded in clean ocean water in 14 days, the degradation was 100% in 5 days for DBP and 68% in 14 days for DEP with impure ocean water. Hattori et al. (1975) observed 100% decomposition of DEP after 6 days and 100% decomposition of DMP after 8-11 days in river water initially spiked with 25 mg/ℓ of the ester. DEHP degraded only ~40% after 2 weeks in river water. The higher degradation in impure water was attributed to the presence of higher concentrations of nutrients. Longer chain phthalate esters decomposed faster than DMP and DEP in clean ocean water, a finding not further explained (Hattori et al., 1975).

In aquatic sediments under anaerobic conditions, biodegradation of short chain alkyl esters appears to be slow and degradation of the longer chain esters has been observed to be very slight or undetectable (Johnson et al., 1984; Johnson and Lulves, 1975; Horowitz et al., 1982; Shelton et al., 1984). Johnson and Lulves (1975) observed 61 and 98% anaerobic mineralization of DBP in 14 and 30 days, respectively. Under the same conditions, no detectable degradation of DEHP was measured after 30 days. Johnson et al. (1984) measured 10% anaerobic mineralization of radiolabeled DEHP after 28

days and <1% mineralization of DIOP. Optimal degradation of long chain phthalates occurred at high concentrations in nutrient-rich aquatic sediments with temperatures above 22°C. Such environmental conditions are typical of sewage treatment ponds, wetlands, eutrophic lakes and enriched streams during summer. Winter conditions, particularly at northern latitudes and environmentally realistic (low, <1 µg/ℓ) concentrations would adversely affect biodegradation (Johnson et al., 1984).

Volatilization and leaching are two common modes of PAE transport through the environment. When PAEs are used as plasticizers in polymers, the link between the plasticizer and the polymer involves a physical interaction rather than a chemical reaction. The polar groups of PAEs adhere to the residual free PVC dipoles, but are not chemically bound. Thus, the PAEs are potentially free to be removed by volatilization and leaching. For example, Atlas et al. (1982) measured the mass-transfer coefficient of DBP to be 0.104 cm/hour in stirred (200-300 rpm) seawater free of interfering organic contaminants at 23°C. At a depth of 4.5 cm, the volatilization half-life of DBP has been calculated to be 30 hours following the method of Dilling (1977). Henry's Law constants (H) for some PAEs, are listed in Table II-3. Lyman et al. (1982) generalized volatility according to ranges of (H). The information presented in Table II-3 suggests that volatilization from water would not be a rapid but still a possibly significant removal process for these PAEs. Volatilization of DEHP (27.1%) and DEP (4.5%) from PVC occurred after heating PVC material for 24 hours at 87°C over activated carbon (Darby and Sears, 1969). In another study, Graham (1973) found that the air inside new automobiles contained ≤0.72 µg/ℓ phthalates due to volatilization from plastics (upholstery, seatcovers, automobile mats, automobile tops).

TABLE II-3

Henry's Law Constants for PAEs*

Compound	Constants atm•m³/mol
DMP	1.3×10^{-5}
DEP	5.5×10^{-5}
DBP	2.9×10^{-4}
DOP	2.3×10^{-5}
DEHP	1.0×10^{-3}
BBP	1.5×10^{-4}

*Calculated using vapor pressure and water solubility data listed in Table II-1.

The rate of environmental leaching is affected by the formation of various complexes. Evidence suggests that complexation of phthalates in natural water with organic substances may be one mode of transport of phthalates (Khan, 1980; Ogner and Schnitzer, 1970; Matsuda and Schnitzer, 1971). Phthalate esters have been observed readily interacting with fulvic acid, a widely occurring humic substance found in soils and waters. The phthalates appear to adsorb to the surface of the fulvic acid molecule rather than react with it. The fulvic acid-phthalate complex is very soluble in water; thus, mobility of otherwise insoluble phthalate esters is modified. Extent of solubilization appears to vary with phthalate size. Equivalent quantities of fulvic acid will solubilize 4 times as many equivalents of di(2-ethylhexyl) phthalate as of di-n-butyl phthalate (Matsuda and Schnitzer, 1971). Hydrated phthalates, for example, are more readily extracted from PVC tubing than nonhydrated forms (Wildbrett, 1973). Theory suggests that immigrating water molecules into the tubing adhere to the unsolvated phthalates molecules, which are ultimately responsible for the plasticizing effect in PVC. This prevents the molecules from adhering to residual free PVC dipoles and therefore permitting mobility (Wildbrett, 1973). Furthermore, dialkyl phthalates and the widely occurring humic and fulvic acid form a stable, soluble complex that allows transport in water. Surfactants are used for solubilizing phthalates from stream beds and landfills (Ogner and Schnitzer, 1970). Very little DEHP is extracted by water because of DEHP's low solubility in water. Ethanol significantly increases the amount of DEHP extracted, while pH has little or no effect. The mean DEHP concentration extracted from ethanol solutions of 5, 10, 40 and 70% were 2, 6, 29.8 and 322.7 mg/ℓ respectively (Lawrence and Tuell, 1979).

Hydrolysis does not appear to play an important role in the removal of PAEs from the environment. Glendhill et al. (1980) observed >5% hydrolysis of 1 mg/ℓ n-butyl benzyl phthalate in 28 days. Wolfe et al. (1980) estimated second-order rate constants for alkaline hydrolysis of phthalates at pH 10-12 and 30°C. Rate constants varied with the size and complexity of the phthalates and ranged from 1.1×10^{-4} M^{-1} sec^{-1} for DEHP to 6.9×10^{-2} M^{-1} sec^{-1} for DMP. Thus, corresponding estimated half-lives at pH 7 range from 3.2-2000 years.

Experimental data regarding oxidation and photolysis of PAEs in water were not located in the available literature. However, calculated predictions indicate that these processes would not be environmentally important (Mabey et al., 1982; Callahan et al., 1979).

Adsorption

Sullivan et al. (1982) studied the adsorption of DBP and DEHP onto clay minerals, calcite and sediment samples from seawater. Results indicate that adsorption increases with increased salinity or decreased solubility of phthalates. Adsorption onto the clay minerals and calcite appeared to be a reversible process, whereas adsorption onto sediments was irreversible. This suggests that marine sediments may act as a final repository of PAEs (Sullivan et al., 1982). Mabey et al. (1982) calculated sediment-water partition coefficients for phthalates, indicating adsorption is likely for all PAEs with adsorption tendency increasing with size and branching of the ester chain. Sediment adsorption coefficents range from 98 for DMP to >150,000 for DBP and the larger PAEs including BBP. Gledhill et al. (1980)

observed significant partitioning of BBP to sediments in a simulated lake microcosm. The average ratio of this compound measured in sediments versus water was 571:1.

The contention that phthalates will be absorbed significantly onto sediments in aquatic ecosystems is supported by the observation that phthalates are commonly found in bottom sediments from both streams and seas at levels ranging from <0.1-316 ng/ℓ (Giam et al., 1978).

Summary

PAEs are colorless liquids at standard temperature and pressure. They are characterized by low volatility, low solubility in water and high solubility in oils and organic solvents. PAEs are formed by the reaction of phthalic acid with a specific alcohol. Industrially manufactured PAEs, however, are often formed from phthalic anhydride rather than the acid.

Analysis of PAEs is complicated due to matrix interferences and limited analytical methods and analysis procedures. The most precise analytical method is by gas chromatography with an electron capture detector.

PAEs are produced by reacting phthalic anhydride with an excess amount of the corresponding alcohol(s) in the presence of an esterfication catalyst. The commercial products are usually >99% pure. Total U.S. production volume of PAEs amounted to 1179 million pounds in 1984. They are used predominantly as plasticizers for polyvinyl chloride resins. To a lesser extent, PAEs are used as plasticizers for other vinyl resins,

cellulose ester plastics, synthetic elastomers and other polymers. Nonplasticizer uses include pesticide carriers, cosmetics, fragrances, munitions, industrial oils and insect repellants.

Biodegradation of PAEs is the primary method of removal from the environment. PAEs are reported to be metabolized in the aquatic environment by a variety of pure microorganisms and degraded by mixed microbial systems. The microbial degradation rates vary widely depending upon environmental conditions such as temperature, pH, amount of oxygen present and the phthalate structure. Biodegradability of phthalates in freshwater decreases with increasing size and complexity of the phthalate ester chains. Under anaerobic conditions biodegradation of short-chain esters is possible but slower than aerobic conditions, while degradation of the longer-chain esters under anaerobic conditions is very slight or undetectable. Hydrolysis, oxidation and photolysis are not expected to be a significant removal mechanism of PAEs.

Volatilization and leaching are two common modes of PAE transport through the environment. Estimated Henry's Law constants suggest that volatilization from water would not be rapid but could possibly be a significant removal process. The rate of leaching is affected by the formation of various complexes. Complexation with the widely occurring humic and fulvic substances causes solubilization of PAEs in water, thus modifying their mobility.

Results from sediment absorption studies in saltwater suggest that adsorption increases with increased salinity or decreased solubility of

PAEs. Adsorption onto clay minerals and calcite appears to be a reversible process, whereas absorption onto sediments may act as a final repository of PAEs. Calculated sediment water partitioning coefficients indicate absorption is likely for all PAEs, while absorption tendency increases with the size and complexity of the ester chain.

III. TOXICOKINETICS

Introduction

The route of administration of PAEs can affect the eventual absorption, distribution, metabolism and elimination of these compounds. Orally administered PAEs are hydrolyzed in and absorbed from the GI tract as the monoester form. Hydrolysis is greater for lower molecular weight esters than for higher molecular weight esters. Once absorbed, PAEs or their metabolites are distributed throughout the body. Initially the majority of these compounds accumulate in the liver. Deposition of PAEs is mostly in fat, the GI tract, kidneys and liver. PAEs are primarily excreted in the urine; however, elimination through feces and bile also occurs.

Absorption

Most orally administered PAEs are hydrolyzed in and absorbed from the GI tract as monoesters (Pollack et al., 1985a). Absorption also occurs after dermal, i.p., i.v. or inhalation exposures. In *in vitro* experiments with DEHP, DBP, DEP and DMP in the presence of intestinal preparations from rats, ferrets, baboons and humans, the diesters were hydrolyzed to their monoesters. In these studies, DEHP required the longest time for *in vitro* hydrolysis to the monoester by intestinal preparation (Lake et al., 1977). Alkyl-chain length and PAE concentration have been shown to affect hydrolysis rates (Table III-1).

DEHP. Intestinal absorption of DEHP and DBP by rats (strain not specified) after administration by oral gavage has been estimated from analysis of urinary excretion products (Kluwe et al., 1982a). Absorption of DEHP appeared to be less complete than that of DBP with only 40-50% of the 3

TABLE III-1

Hydrolysis of Phthalic Acid Esters by Rat Intestinal Contents[a,b]

Compound[c]	Concentration (mg/mℓ)	Portion Metabolized[d] (%)
DMP	1	60
DBP	1	80
DEHP	1	20
DEHP	0.1	100

[a]Source: Kluwe, 1982a

[b]The chemicals were incubated for 16 hours at 37°C under an N_2 atmosphere in 20% (v/v) suspensions of gut contents in phosphate-buffered Ringers solution containing 1% (w/v) D-glucose.

[c]DMP = Dimethyl phthalate; DBP = di-n-butyl phthalate; DEHP = di(2-ethylhexyl) phthalate.

[d]Percent metabolized in 16 hours

or 1000 mg/kg dose recovered in urine, respectively. However, >90% of the dose appeared in the urine of rats following the ingestion of 10 or 2000 ppm (0.5 or 100 mg/kg/day, respectively, assuming 5% food consumption and 350 g rats) via feed (Table III-2) (Kluwe, 1982a).

Esterases that are capable of hydrolyzing diester phthalates have been found in rat intestinal mucosal cells as well as extracellularly in the intestinal contents (Rowland, 1974; Rowland et al., 1977). Wallin et al. (1974) demonstrated that a small portion of orally administered DEHP may be absorbed from the GI tract as the intact compound.

Albro et al. (1982) and Albro (1986) observed an absorption threshold for a series of single oral doses of DEHP in Fischer rats. Animals received 1.8-1000 mg/kg of ^{14}C-DEHP in cottonseed oil. As the dosage increased, a threshold (121 μg/g DEHP in the liver) was reached above which a steady increase in the amount of unhydrolyzed DEHP or intact diester reached the liver. This may be due to saturation of esterases in the GI tract. Dosages below this threshold result in absorption of hydrolyzed diesters. Administration of DEHP in the diet resulted in intact DEHP reaching the liver at dietary levels exceeding 4300 ppm (430 mg/kg/day calculated using the authors' assumption of 10% food consumption). In contrast to the results observed in rats, Albro et al. (1982) did not detect an absorption threshold in either CD-1 or B6C3F1 mice administered ≤1000 mg/kg of DEHP.

Rhodes et al. (1986) reported that the excretion profile and tissue levels of radioactivity following oral administration of ^{14}C-DEHP demonstrated considerably reduced absorption in the marmoset compared with the rat. The urinary metabolite pattern in the marmoset was qualitatively

TABLE III-2

Estimation of Intestinal Absorption of Phthalic Acid Esters in Rats[a]

Compound	Dose[b]	Time[c] (days)	Percentage of Dose Excreted in Urine
DBP	60 mg/kg	2	90
	270 mg/kg	2	90
	2310 mg/kg	2	90
DEHP	3 mg/kg	4	40
	1000 mg/kg	1	50
	10 ppm[d] (0.5 mg/kg/day)	NR	>90
	2000 ppm[d] (100 mg/kg/day)	NR	>90

[a]Source: Kluwe, 1982a

[b]Oral gavage, unless specified otherwise

[c]Period of sample collection, between exposure and termination

[d]Concentration incorporated into feed

NR = Not reported

similar to, but quantitatively different from, that in the rat. Following an oral dose of 2000 mg/kg (in corn oil), marmoset tissue is exposed to approximately equivalent levels of DEHP and metabolites to that expected for rat tissues following an oral dose of 200 mg/kg (in corn oil). This suggests that DEHP is not readily hydrolyzed by marmoset lipases and therefore not readily absorbed by this species.

The intestinal absorption of DEHP has been studied in two human subjects. In one human subject, 4.5% (as metabolites) of a single oral dose of 10 g DEHP was recovered in the urine after 24 hours. Similarly, a second subject received 5 g DEHP orally, and 2% (as metabolites) of the dose was recovered in the urine after 24 hours (Shaffer et al., 1945).

BBP. Data regarding the absorption of BBP could not be located in the available literature. Systemic effects observed after oral exposure to BBP, consistent with those observed following exposure to other PAEs, indicate that absorption of BBP does occur.

DBP. Intestinal absorption of DBP in rats (strain not specified) has been estimated following oral gavage administration (see Table III-2). Greater than 90% of the 60 mg/kg to 2310 mg/kg range of DBP dosages was found in the urine (as the parent compound or its metabolite) within 2 days after gavage administration (Kluwe, 1982a). Kaneshima et al. (1978) also found a small amount of intact DBP in the bile of rats given oral doses of the compound. However, it is hypothesized that in most cases PAEs are absorbed from the intestine as monoesters rather than diesters. Absorbtion following the administration of DBP by other exposure routes is not well documented.

DEP. Data regarding the absorption of DEP could not be located in the available literature. Systemic effects observed after oral exposure to DEP (consistent with those observed following exposure to other PAEs) indicate that absorption of DEP does occur.

DMP. Reports of the absorption of DMP are limited to one Russian article on the dermal absorption of DMP for rats and humans. For rats, maximum levels in the blood were reached in 0.5 hours after application to the skin. The metabolites phthalic acid and monomethyl phthalate were found in urine, organs (not otherwise specified) and blood. Similar results were reportedly observed in human volunteers; however, the experimental details were not provided in the abstract (Gleiberman et al., 1978).

Distribution

Once absorbed, PAEs or their metabolites are distributed to various tissues and organs. Kluwe (1982a) has provided a thorough overview of this topic. In general, orally administered PAEs are biotransformed in the intestine to the monoester. Initially these compounds accumulate in various organs, predominantly in the liver. These materials are excreted almost completely within days demonstrating that little long-term accumulation occurs. In the case of humans with compromised kidney function who are on hemodialysis, phthalic acid does accumulate (Pollack et al., 1985b). As Table III-3 indicates, most of the orally administered PAEs are found in adipose tissue, GI tract, kidney or liver.

DEHP. A factor essential in understanding differences in PAE disposition is the role of the route of administration. Distribution of labeled DEHP has been studied after intravenous administration. Daniel and Bratt

TABLE III-3

Distribution of Orally Administered Phthalic Acid Esters[a]

Compound	Species	Dose (mg/kg)	Time[b] (days)	Repository Organs
DBP	rat	60	1	Intestine, adipose, liver, kidney, muscle
	rat	270	1	Liver, kidney, adipose
			2	None
DEHP	rat	500	1	Intestine, stomach, liver, kidney, adipose
	rat	800	1	Liver, kidney, adipose, muscle, testis
			4	Adipose

[a]Source: Kluwe, 1982a

[b]Time between administration and examination

(1974) found 60-70% of a single i.v. dose of radiolabeled DEHP (emulsified in oleic acid) in the liver, lungs and spleen of rats. The compound and metabolites disappeared from the blood rapidly and were detected in these organs within 2 hours. Subsequently, an elimination half-life of 1-2 days from these distribution sites was estimated.

Examination of tissues from two deceased patients who had received transfusions of blood stored in PVC blood bags, revealed DEHP in the spleen, liver, lung and abdominal fat at concentrations ranging from 0.025 mg/g (dry weight) in the spleen to 0.270 mg/g (dry weight) in the abdominal fat (Jaeger and Rubin, 1970).

Tissues were also analyzed for BGBP (butyl glycolyibutyl phthalate) and DEHP, using the isolated perfused rat liver technique. BGBP was recovered from the perfusate as a water-soluble metabolite. DEHP was cleared from the perfusion medium after 60 minutes. Upon analysis of the liver, ~90% of the total recoverable dose remained as unmetabolized compound. The investigators concluded that DEHP is accumulated by the liver primarily as the unmetabolized parent compound (Jaeger and Rubin, 1970).

When whole body autoradiography techniques were utilized in mice after a single i.v. injection of ^{14}C-DEHP (2.293 μg) using sterile mouse plasma as the solubilizing substance, radioactivity was detected in the kidney and liver initially, followed by accumulations in the urine, bile and intestine. After 168 hours (7 days), radioactivity was found in the intestinal lumen (due to secretion of the compound from the liver into the bile), but no radioactivity was detected in the spleen or lung (Waddell et al., 1977).

Lindgren et al. (1982) also investigated the distribution of labeled DEHP with whole body autoradiography. C57Bl mice received ^{14}C-DEHP by oral intubation (soybean oil vehicle) or injection (absolute ethyl alcohol vehicle in the tail vein). Although DEHP administered i.v. was labeled at either the carbonyl group (dose level 3.6 mg DEHP/kg) or at the position of an alcohol (2-ethylhexyl-1-^{14}C) (dose level 9.6 mg DEHP/kg), the distribution of the compound was similar with both labeled forms. Within 4 hours of a single i.v. injection, high levels of activity were found in the gall bladder, intestinal contents, urinary bladder, liver, kidney and brown fat. Lower levels were observed in the white fat, myocardium, muscles, blood, bone, cartilage, testicles and nervous system. The concentrations of DEHP remained high in the gall bladder, intestinal contents, urinary bladder and brown fat 24 hours after the single injection. In mice that were pretreated with either DEHP, sodium phenobarbital or 3-methylcholanthrene before receiving oral doses of labeled DEHP once daily for 5 consecutive days, the concentration of ^{14}C-DEHP in the brown fat was higher than levels found in mice treated with DEHP alone. Further, mice orally dosed with DEHP and then sacrificed at intervals between 5 and 30 days retained the carbonyl-^{14}C-DEHP (but not the 2-ethylhexyl-1-^{14}C-DEHP) in the skin, cartilage and tendons. Low concentrations of DEHP, labeled at either site, were observed in the bone. The authors state that the mechanisms that may underlie accumulation are unresolved. Lindren et al. (1982) attributed high levels in brown fat to induction of mixed function oxidases causing an increased production of DEHP metabolites with affinity to brown fat.

A single dose of labeled DEHP administered i.v. in saline solution in mice resulted in accumulation primarily in the lungs with lesser amounts

occurring in the brain, fat, heart and blood. There was no apparent preference for fatty tissue (Dillingham and Autian, 1973).

In experiments performed by Tanaka et al. (1975), ^{14}C-DEHP (500 mg/kg as a 25% solution) was administered p.o. (solubilized in Tween 80) or i.v. (as a dispersion prepared by sonication of DEHP in saline) to groups of male Wistar rats. DEHP was labeled with ^{14}C at the carbonyl carbon. After oral dosing, liver and kidney concentrations of the compound reached a maximum in 2-6 hours with peak blood levels occurring after 6 hours. Detection of the radioactivity in the liver after the first hour following the i.v. injection revealed that 70-80% of the original dose was deposited in the liver. These initial radioactivity levels in the liver decreased to 50% after 2 hours and 0.17% after 7 days. The results from both p.o. and i.v. administrations demonstrated high levels of radioactivity occurring in the intestine, and lesser amounts in other organs and tissues. However, the testicles and brain showed little affinity for the compound.

In a study performed by Oishi and Hiraga (1982), Wistar rats received a single oral dose of 25 mmol/kg (9.77 g/kg) DEHP by gastric intubation (vehicle not stated). The animals were then examined after 1, 3, 6, 24, 48 and 96 hours. Blood and tissue sample analyses revealed that the concentrations of DEHP and its hydrolysis product, MEHP, reached peak levels within 6-24 hours after dosing. The peak concentrations in the heart and lungs occurred within 1 hour, while fat levels of DEHP and MEHP increased for 2 days. The highest ratio of MEHP/DEHP (mol %) was found in the testes (~210%) while all other tissues sampled exhibited a ratio of ~113% or less.

Minimal amounts (≤ 1 μg/g) of both compounds were detected in the kidney and brain. The lung contained only MEHP while low levels of DEHP were found in the spleen.

The effects of ^{14}C-DEHP in the diet were investigated so that tissue accumulation of DEHP in rats could be examined (Daniel and Bratt, 1974). Groups of 24 female rats were fed diets containing either 1000 or 5000 ppm DEHP (50 or 250 mg/kg/day, assuming 350 g rats consume 0.05 kg food/day) for 35 and 49 days, respectively. Radioactivity was then monitored in the liver and abdominal fat after the food was consumed. The labeled compound was found to increase in these tissues until steady-state concentrations were achieved. Steady-state levels were reached after 1 week in the liver tissues and after 2 weeks in adipose tissue. Upon cessation of DEHP administration, radioactivity in the liver was decreased below the level of detection within 3 weeks. The levels in adipose tissue remained at nearly one-third of the steady-state concentrations after 3 weeks (Daniel and Bratt, 1974).

Jacobson et al. (1977) also demonstrated that DEHP or its metabolites achieve steady-state levels in experiments using rhesus monkeys. The animals received transfusions of blood contaminated with DEHP to yield doses ranging from 6.6-33 mg/kg. The compound or its metabolites were retained in trace amounts (liver, testis, heart and fat) for ≤ 14 months after treatment. As pointed out by both Daniel and Bratt (1974) and Jacobson et al. (1977), there is a steady-state concentration that is reached, after which DEHP (or metabolites) is then rapidly eliminated from the organs or tissues through various routes, thus preventing significant accumulation over long periods of exposure.

Transfer of DEHP and its metabolites from maternal to fetal tissues has been investigated by Singh et al. (1975). In the study, one group of 13 pregnant Sprague-Dawley rats was injected i.p. with a single 5 mℓ/kg (250 mg/kg bw) carboxy-labeled ^{14}C-DEHP dose on day 5 of gestation. A second group of 10 pregnant rats was injected with a single 5 mℓ/kg ^{14}C-DEHP dose on day 10 of gestation. One rat injected on day 5 of gestation was asphyxiated by an overdose of ether 72 hours after the ^{14}C-DEHP injection. The remaining rats were asphyxiated at 24-hour intervals (one rat/24-hour interval) through day 20 of gestation. Rats injected on day 10 of gestation were sacrificed every 24 hours through day 20 of gestation. Radioactivity was detected in the maternal blood, placentas, amniotic fluid and fetal tissue. None of the fetal tissue levels exceeded the maternal blood levels. Less than 1% of the injected dose was detected in the fetal tissue at any of the measured times. Concentrations of radioactivity diminished quickly in amniotic fluid and maternal blood as indicated in Table III-4. The half-life for DEHP was calculated as 2.33 days. Fetal concentrations ranged from 5.9×10^{-4} to 4×10^{-6} mol/kg. Specific metabolites were not identified in this paper. This investigation demonstrates that ^{14}C-labeled material (^{14}C-DEHP and its metabolites) is distributed to the developing rat fetus throughout organogenesis. Further, the authors conclude that the presence of DEHP and it metabolites may act directly on embryonic tissues to induce developmental effects.

Bratt and Batten (1982) observed clear species and sex differences in the tissue retention of DEHP. Rats and marmosets were given 1960 mg/kg/day of ^{14}C-DEHP (^{14}C ring labeled) orally for 14 days. In the rat, the females retained higher concentrations of the ^{14}C-radiolabel in the liver and kidney (286 and 176 μg/g tissue, respectively) than the males (216 and

TABLE III-4

Distribution of ^{14}C-DEHP in Rats Injected i.p. on Either Day 5 or 10 of Gestation[a]

Gestation Day	DEHP Injection Day 5[b]				DEHP Injection Day 10[b]			
	Maternal Blood	Placenta	Amniotic Fluid	Fetal Tissue	Maternal Blood	Placenta	Amniotic Fluid	Fetal Tissue
8	95,098	NR	2577	470				
9	70,285	NR	1177	556				
10	46,297	NR	200	641				
11	22,439	NR	539	890	114,656	NR	6186	13,136
12	20,326	1180	375	176	118,338	NR	983	5,461
13	27,993	1386	683	579	67,243	4447	616	1,263
14	26,837	1672	225	437	30,278	3023	660	1,224
15	11,656	1215	254	453	31,813	5224	1022	2,550
16	10,401	2732	346	1453	26,510	7551	965	2,971
17	8,003	1723	352	2223	19,139	3940	562	2,519
18	6,000	2075	239	1802	24,793	3821	733	4,705
19	6,402	1327	182	1938	15,491	2914	458	1,526
20	4,192	1146	232	1641	6,022	3085	275	726

[a]Source: Singh et al., 1975

[b]Total counts (dpm) in tissues assuming total blood in the rat to be 7% of its body weight.

NR = Not reported

115 μg/g tissue, respectively). In addition, male rats retained 36 μg DEHP/g tissue in the testis. A similar pattern was observed in the marmoset. Female marmosets retained 47 and 35 μg DEHP/g in the liver and kidney, respectively, whereas male marmosets retained 29 and 15 μg DEHP/g in the liver and kidney, respectively. Testis concentrations reached 8 μg/g tissue in the marmoset. The rats of both sexes retained higher tissue concentrations of ^{14}C-radiolabel than did the marmosets.

Similar results were also reported by Rhodes et al. (1986) in a comparison of the blood and tissue levels of DEHP and its metabolites in the rat and marmoset. The animals were administered 2000 mg/kg/day of ^{14}C-DEHP (labeled in the phenyl ring) for 14 days. The level of ^{14}C-radiolabel in the marmoset tissues was only 10-20% that of the rat. In both the rat and marmoset the liver retained the highest level of ^{14}C-radiolabel.

Lindgren et al. (1982) examined the distribution of ^{14}C-DEHP administered to pregnant C57Bl mice at gestation day 8 and 16 by oral intubation. Dose levels administered at day 8 of gestation corresponded to 7.7 mg DEHP/kg (2-ethylhexyl-1-^{14}C) and 2.9 mg DEHP/kg (carbonyl-^{14}C). Dose levels administered at day 16 of gestation corresponded to 4.8 and 1.8 DEHP/kg of (2-ethylhexyl-1-^{14}C) and (carbonyl-^{14}C) labeled DEHP, respectively. Uptake of the ^{14}C labeled DEHP was not quantified. Whole body autoradiography revealed that at early gestation (8 days), uptake occurred in the yolk sac with high concentrations of (carbonyl-^{14}C) labeled DEHP in the gut at 4 hours after treatment. Twenty-four hours after administration of (2-ethylhexyl-1-^{14}C) labeled DEHP, activity in the neuroepithelium was observed. At late gestation (16 and 17 days), accumulations of either ^{14}C

labeled DEHP were also high in the yolk sac. The fetuses on days 16 and 17 of gestation were found to have high concentrations (levels not quantified) of either ^{14}C labeled DEHP in the renal pelvis, urinary bladder and intestinal contents. Lesser amounts (levels not quantified) were detected in the liver and the mineralized portions of the fetal skeleton. Some skeletal uptake of DEHP was also noted.

The placental transfer of DEHP was examined in guinea pigs by Kihlström (1983). The author utilized a placental perfusion technique and determined that the solution employed as a perfusion medium may affect the placental transport of DEHP. The level of DEHP administered was not reported. The maternal liver uptake and total placental uptake of DEHP was calculated after a constant plasma concentration was reached by catheter infusion into the vena jugularis. Maternal hepatic uptake was estimated to be 41% of the dose, while total placental uptake was ~13-15% of the dose. A significant difference ($p<0.001$) of 0.21 ± 0.09 and 0.47 ± 0.10 ppm of the total dose/mg was found between the DEHP concentrations in the fetal plasma and that of the final perfusion media, respectively, indicating that the compound was distributed in the fetal tissues. In addition, placental tissue concentrations of DEHP were much larger (7.5 ± 2.5 ppm of total dose/mg tissue) than the levels in the perfusion plasma (0.47 ± 0.10 ppm of total dose/mg plasma), indicating that the greater part of the DEHP taken up by the placental tissues does not enter into the fetal circulation.

<u>BBP</u>. The distribution of BBP has been studied in rats following both i.v. and oral administration. Eigenberg et al. (1986) evaluated the disposition of BBP after an i.v. dose of 20 mg/kg to male Fischer-344 rats.

Brain, lung, liver, kidney, spleen, testes, small intestines, renal fat, muscle (thigh) and skin (abdominal) were removed and examined. BBP was rapidly distributed to the tissues and eliminated. The initial half-life was <30 minutes and the terminal half-life was 4.5-7.3 hours. Elimination from the blood and fat was mono-exponential whereas elimination by the kidney, muscle, skin and small intestine followed a biexponential decay curve. Since BBP is rapidly metabolized (see Metabolism Section) and eliminated, it is not sequestered in fat.

Lake et al. (1978) observed similar results in male Sprague-Dawley rats. Oral doses of 16, 160 or 1600 mg/kg were administered by oral intubation. At the end of 5 days animals were sacrificed and examined. Radioactive residues were present in the liver, kidney, small intestine and total gut contents. However, the residues present were <1% of the administered dose. There was no evidence of tissue accumulation.

DBP. Tanaka et al. (1975, 1978) compared the distribution of ^{14}C-DBP (labeled in the carbonyl moiety) and ^{14}C-DEHP with male Wistar rats after single i.v. or p.o. doses. Few differences were observed in the distribution pattern of DBP compared with that of DEHP. Following i.v. administration DBP did not accumulate in the liver to the same extent as DEHP. After 1 hour ^{14}C levels in the liver were 6% of the total i.v. dose for DBP while DEHP had been detected at 76% of the total i.v. dose. Retention of DBP in the heart, lung and spleen 24 hours after oral or i.v. exposure appeared to be shorter than DEHP in these organs. Affinity for adipose tissue appeared to be higher following i.v. or oral administration for DEHP than for DBP after 24 hours.

The distribution of DBP has been studied with rats administered the compound in the diet or by gavage (corn oil vehicle). Williams and Blanchfield (1975) added 1000 mg/kg of DBP-7-^{14}C to the diets of 24 male Wistar rats for 12 weeks. No substantial accumulation of DBP or MBP was detected at 4, 8 or 12 weeks in any of the organs and tissues. Four hours after a single (0.27 or 2.31 g/kg ^{14}C-DBP) intubated dose was administered to rats, the label was detected throughout the body. Yet, within 48 hours the tissues and organs contained only traces of radioactivity. Clearance of the labeled DBP was more rapid at the lower dosage.

<u>DEP</u>. Singh et al. (1975) investigated the maternal-fetal transfer of carboxy-labeled ^{14}C-DEP in rats. Thirteen pregnant Sprague-Dawley rats were injected i.p. with a single 1.0116 mℓ/kg (51 mg/kg bw) ^{14}C-DEP dose on day 5 of gestation. Another group of 10 rats was injected with the same amount of ^{14}C-DEP on day 10 of gestation. The group of rats injected on day 5 of gestation were sacrificed by an overdose of ether 72 hours after the ^{14}C-DEP injection and then at 24-hour intervals through day 20 of gestation. Rats injected on day 10 of gestation were sacrificed in the same manner every 24 hours through day 20 of gestation. As with DEHP, radioactivity was detected in maternal blood, placentas, amniotic fluid and fetal tissue at both gestational stages (5 and 10 days) and <1% of the injected dose was detected in the fetal tissue at any of the measured times. The concentrations of radioactivity diminished quickly in maternal blood as indicated in Table III-5. Based on a first-order excretion curve, the half-life for DEP was calculated to be 2.22 days. Fetal concentrations ranged from 1.5×10^{-4}-2.8×10^{-6} mol/kg. Specific metabolites of DEP were not identified in this study. DEP and its metabolites were present in the

TABLE III-5

Distribution of ^{14}C-DEP in Rats Injected i.p. on Either Day 5 or 10 of Gestation[a]

	DEHP Injection Day 5[b]				DEHP Injection Day 10[b]			
Gestation Day	Maternal Blood	Placenta	Amniotic Fluid	Fetal Tissue	Maternal Blood	Placenta	Amniotic Fluid	Fetal Tissue
8	29,811	NR	11	333				
9	27,180	NR	21	562				
10	19,422	NR	61	372				
11	9,204	447	131	145	50,145	871	471	317
12	14,376	390	198	124	41,497	1434	824	216
13	6,258	412	128	107	33,645	1549	428	332
14	7,491	572	158	210	27,423	1424	390	333
15	4,806	613	231	228	27,081	824	898	887
16	3,804	1245	167	507	18,546	799	526	664
17	3,444	1313	176	672	11,257	800	583	1808
18	3,661	1347	166	947	6,068	1775	364	1820
19	2,479	831	122	916	3,892	1473	257	1695
20	2,848	965	116	1185	2,674	856	101	1176

[a]Source: Singh et al., 1975

[b]Total counts (dpm) in tissues assuming total blood in the rat to be 7% of its body weight.

NR = Not reported

developing rat fetus during organogenesis. Singh et al. (1975) suggest that the presence of DEP and its metabolites may act directly on embryonic tissues in the induction of teratogenicity.

DMP. Data regarding the distribution of DMP could not be located in the available literature.

Metabolism

Dialkyl phthalates are hydrolyzed to monoesters in the intestine and other organs and tissues before and after absorption. The rate of hydrolysis is greater for the lower molecular weight esters such as DMP and DBP (see Table II-1 for weights). Although both ester linkages of PAEs can be hydrolyzed to produce phthalic acid, only small fractions of the long-chain alkyl phthalates undergo such complete conversion. The metabolic profiles of single doses of phthalates may differ from profiles after multiple exposures; some phthalates such as DEHP and MEHP have been shown to induce their own metabolism. Thus, duration of exposure, the dose level administered and the status of the animal with respect to the metabolic pathway of peroxisomal proliferation must be considered when evaluating studies on the metabolism of phthalates.

DEHP. Numerous studies have focused upon the metabolic profile of DEHP. Albro et al. (1973) identified the first step in the rat metabolism of orally administered DEHP in rats as the conversion of the diester to the monoester, MEHP. Two distinct alcohol intermediates are formed by ω- and ω-1 oxidation of the monoester sidechain. Just as is the case for DBP metabolism as noted by Albro and Moore (1974), oxidation of these alcohols

results in the generation of carboxylic acid (which can be further oxidized to a ketone.) Figure III-1 shows a number of products that can be formed from metabolism of orally ingested DEHP in rats. Albro et al. (1983b) postulated that oxidation of the aliphatic side chain of DEHP or MEHP may involve placement of the hydroxyl group at positions more distant than ω-1 from the terminal methyl group. Based upon the discovery of highly polar metabolites in the urine of rats, given two gavage administrations of DEHP or MEHP, the authors then hypothesized that attacks by oxygen species may occur concurrently at two sites, or that an oxidized metabolite may receive a second oxidation.

Sjöberg et al. (1985a) supported the Albro et al. (1983b) hypothesis and further studied the four major metabolites of DEHP. The four metabolites studied were MEHP, mono-(5-carboxy-2-ethyl pentyl) phthalate, mono-(2-ethyl-5-oxohexyl)phthalate and mono-(2-ethyl-5-hydroxyhexyl) phthalate and the metabolites will be referred to as MEHP, Met V, Met VI and Met IX, respectively. The primary metabolite MEHP was studied separately. Thirty-four male Sprague-Dawley rats (40 days old) were administered a single cannulated infusion over a 3-hour period of either 5, 50 or 500 mg/kg bw DEHP. Blood samples were drawn 1, 2, 3, 3.5, 4, 6, 8, 11, 14, and 24 hours after the infusion. Elimination patterns of DEHP and MEHP were similar in the groups administered 5 and 50 mg/kg DEHP. Plasma concentrations of DEHP were much higher at all times than MEHP, and MEHP plasma levels were much higher than Met V, VI and IX. Plasma concentrations of Met V, VI and IX could not be detected 6 hours after the infusion of 5 mg/kg DEHP. Sjöberg et al.

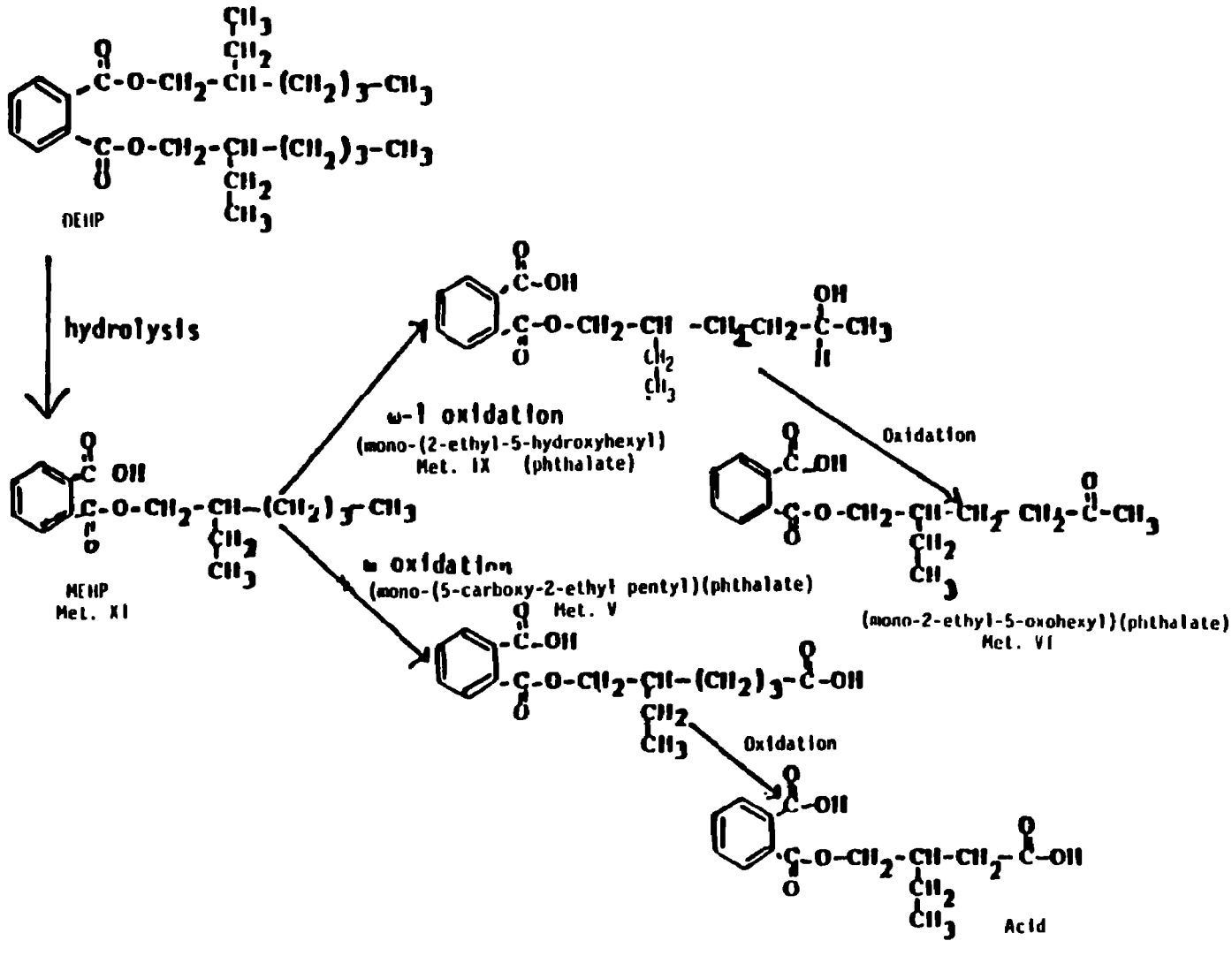


FIGURE III-1

Routes of Metabolism of DEHP

Source: Adapted from Albro et al., 1973; Sjöberg et al., 1985a
(For more detail see Figure III-4)

(1985a) suggested that the parallel decrease observed in the plasma concentrations of DEHP, MEHP and the metabolites IX and V (Figures III-2 and III-3) indicate that the elimination of DEHP is the rate-limiting step in the depositions of these metabolites. The shape of the plasma concentration-time curve also indicated that the elimination of MEHP was rate-limited by its formation. The investigators stated this was verified by the observation that the clearance of MEHP when given separately was higher than that of the parent compound.

Although several species of animals have been observed to excrete glucuronide conjugates of MEHP upon exposure to DEHP, rats are an exception (Tanaka et al., 1975; Williams and Blanchfield, 1975; Albro et al., 1982). Table III-6 illustrates the rat's inability to excrete the MEHP glucuronide, but not MBP derivatives in comparison to other mammals.

Studies performed by Lake et al. (1984a) demonstrated that a single orally administered dose of ^{14}C-DEHP at 100 or 1000 mg/kg was metabolized to a greater extent in the rat than in the hamster. Although similar amounts of radioactivity were recovered in the urine and feces of both species, fecal extracts contained only unchanged DEHP in the hamster while in the rat, ~50% of the radioactivity occurred as metabolites (specific metabolites not identified), possibly including MEHP. After 96 hours only negligible amounts of radioactivity were present in either the liver, kidney or total gut contents of both species.

Lake et al. (1976) examined urine samples from rats and ferrets treated orally with ^{14}C-DEHP. In ferrets the compound was hydrolyzed to MEHP and

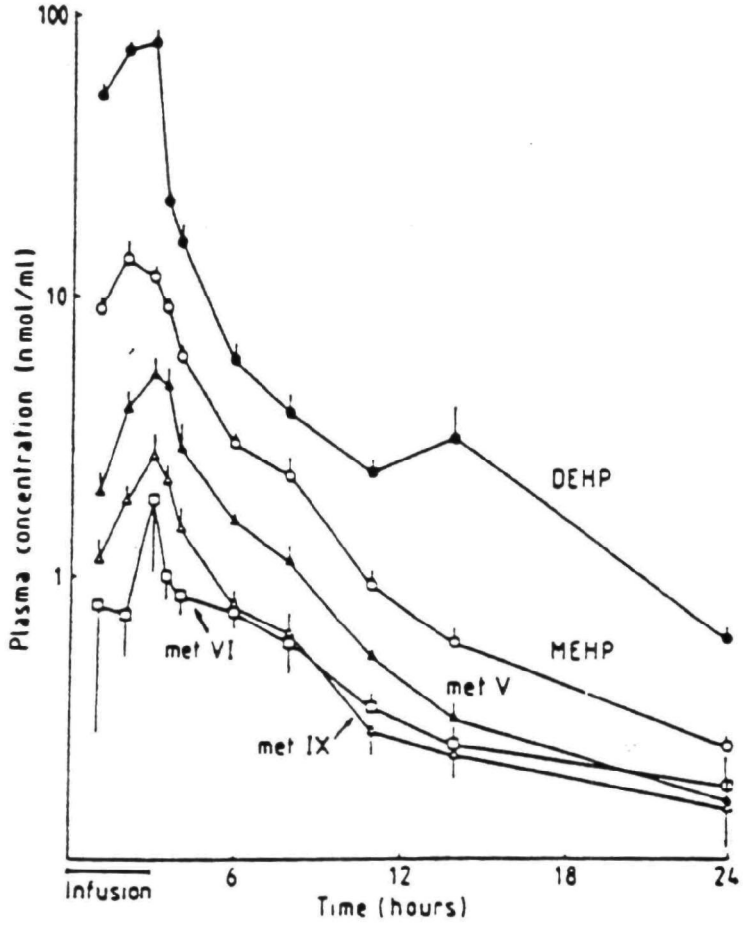


FIGURE III-2

The Mean Plasma Concentration-Time Curves of DEHP and its Investigated Metabolites in Rats Infused with 50 mg/kg DEHP

Source: Sjöberg et al., 1985a

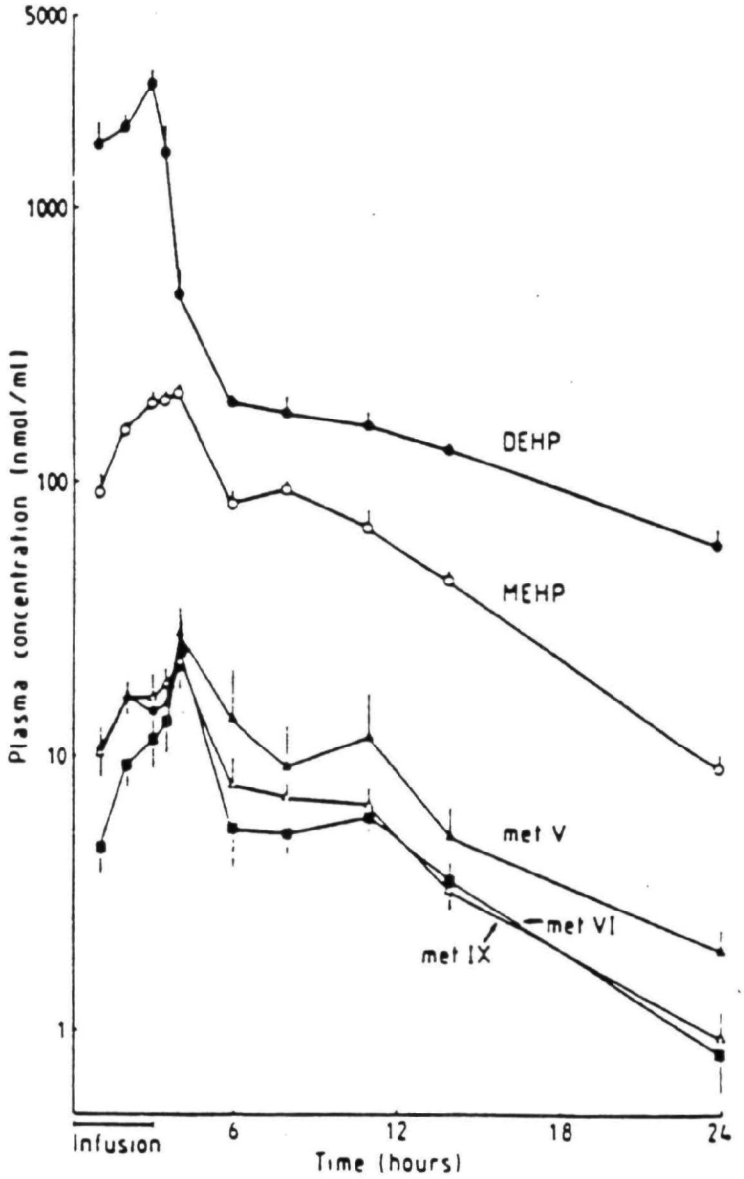


FIGURE III-3

The Mean Plasma Concentration-Time Curves of DEHP and its Investigated Metabolites in Rats Infused with 500 mg/kg DEHP

Source: Sjöberg et al., 1985a

TABLE III-6

Synthetic Metabolism Of Phthalic Acid Esters*

Compound	Species	Route	Dose	Conjugated Metabolites
DBP	rat	gavage	500 mg/kg	MBP-glucuronide
	rat	gavage	60 mg/kg	MBP-glucuronide derivatives
	guinea pig	NR	NR	MBP-glucuronide derivatives
	hamster	NR	NR	MBP-glucuronide derivatives
DEHP	rat	various	various	None
	ferret	gavage	600 mg/kg	MEHP glucuronide derivatives
	monkey	i.v.	NR	MEHP glucuronide derivatives
	human	i.v.	94-171 mg	MEHP glucuronide derivatives

*Source: Kluwe, 1982a

NR = Not reported

ultimately excreted in the urine as free and glucuronide conjugated MEHP derivatives. Metabolism in the rat also produced MEHP derivatives; however, glucuronide conjugates of MEHP were absent from the urine indicating the rat's inability to excrete glucuronide conjugates of MEHP. Otherwise, the authors stated that the two species metabolized DEHP similarly. In addition, Rhodes et al. (1986) demonstrated that the metabolism of DEHP by marmoset monkeys is comparable with that of other primates and shows the same characteristic differences from the rat as other primate species.

In another study, Lake et al. (1977) compared species metabolism of DEHP and other PAEs in hepatic tissue preparations. The authors compared male Sprague-Dawley rats, male albino ferrets and male olive baboons. In the liver homogenates from baboons and ferrets the order of hydrolysis of the tested diester PAEs to their monoester forms was DEHP < DBP < DEP < DMP. The rates of hydrolysis in preparations from rats were in the order of DEHP < DBP < DMP < DEP. The rates of hydrolysis for DEHP were slower (statistical analysis not reported) than the other PAEs in all three species examined. Diester hydrolase activity in liver homogenates generally increased in the order ferret, rat, baboon. The authors stated that the baboon, rat and ferret would be suitable for assessing toxicity in man since the results show species similarities in their hydrolysis of PAEs.

Lhuguenot et al. (1985) investigated the metabolism of DEHP and MEHP after multiple administration in rats. Male Alderley Park (Wistar derived) rats were gavaged with daily doses of 50 or 500 mg/kg DEHP or MEHP in corn oil for 3 consecutive days. Rats were grouped three/dose group for each

chemical. Urine samples were collected from each animal at 24-hour intervals for 4 days. Water-soluble conjugates were not detected in rat urine after DEHP or MEHP administration. A novel metabolite (XII), however, was detected in the urine after administration of both compounds (Figure III-4). The proportion of the daily doses excreted in the urine reached a steady-state within 48 hours of multiple exposure to DEHP or MEHP. At 50 mg/kg DEHP and MEHP there were essentially no changes in the metabolic profile when expressed as a percentage of total metabolites. At the 500 mg/kg dose level quantities of metabolites I and V were increased 6.4- and 2.5-fold, respectively. When individual metabolites were expressed as percentages of total metabolites at the 500 mg/kg dose level, metabolites I and V increased with time while the proportion of metabolites IX and VI decreased with time. Multiple dosing with 500 mg/kg MEHP increased the quantity of metabolite I 4.1-fold. Small decreases in metabolites VI and IX were also observed. After 3 consecutive days of 500 mg/kg MEHP treatment, hepatic peroxisomal ß-oxidation (as measured by the enzyme palmitoyl-CoA) increased 4-fold. The authors concluded that the metabolism of metabolite V is by peroxisomal ß-oxidation of the ω-oxidated MEHP since at the 500 mg/kg dose level there was a 4-fold increase of peroxisomal ß-oxidation and hence an increase in metabolite I. A 2-fold decrease in peroxisomal ω-1 oxidation products was also observed. The increase in ω-oxidation in the absence of an increase in ω-1 oxidation, may imply the involvement of a cytochrome P-450 with a high specificity for ω-hydroxylation. Lhuguenot et al. (1985) also confirmed that MEHP is metabolized by the same pathways as DEHP in rats. Peroxisomal ß-oxidation enzyme system is important since peroxisomes contain enzymes involved in fatty acid ß-oxidation, which generates hydrogen peroxide. Turnbull and Rodricks (1985) and Rodricks and Turnbull (1987)

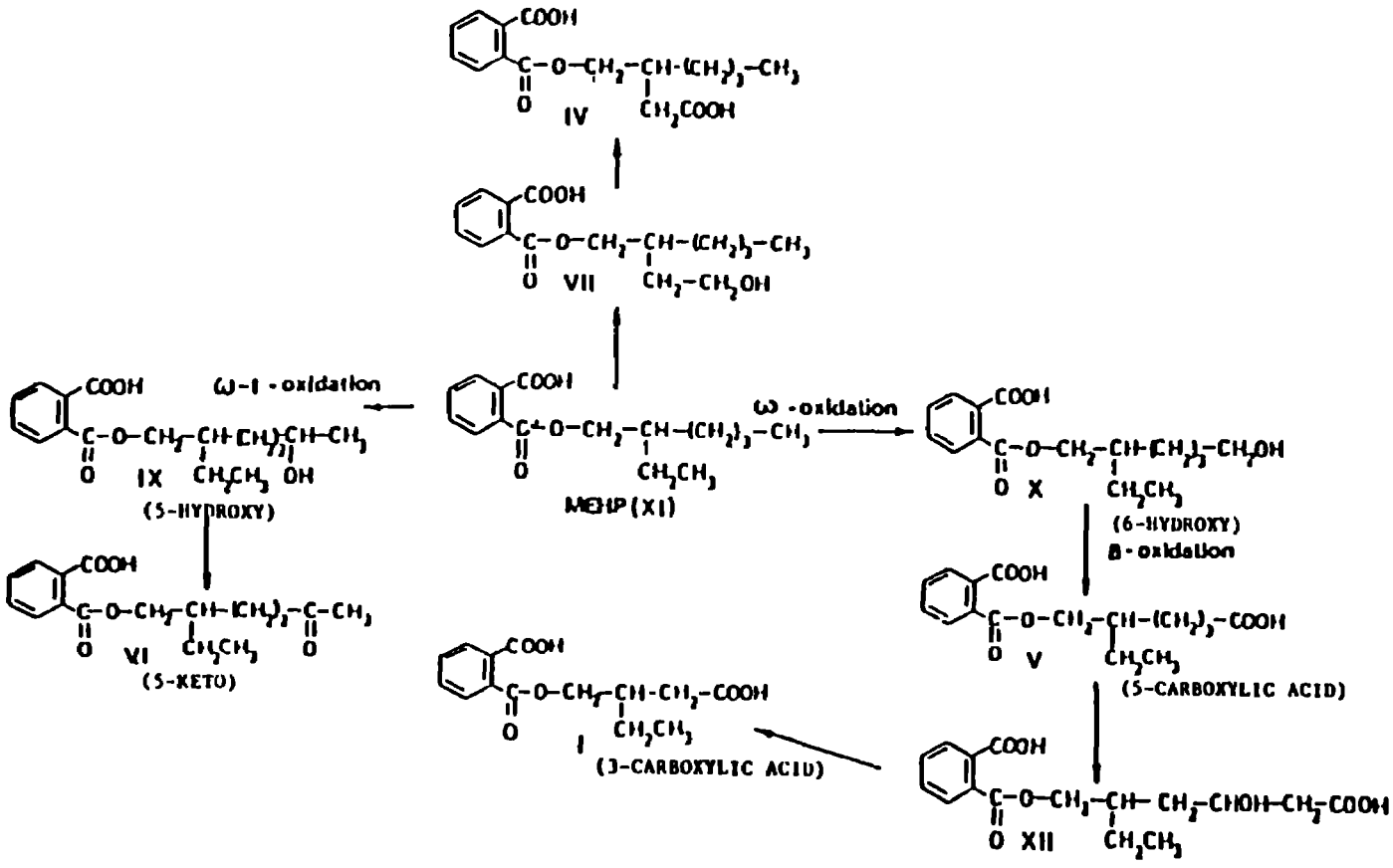


FIGURE III-4

Routes of Metabolism of MEHP in Rats
(metabolites are numbered according to Figure III-1)

Source: Adapted from Lhuguenot et al. (1985), Albro et al. (1973) and Albro and Moore (1974)

hypothesized that the process of hydrogen peroxide formation by the peroxisomes is responsible for DEHPs carcinogenic effects. For a more detailed explanation of the mechanisms involved see Chapter VII.

Short et al. (1987) demonstrated that monkeys have a lower capacity to metabolize DEHP by β-oxidation than rats. Fischer 344 rats (12 males/group) were fed diets containing 1000, 6000 or 12,000 ppm DEHP (50, 300 or 600 mg/kg/day assuming 350 g rats consume 5% of their body weight). Three subgroups were formed for each dose and received the above diets for 0, 6 or 20 days. They received a similar dietary level of ^{14}C-carbonyl labeled DEHP for 1 day. Cynomolgus monkeys (2 males/group) received 100 and 500 mg/kg/day by gavage for 21 days. Each monkey then received a single dose of ^{14}C-DEHP followed by three additional daily dosages of DEHP. In rats, urinary elimination of metabolite 1 was constant with all dietary levels on day 0; however, it increased with all dose levels by day 6. The metabolites are numbered according to Figures III-1 and III-4. The increased percentage of metabolite I in the urine persisted for 20 days. In contrast, metabolite V increased with dietary level on day 0 but decreased with dietary level from days 6 to 20. Urinary levels of MEHP and metabolite X in monkeys appeared to increase with repeated doses of DEHP. There was no increase in the conversion of metabolite X to metabolites V and I. The output of metabolites IX and VI was unchanged or slightly decreased suggesting little change in the ω-1 oxidation pathway.

In a second portion of this study, Short et al. (1987) did not find any treatment-related evidence of hepatic peroxisomal proliferation in monkeys at DEHP levels ≤500 mg/kg/day. Whereas, exposure to similar levels (11,

105, 667, 1223 and 2100 mg/kg/day) of DEHP in rats produced hepatic peroxisomal proliferation. It is difficult to compare exposure levels since monkeys were administered bolus doses and rats were administered feed. However, Short et al. (1987) stated that the doses are in a comparable range. The authors concluded that urinary levels of metabolite I serve as a useful marker to detect peroxisomal induction activity and hence may serve as a marker for making interspecies comparisons. As a result rats may not provide a good basis for predicting the possible carcinogenicity in higher primates if peroxisome proliferation is indeed the mechanism or one of the mechanisms of action.

Schmid and Schlatter (1985) found that a single oral dose of DEHP taken by two volunteers (30 mg each) was excreted in the urine as DEHP metabolites within 24 hours. Only 11 and 15% of the dose was eliminated as metabolites in the urine with the remainder most likely eliminated in the feces (details not provided). The urinary metabolites [derivatives of mono(2-ethylhexyl) phthalate] were enzymatically hydrolyzed and methylated for identification. The quantitative distribution of conjugated and free metabolites determined was by gas liquid chromatography-mass spectrometry. Twelve metabolites were detected, the four major ones being free and conjugated forms of the monoester (MEHP) and its 5-carboxylic acid (metabolite V), 5-keto (metabolite VI) and 5-hydroxy (metabolite IX) derivatives (see Figures III-1 and III-4). These same few metabolites were also observed in rats (Sjöberg et al., 1985a,d, 1986a), thus indicating that humans and rats share common metabolites. Two of the major metabolites involved oxidation of the terminal (C-6) methyl group to the corresponding acid, a biotransformation process that also occurs in the rat (Watts, 1985).

Rowland (1974) found that Wistar and Sprague-Dawley rat intestinal and caecum contents degraded DEHP. However, there were differences between the two strains. The authors stated that DEHP was degraded to a single metabolite, which was identified as MEHP. Wistar rats were fed diets of 2% w/w ^{14}C carboxyl labeled DEHP and were found to degrade DEHP at the rate of ~1300 μg/g intestinal contents/16 hours or 700 μg/g caecal contents/16 hours. The maximum rate of degradation occurred at pH 7.0, which is the approximate pH values of the intestines and caecum in rats. When a mixture of the antibiotics tetracycline hydrochloride, neomycin sulphate, chloramphenicol and streptomycin sulphate were added to the incubation mixture at 2 mg/mℓ each, the breakdown of DEHP by the caecal contents was reduced from ~700 to 300 μg/g caecal content/16 hours. The mixture of antibiotics had a similar effect on DEHP degradation in the contents of the small intestines (Rowland, 1974).

In the same experiment, degradation of DEHP by caecal and intestinal contents increased ~3-fold in Sprague-Dawley rats fed 2% w/w DEHP when compared with rats fed a standardized diet. The increased rate of DEHP metabolism by the small intestine was >60% in the DEHP-fed rats as compared with only 18% by the intestinal content of rats fed only the standardized diet. Addition of the mixture of antibiotics tetracycline hydrochloride, neomycin sulphate, chloramphenicol and streptomycin sulphate at 2 mg/mℓ each had no effect on the rate of DEHP breakdown by the caecal or intestinal contents. Changes in the microbial flora of the alimentary tract were compared with the controls in the Sprague-Dawley rats. In both regions the total number of bacteria were lowered by a factor of 10 in the DEHP-fed rats. The microbial flora in the proximal small intestines of DEHP-fed rats consisted of bifidobacteria, bacteroides and lactobacilli, whereas the

proximal small intestines of the controls harbored a wide variety of bacterial types. The differences in the microbial flora between the caecum and the distal region of the small intestines of DEHP-fed rats and controls were negligible.

Rowland (1974) concluded that gut flora "play only a minor role" in the metabolism of DEHP in the alimentary tract of Sprague-Dawley rats since in the presence of antibiotics the small intestines and caecum still metabolized DEHP at ~50% of the control rate. The author also found that the increase in rate of DEHP degradation and change in microbial flora in the alimentary tract did not occur in the Wistar rats fed DEHP. No explanation was given for these findings and no other data was provided for the rate of metabolism of DEHP-fed Wistar rats. Thus, the results support the hypothesis that the increased rate of DEHP degradation is due to enzyme induction in the mucosal cells of the intestine.

In another study Rowland et al. (1977) confirmed that phthalate esters are metabolized to the corresponding monoester by the GI contents in rats and in cultured human feces. However, the rates of hydrolysis were greatest in the presence of rat small intestine contents and much slower with caecal or stomach contents. The percentage of metabolized DEHP by 16 hours post-treatment in the stomach, small intestine, caecum and human feces were 1.0 ± 0.2, 22.1 ± 0.5, 6.9 ± 1.0 and 0.6 ± 0.2, respectively. Each value represents the mean $\pm$ SEM of four incubations.

The metabolism of ^{14}C-labeled DEHP in the tissue homogenates of young (45 day old) and old (630 day old) male Sprague-Dawley rats has been studied by Gollamudi et al. (1983). The metabolite MEHP was identified in liver,

kidney and lung homogenates incubated in a mixture of 0.5 μCi ^{14}C-labeled DEHP and a concentration of unlabeled compound to yield a final concentration of 1 mM DEHP. An unidentified metabolite was present in the homogenates of the 630-day-old rats but not 45-day-old rats. The formation of MEHP was decreased by 14% ($p<0.001$) (expressed as dpm/mg protein) in the liver homogenates of the old rats. In contrast, the lung and kidney homogenates did not show any significant change in MEHP formation (measured as dpm/mg protein).

Pollack et al. (1985a) investigated the differences in the route of administration of DEHP in rats following single or multiple injections by i.p., i.a. or p.o. administration, and found that the formation of monoethylhexyl phthalate (MEHP) from DEHP was route-dependent. Following p.o. administration of DEHP, 80% was converted to MEHP, while only 1% MEHP was seen following single doses by i.a. or i.p. Agarwal (1986) stated that i.p. administration converts DEHP to MEHP much slower because of the limited hydrolyzing capacity of visceral organs.

Gollamudi et al. (1985) investigated the rates of DEHP hydrolysis in the tissues of adult Sprague-Dawley male and female 45-day-old rats, fetuses on day 19 of gestation, newborns within 12 hours of parturition, pregnant dams and the placenta on day 19 of gestation. Placenta and fetuses were removed on day 19 of gestation for the hydrolysis study. Tissues from the fetuses of each rat were pooled. Neonates were sacrificed within 12 hours of birth. The conversion of DEHP to MEHP by the liver and for placenta preparations (analyzed as organ-to-whole-body metabolism) was significantly ($p<0.05$) less active in the placenta < fetus < neonate than in the adult male and female rats and pregnant dams, whereas the conversion by the lung

and kidney preparations (analyzed as organ-to-whole-body metabolism) was in the order of fetus < neonate < placenta < adult female < adult male < pregnant dams. The tissues of the fetuses and neonates showed significant ($p<0.05$) DEHP hydrolysis activity.

Peck et al. (1979) examined humans receiving DEHP-laden platelet concentrates. Urinary metabolites included MEHP, eight oxidized derivatives of the monoester (the predominant species being mono-2-ethyl-3-carboxyl-propyl phthalate) or 5-ethyl-isohexanol monoester of phthalic acid and trace amounts of intact DEHP (Peck and Albro, 1982). In contrast to the rats inability to excrete the MEHP glucuronide conjugates (Tanaka et al., 1975; Albro et al., 1982; Williams and Blanchfield, 1975), ~90% of the metabolites were excreted in the human urine as glucuronide conjugates, while the remaining ~10% was excreted in feces (Peck et al., 1979).

Both primates and humans exhibited similar metabolic profiles. Peck and Albro (1982) described DEHP metabolism in studies with African green monkeys. The experiments simulated human blood transfusion exposures to DEHP by impregnating PVC plastic strips with ^{14}C-carbonyl labeled DEHP. The strips were immersed in plasma, which in turn was infused into the monkeys. The predominant metabolic products in the urine included the 5-ethyl-isohexanol monoester of phthalic acid and MEHP. As in the previously described study by Peck et al. (1979) with humans, >90% of the urinary metabolites in the monkeys were glucuronide conjugates.

Evidence submitted by Albro et al. (1982) indicated that mice, guinea pigs and hamsters also excrete glucuronides of MEHP following single oral

exposures to (carbonyl labeled) [7-^{14}C]-DEHP (cotton seed oil vehicle). In each species these conjugates of DEHP metabolites comprised at least 64% of the urinary metabolites detected.

von Däniken et al. (1984) found that when rats (F344) and mice (NMRI) were pretreated with DEHP (10 g/kg) in the diet for 2-3 weeks and then given radiolabeled ^{14}C-DEHP by gavage (in olive oil), the metabolism of the subsequent dose of ^{14}C-carboxyl labeled DEHP was increased. Exhaled $^{14}CO_2$ from the degradation products of ^{14}C-DEHP was generated over a shorter time period for pretreated animals as compared with nonpretreated rats. Liver DNA was isolated 16 hours after treatment with ^{14}C-carboxylate labeled DEHP and analyzed for radioactivity. No evidence for covalent binding of ^{14}C-labeled DEHP or metabolites to liver DNA in either species was detected.

In studies where 200 mg/kg ^{14}C-carbonyl labeled DEHP was administered intravenously to groups of rats, the blood levels of radioactivity were used to estimate the biphasic disappearance of DEHP. The half-life values correspond to 9 and 22 minutes (Schultz and Rubin, 1973). Within 1 hour, 8% of the dose was detected as water soluble metabolites in the liver, intestinal contents and urine. After 24 hours, 54.6% of the dose was found as the water soluble metabolites in the intestinal tract, excreted feces and urine. Only 20.5% was recovered in organic extractable form.

Rubin (1976) injected rats intravenously with an emulsified form of DEHP (dose not reported) resulting in biexponential disappearance of blood DEHP. Blood half-lives of 3.5 and 35 minutes were determined. However, when the

DEHP was solubilized without surfactant, disappearance was monoexponential with a half-life of 19 minutes. Further studies in humans with DEHP solubilized without surfactant also yielded a monoexponential rate of compound disappearance where the mean half-life was calculated at 28 minutes.

BBP. Only one study was found regarding the metabolism of BBP. Eigenberg et al. (1986) identified the major urinary metabolites after rats were administered oral doses of 2, 20, 200 and 2000 mg/kg BBP. Urinary metabolites consisted of monophthalate (MP), monophthalate-glucuronide (MP-glucuronide) and "unidentified" metabolites. At 200 mg/kg BBP the amount of free MP and the ratio of free to conjugated MP was greater than at 2 and 20 mg/kg. At 2000 mg/kg BBP there was a shift to primarily fecal elimination (72%) with only 22% of the dose excreted in the urine.

Four hours after i.v. administration of 20 mg/kg BBP, rats excreted 55% of the total ^{14}C dose in the bile and 34% of the total ^{14}C dose in the urine (Eigenberg et al., 1986). Biliary metabolites were identified as large quantities of monobutyl phthalate glucuronide (MBuP-glucuronide) and monobenzyl phthalate glucuronide (MBeP-glucuronide), (26 and 13% of the dose, respectively), trace amounts of free MBuP and MBeP (1.1 and 0.9% of the dose, respectively) and unidentified metabolites (14% of the dose).

Eigenberg et al. (1986) concluded that the BBP-treated rats major urinary metabolites were MP and MP-glucuronide, in contrast to the rats inability to excrete the glucuronide conjugates of MEHP upon exposure to DEHP (Tanaka et al., 1975; Williams and Blanchfield, 1975; Albro et al., 1982). As the oral dose of BBP increased, there appeared to be a decrease

in the ratio of MP-glucuronide to unconjugated MP metabolites. After i.v. administration, reduced amounts of glucuronide were observed.

DBP. The urinary metabolites of orally administered DBP were studied by Albro and Moore (1974). Doses of 0.2 mℓ DBP (599 mg/kg/day assuming 0.350 kg rats) were administered by gavage to adult male CD rats at 24-hour intervals. Urine samples were collected ≤48 hours after the initial dosing. DBP was converted to six metabolic products with a total of 24.6% of the phthalate moiety recovered 48 hours after the first feeding and 24 hours after the second feeding. DBP was detected, to a lesser extent (0.1%), as the intact ester in rat urine. Each metabolite could be resolved by HPLC; however, the complete structures could not be identified. Metabolism of DBP was characterized largely by hydrolysis of one ester bond and terminal (ω) and subterminal (ω-1) oxidation to primary and secondary alcohols, which were ultimately oxidized to acid and ketone species, respectively.

Urinary metabolites of ^{14}C-DBP in the rat, guinea pig and hamster were determined as MBP, the MBP glucuronide, phthalic acid, unchanged DBP and ω- and ω-1 oxidation products of MBP (Tanaka et al., 1978). Hydrolysis of DBP was found to occur primarily in the liver with some contribution from the intestinal microflora. Similarly, Williams and Branchfield (1975) identified the urinary metabolites of a single oral dose of ^{14}C-DBP in the rat to be phthalic acid, MBP and two other methylated metabolites.

In experiments by Foster et al. (1982), the major urinary metabolite detected in both species after p.o. treatment (no vehicle stated)

of male Sprague-Dawley rats and DSN hamsters with ^{14}C-labeled DBP (2 g) or MBP (800 mg) was the MBP glucuronide. Most of the MBP (17.4% in rats and 6.3% in hamsters) and metabolites (as measured by HPLC) were excreted as glucuronide conjugates (47.8% in rats and 56.9% in hamsters) and not as the free acid. Further studies indicated that after oral administration of DBP or MBP, the levels of free unconjugated MBP in the urine were 3- to 4-fold higher in the rat than in the hamster. Intestinal esterase activities were comparable in the two species, but testicular β-glucuronidase activity was significantly higher ($p<0.001$) in the rat than in the hamster. The increased level of β-glucuronidase activity in the testicular tissue of rats suggests that the levels of free MBP available to hamsters testes would be much lower than in the case of the rat. MBP produced cell injury to cultured sertoli and germ cells much more effectively than DBP (Gray, n.d.). Increased free MBP may account for species susceptibility to MBP- or DBP-induced testicular damage. In conclusion, the authors state that the major urinary metabolite of MBP or DBP is the MBP glucuronide and not the free acid.

Rowland et al. (1977) examined the rate of DBP hydrolysis to the monoester in suspensions of human feces or raw-gut contents from Wistar rats. The rates of hydrolysis were greatest in the presence of rat small intestine contents and much slower with caecal or stomach contents. The percentages of metabolized DBP in 16 hours by the stomach, small intestine, caecum and human feces were 0.5 ± 0.07, 80.8 ± 2.3, 22.9 ± 1.3 and 6.2 ± 0.2, respectively. At concentrations of ≤ 200 µg/mℓ DBP was metabolized ~70% in 30 minutes. Both the age and sex of the rats influenced the rates of DBP metabolism. Young male rats, 26 days old, metabolized DBP more slowly than 33-day-old male rats or 26-day-old female rats 30 minutes after exposure.

Female rats between 33 and 40 days old metabolized 15.7±2.5 and 25.5±3.3% DBP in 30 minutes, respectively, whereas male rats at the same ages metabolized 34.5±2.4 and 34.4±2.2, respectively.

DEP. One study was found regarding the metabolism of DEP. Rowland et al. (1977) examined the rates of hydrolysis of DEP to the monoester in suspensions of human feces or raw-gut contents from Wistar rats. The specific monoesters formed, however, were not identified. The rates of hydrolysis were greatest in the presence of rat small intestine contents and much slower with caecal or stomach contents. The percentages of metabolized DEP in 16 hours by the stomach, small intestine, caecum and human feces were 2.5±0.2, 36.4±2.1, 11.5±0.5 and 3.0±0.1, respectively.

DMP. Kaneshima et al. (1978) studied the effects of a single oral dose of 500 mg/kg ^{14}C-DMP in 50% ethanol upon the biliary excretion of rats. Several metabolites were detected. An extract of the bile contained DBP, MBP, phthalic acid and an unidentified substance. A glucuronide of MBP and traces of other glucuronides were also discovered upon further analysis using TLC.

Albro and Moore (1974) studied the urinary metabolites of DMP. Adult male CD rats were administered 0.1 mℓ DMP (17 mg/kg/day assuming 0.350 kg rats consume 5% body weight) by gavage at 24-hour intervals. Urine samples were collected ≤48 hours following the initial dosing of DMP. A sample of urine obtained after 24 hours contained the following metabolites: 14.4% free phthalic acid, 77.5% monomethyl phthalate and 8.1% dimethyl phthalate intact. The metabolites were identified by GLC and TLC.

Rowland et al. (1977) examined the rate of DMP hydrolysis to the monoester in suspensions of human feces or raw-gut contents from Wistar rats. The rates of hydrolysis of DMP to the monoester were greatest in the presence of rat small intestine contents and much slower with caecal or stomach contents. The percentages of metabolized DMP in 16 hours by the stomach, small intestine, caecum and human feces were 21.2±1.1, 61.1±0.9, 15.9±0.4, 8.3±0.2, respectively.

Excretion

The PAEs and their metabolites are eliminated from the body through urinary, fecal and biliary excretion routes. The greater part of the metabolites of the administered esters are excreted in the urine. Most studies of excretion have utilized the compounds DEHP and DBP as is shown in Table III-7.

DEHP. Excretion of phthalates has been predominantly studied using DEHP. Schultz and Rubin (1973) found ~13% of a single oral dose of 200 mg/kg ^{14}C-carbonyl labeled DEHP (in corn oil) in the organic solvent extracts in the urine, feces and large intestine contents of rats. The urine contained 62% in water extracts. Daniel and Bratt (1974) reported that upon a single oral exposure to 2.9 mg/kg ^{14}C-carbonyl labeled DEHP, rats excreted 42% and 57% of the dose in the urine and feces, respectively, in 7 days. In another portion of the study, rats were fed 1000 ppm DEHP (50 mg/kg/day assuming rats weighing 180 g consume 0.05% of their body weight) for 7 days and then given a single oral dose of 2.9 mg/kg ^{14}C-labeled DEHP. Rats excreted 57% of the radioactivity in the urine and 38% in the feces in 4 days. Biliary-cannulated rats excreted 14 and 9% of the 2.6 mg/kg ^{14}C-DEHP labeled dose in 4 days in urine and feces, respectively.

TABLE III-7

Summary of Biliary, Fecal and Urinary Excretion of DBP or DEHP in Rats[a]

Compound	Dose	Time[b]	Exposure Route	Portion of Dose, %[c]		
				Bile	Feces	Urine
DBP	60 mg/kg	24 hours	gavage	40	5	88
	500 mg/kg	6 hours	gavage	5	NR	NR
	2.31 g/kg	48 hours	gavage	NR	5	82
	50 mg/kg	5 hours	i.v.	10	NR	NR
DEHP	2.6 mg/kg	4 days	gavage	14	56	42
	1.0 g/kg	4 days	gavage	NR	40 (8)	60
	10 ppm[d]	NR	feed	NR	4 (0)	96
	2000 ppm[d]	NR	feed	NR	9 (6)	91
	50 mg/kg	7 days	i.v.	NR	28	49

[a]Source: Kluwe, 1982a

[b]Time of collection post treatment

[c]Metabolites in parentheses

[d]Concentration incorporated into feed

NR = Not reported

Lake et al. (1975) found that after a single oral dose of 2000 mg/kg ^{14}C-carbonyl labeled DEHP to Wistar rats no radioactivity was detected in the urine and feces and only <0.1% of the radioactivity remained in the tissues and organs after a 4-day period. A similar rate of elimination was determined in rats pretreated with DEHP for 6 and 13 days.

Teirlynck and Belpaire (1985) studied the disposition of DEHP and MEHP in male Wistar rats. Three hours after a single oral dose of 2800 mg/kg DEHP, plasma concentrations of 8.8 µg/mℓ DEHP and 63.2 µg/mℓ MEHP were observed. The ratio of the area under the plasma concentration-time curve of MEHP:DEHP was 16.1. MEHP had a half-life of 5.2 hours. When ^{14}C-DEHP was administered, 19.3% of the radioactivity was excreted in the urine within the first 72 hours. The remaining radiolabel was excreted in the feces. The urinary excretion rate indicated a half-life of 7.9 hours. Multiple dosing with 2800 mg/kg DEHP for 7 consecutive days produced no accumulation of either DEHP or MEHP.

Tanaka et al. (1975) observed that in Wistar rats given labeled DEHP p.o. (500 mg/kg, 25% solution in Tween 80) or i.v. (emulsified with a solution of 4% BSA in saline), ~80% of the dose was excreted in the urine and feces in 5-7 days. Intact DEHP was not detected in the urine after a single oral dose. However, after repeated oral administration of 500 mg/kg DEHP for 20 days, the intact compound was identified in the rat urine. The authors concluded that DEHP may accumulate in the body until the rates of absorption and excretion achieve a steady state, after which the intact diester will occur in the urine.

Albro et al. (1982), studied the effect of DEHP exposure on excretion. Three groups of male Fischer 344 rats received ^{14}C-DEHP (in cotton seed oil) by gavage at one of three dosage levels (1.8, 18 or 180 mg/kg bw/day DEHP) for 10 days. After an initial 4-day acclimation period, excretion was found to be independent of the dose indicating that the overall elimination mechanism was not affected by the doses given. Rats receiving 180 mg/kg/day DEHP eliminated ^{14}C at a rate 100 times that of rats receiving 1.8 mg/kg/day DEHP.

Tissue half-lives of DEHP and MEHP were determined in male Wistar rats after a single oral administration of DEHP (25 mmol/kg or 9.8 g/kg) by gastric intubation (Oishi and Hiraga, 1982). Both compounds disappeared exponentially from the blood, liver, testes, heart, spleen, lung and epididymal fat with $t_{1/2}$ values of 23-68 hours for MEHP and 8-156 hours for DEHP (Table III-8). The longest half-lives for both DEHP and MEHP occurred in the epididymal fat followed by disappearances from the testes for MEHP and the liver for DEHP. In the present study MEHP exhibited the long half-life and the highest ratio of MEHP/DEHP in the testes. In addition, with the exception of epididymal fat and lungs, the biologic half-life of MEHP was slightly longer than DEHP.

In studies with African Green monkeys described by Peck and Albro (1982), i.v. administration of ^{14}C-DEHP resulted in an accumulation of >50% of the injected ~3 mg dose in the urine after 5 hours. Within 24 hours, >70% of the dose was excreted in the urine. Fecal excretion was found to account for >5% of the administered dose after 4 days.

TABLE III-8

MEHP/DEHP Ratios and Biological Half-Lives of DEHP and MEHP at 6 Hours After Administration[a]

	MEHP/DEHP Ratio[b] (mol%)	Biological Half-Life[c]	
		MEHP	DEHP
Blood	113±23	23.8	18.6
Liver	79±17	31.9	28.4
Testes	210±4.8	49.9 (6<t<48)	8.28 (24<5<96)
Heart	46±0.57	28.8	15.2
Spleen	d/	22.6	d/
Lung	d/	d/	1.49 (1<t<6) 25.3 (6<t<96)
Epididymal fat	87±24	67.6 (24<t<96)	156 (48<t<96)

[a]Source: Oishi and Hiraga, 1982

[b]Values of MEHP/DEHP ratio were mean ± SD of three rats.

[c]Biologic half-lives were calculated from least squares fit of chemicals during 6-96 hours except for lung, fat and testes.

[d]Present but in amounts too small to be estimated.

Rhodes et al. (1986), studied the comparative pharmacokinetics of DEHP in the Wistar rat and the marmoset monkey following oral, i.v. or i.p. administration. In both the rat and marmoset, the proportion of dose excreted in the urine or feces (2:1, respectively) in either male or female animals, was similar following single or multiple oral administrations of ^{14}C-DEHP at 2000 mg/kg bw.

After single oral doses by gavage of 100 mg/kg ^{14}C-DEHP, both Fischer 344 rats and cynomolgus monkeys eliminated ~30% of the dose in the urine during the first 24 hours (Short et al., 1987). Intact DEHP was not detected in the urine; however, 20% was recovered in the feces of rats and 34.3% was recovered in the feces of monkeys. In addition, ~50% of the dose was recovered in the feces within 48 hours after treatment. Multiple doses of 1000, 6000 or 12,000 ppm (105, 667 or 1223 mg/kg/day, respectively) in rats resulted in 50-70% of the radioactivity being excreted in the urine and 20-40% in the feces. The amount of radioactivity in the urine tended to increase with dose while the amount in the feces tended to decrease. The average amount of recovery in the rats urine ranged from 88-96% of the administered dose in all groups. Multiple gavage doses of either 100 or 500 mg/kg ^{14}C-DEHP to cynomolgus monkeys resulted in an average decreased radioactivity of 40% to 10% in the urine and 40-60% in the feces, respectively.

Schmid and Schlatter (1985) found that 30 mg DEHP taken orally by two volunteers was excreted after 24 hours in the urine at 10 and 15% of the dose. DEHP taken by the same volunteers for 4 days at 10 mg daily gave no evidence of accumulation; 10 and 25% of the total dose was recovered in the

urine after 48 hours. On the basis of this the investigators estimated a half-life of 12 hours, and concluded that accumulation of DEHP in the body is unlikely to occur. Unfortunately, fecal analysis that could have supported this hypothesis were not performed. Generally these data compare well with those of Peck et al. (1979) for human patients that received infusions of DEHP-contaminated blood.

Peck et al. (1979) followed the excretion of DEHP and its metabolites from two patients receiving DEHP-laden platelet concentrates. In one individual, 57% of 94.7 mg DEHP that was infused over 4 hours was detected in the urine 8.5 hours later. A second subject received 174.3 mg DEHP in 1.5 hours. Within 24 hours of administration, over 60% of the dose was recovered in the urine.

BBP. BBP was rapidly excreted after single oral doses of 2, 20, 200 or 2000 mg/kg to male Fischer 344 rats (Eigenberg et al., 1986). This phthalate undergoes extensive enterohepatic circulation. The majority of the dose (~75%) was eliminated in the urine and ~20% eliminated in the feces; >92% of the dose was excreted by the fourth day. At 2000 mg/kg there was a shift to primarily fecal elimination (72% of the dose after 4 days). Eigenberg et al. (1986) stated that increased fecal elimination at the highest dose may be due to incomplete absorption during enterohepatic circulation. Four hours after a single i.v. injection (20 mg/kg BBP) 55% of the total dose was excreted into the bile and 34% in the urine.

Lake et al (1978) found similar results when single oral doses of 16, 160 and 1600 mg/kg BBP was administered to male Sprague-Dawley rats. Approximately 80% of the administered dose was excreted in the urine within 5 days; most of the remainder was excreted in the feces (~17%).

DBP. After single oral doses of 0.1 and 0.13 g/kg DBP-7-^{14}C to male Wistar rats, 96% of the radioactivity was excreted in the urine. Most of the original dose (80-90%) was recovered in the urine (Williams and Blanchfield, 1975). Tanaka et al. (1978) found that DBP was rapidly absorbed, metabolized and excreted after either oral or i.v. administration to rats. Within 48 hours, >90% of the administered DBP was excreted in the urine, regardless of the exposure route. Excretion in the feces was minimal (Tanaka et al., 1978).

Kaneshima et al. (1978) also looked at excretion of ^{14}C-radiolabel (position of label not reported) in the bile after i.v. or oral administration of ^{14}C-DBP to rats. About 10% of a 50 mg/kg dose was recovered in the feces (though it could be the bile; the paper was not clear) within 5 hours after injection and 4.5% of a 500 mg/kg dose was detected within 6 hours after ingestion. The results of these studies upon excretion have led to postulations that hepatobiliary excretion of DBP metabolites may be saturated at high doses or they may occur only after a specific period of time following absorption (Kluwe, 1982a).

DEP. Data regarding the excretion of DEP could not be located in the available literature.

DMP. In a DMP metabolite study by Albro and Moore (1974), 14.4% free phthalic acid, 77.5% monomethyl phthalate and 8.1% DMP as intact compound were excreted in the urine after 24 hours. These values are mole percentages of recovered phthalate.

Summary

The fate of PAEs in the body has received considerable attention. Administered doses have been found to be rapidly absorbed from the intestine, skin, peritoneum, blood and lungs. A large percentage of the diesters are hydrolyzed although it is not uncommon to find low levels of the intact compounds present in the excretory products. However, hydrolysis of the diesters appears to be inversely proportional to their alkyl-chain length and concentration. Both dose- and time-dependent quantitative differences in the profile of DEHP urinary metabolites were observed in rats exposed to DEHP or MEHP for 1, 2 or 3 days. The results suggested that DEHP and MEHP are metabolized by similar routes and stimulate their own metabolism by inducing ω-oxidation (cytochrome P-450-mediated ω-hydroxylation) and peroxisomal ß-oxidation. Thus, the duration of exposure, the dose level administered and the status of the animal with respect to peroxisomal proliferation is important when evaluating metabolic studies on PAEs.

Once absorbed, PAEs or their metabolites are deposited throughout the body. Retention or accumulation of PAEs is minimal. Orally administered PAEs are deposited primarily in the liver, intestine, muscle and adipose tissue. However, accumulation in many of these tissues may be a function of the excretion of the compound. Several studies confirm placental transfer as well as fetal tissue uptake.

The route of exposure and structure of PAEs and their metabolites influence their body distribution. Following i.v. administration DBP did not accumulate in the liver to the same extent as DEHP. In addition, retention of DBP in the heart, lung and spleen after oral or i.v. exposure appeared to be shorter than DEHP. Few differences were observed in the distribution pattern of DBP compared with that of DEHP. Information on the distribution of BBP, DEP and DMP is either limited or not available. The study of BBP, DEP and DMP as a function of the routes of administration, as well as the pharmacokinetics and disposition of biologically relevant metabolites, such as MEHP, remain important areas to investigate.

Metabolism of PAEs is governed by their molecular weight and alkyl-chain length. Dialkyl phthalates are hydrolyzed to monoesters in the intestine and other organs following absorption. The rate of hydrolysis is greater for the lower molecular weight esters than for the higher molecular weight esters. Only a small fraction of long-chain alkyl phthalates undergo complete hydrolysis. The hydrolyzed monoesters form glucuronide conjugates in many species. Species differences in PAE conjugation has been observed; for example, DEHP is glucuronated in man and monkey, whereas this does not occur in the rat.

PAEs and their metabolites are eliminated from the body through the urinary, fecal and biliary excretion routes. Though urinary excretion is quantitatively the major route of removal, feces can also be of importance. Most PAEs are excreted as a monoester metabolite (glucuronide conjugate) with a small portion being unchanged parent compounds. Rats are an apparent exception in their inability to form and excrete glucuronide conjugates of MEHP.

Following multiple oral administration of 105, 667 or 1223 mg/kg/day DEHP, the average amount of recovery in the urine of rats ranged from 88-96% of the administered doses. After single oral doses of 100 mg/kg ^{14}C-DEHP, both rats and monkeys eliminated ~30% of the dose in the urine during the first 24 hours. Intact DEHP was not detected in the urine; however, 20% was recovered in the feces of rats and 34.3% was recovered in the feces of monkeys. In one human patient receiving 174.3 mg DEHP, >60% of the dose was recovered in the urine within 24 hours. Only 10-15% of an oral 30 mg dose of DEHP was excreted in the urine of human volunteers. Unfortunately fecal analysis was not performed. Fischer 344 rats rapidly excreted ~75% in urine and ~20% in the feces of single oral doses of 2, 20 or 200 mg/kg BBP. At 2000 mg/kg BBP there was shift to fecal elimination (~72% of the dose after 4 days). After single oral doses of ~0.1 g/kg DBP to Wistar rats, 80-90% of the dose was recovered in the urine. Within 24 hours after an oral dose of 17 mg/kg/day DMP, CD rats excreted the majority of the dose in the urine with 8.1% (mole percentage) as intact compound. Data regarding the excretion of DEP could not be located in the available literature.

IV. HUMAN EXPOSURE

Text to be provided by the Office of Drinking Water.

V. HEALTH EFFECTS IN ANIMALS

Introduction

Since the early 1970s, PAEs have been the subject of extensive toxicologic research. The overall effects of PAEs have been reviewed by several authors (Autian, 1973; Peakall, 1975; Thomas et al., 1978; Thomas and Thomas, 1984). A national conference on the potential health threats of PAEs was held in 1972, the results of which were published in the January 1973 issue of Environmental Health Perspectives. A subsequent issue of Environmental Health Perspectives (1982) was devoted to recent research on phthalate esters following a conference sponsored by the National Toxicological Program (NTP) and the U.S. Interagency Regulatory Liason. The U.S. EPA (1980) published an Ambient Water Quality Criteria Document for PAEs, which summarized literature published through 1979 and developed water quality criteria for ambient water. In 1982, the International Agency for Research on Cancer (IARC) published monographs on several PAEs and related compounds suspected of causing cancer. The Consumer Products Safety Commission reported estimated possible increased risk of cancer to children exposed to DEHP in children's products such as pacifiers, teethers, squeeze toys, plastic baby pants and vinyl fabrics covering playpen pads (CPSC, 1983, 1985). The majority of toxicity studies have focused on DEHP since this compound accounts for ~40% of the phthalates produced for commercial use. Limited information on toxicity is available for several other PAEs.

Short-Term Animal Toxicity

Based on data accumulated from several studies, the acute toxicity of PAEs is considered, qualitatively, to be rather low. All oral, dermal and i.p. LD_{50}s are >1.0 g/kg bw (Table V-1). Oral LD_{50} values reported for

i.p. LD_{50}s are >1.0 g/kg bw (Table V-1). Oral LD_{50} values reported for PAEs range from 1.0 g/kg bw for DEP to 34 g/kg bw for DEHP (see Table V-1). Generally, the acute oral toxicity of the PAE tends to decrease with increasing molecular weight. For any of the tested PAEs, acute toxicity may also vary with species tested. The oral LD_{50}s for DEHP ranged from 26 g/kg bw in rats to 34 g/kg bw in rabbits (Autian, 1973).

Dermal LD_{50}s appear to be approximately twice the oral LD_{50}s. The high dermal LD_{50}s may result, in part, from reduced absorbtion of the administered compounds.. In the case of low molecular weight PAEs, dermal exposure may be decreased by compound volatilization. As a group, PAEs produce little irritation when placed in contact with the skin of animals or humans.

The LD_{50} values for PAEs administered i.p. ranged from 0.7-20 g/kg, again indicating low acute toxicity for these compounds. Toxicity of PAEs is generally greater following i.p. injection than following oral administration. For example, comparison of the oral and i.p. LD_{50} values for the same species indicated that DMP administered i.p. was approximately twice as toxic (on a mg/kg basis) as when administered orally (Autian, 1973). Oral administration of >4 g/kg bw of butylbenzyl phthalate (BBP) to rats proved fatal (Mallette and Von Hamm, 1952). It was unclear as to whether the compound was administered in mineral oil or propylene glycol. The authors reported that animals died between 4 and 8 days after treatment, showing weight loss, apathy and leukocytosis. Histologic examination of the organs revealed toxic splenitis and degenerative lesions of the central nervous system with congestive encephalopathy, myelin degeneration and glial proliferation.

TABLE V-1

Summary Table of Short-term Toxicity Studies of PAEs in Mammals

Exposure/Route	PAE	Critical Effect	Species	Dose/Time	Reference
Oral	DEHP	No effect on mortality	Rat, Fischer 344	0.8-20 g/kg/single	NTP, 1982a
	DEHP	No effect on mortality	Mouse, B6C3F1	1.25-20 g/kg/single	NTP, 1982a
	DEHP	No effect	Rat	25.0 g/kg/single	Krauskopf, 1973
	DEHP	Diarrhea	Rat	110 g/kg/single	Krauskopf, 1973
	DEHP	No deaths	Rat	15.8 g/kg/single	Krauskopf, 1973
	DEHP	No deaths	Mouse	34.5 g/kg/single	Krauskopf, 1973
	DEHP	Death in 8/10	Rat	79.5 g/kg/single	Krauskopf, 1973
	DEHP	No deaths	Rat	5 9 g/kg/single	Krauskopf, 1973
	DEHP	LD_{50}	Rat	26.0 g/kg	Autian, 1973
	DEHP	LD_{50}	Rabbit	34.0 g/kg	Autian, 1973
	DEHP	LD_{50}	Mouse	33.5 g/kg	IARC, 1982
	DEHP	LD_{50}	Guinea pig	26.3 g/kg	IARC, 1982; Krauskopf, 1973
	DEHP	Progressive hepatic changes, increase in relative liver weight, biochemical changes, cellular changes	Rat, albino Wistar	2 g/kg/day for 4, 7, 14 or 21 days	Lake et al., 1975
	DEHP	Hepatic changes, biochemical changes, cellular changes	Rat, Wistar	2.5 g/kg/day for 7 or 21 days	Mangham et al., 1981
	DEHP	Hepatic enlargement (1.5 X control)	Rat	0.3 g/kg/day (0.5% in diet) for 49 days	Daniel and Bratt, 1974
	DEHP	No effect on liver weight	Rat	0.06 g/kg/day (0.1% in diet) for 35 days	Daniel and Bratt, 1974
	DEHP	Decreased body weight gain; a NOAEL in males identified at 77.06 and 126.31 mg/kg/day in females	Rat, F344	38 84, 77.06 154.13, 308.25 and 616.50 mg/kg/day in males; 31.83, 63.16, 126.31, 252.63 and 505.25 mg/kg/day in females/ 14 days	NTP, 1982a
	BBP	LD_{50}	Rat, Fischer 344	2 33 g/kg	IARC, 1982
	BBP	LD_{50}	Mouse, B6C3F1	4.17 g/kg	IARC, 1982
	BBP	LD_{50}	Mouse, B6C3F1	6 16 g/kg	IARC, 1982
	BBP	Mortality with toxic splenitis, degeneration of CNS	Rat, unspecified strain	1.8 g/kg/day for 4-8 days	Mallett and Von Hamm, 1952
	BBP	No effects on hemopoietic system	Rats, Fischer 344	0 625 (375 mg/kg/day) and 1 25% (750 mg/kg/day) for 14 days	Agarwal et al., 1985a

TABLE V-1 (cont.)

Exposure/Route	PAE	Critical Effect	Species	Dose/Time	Reference
Oral (cont.)	DBP	Abnormal biochemical changes, growth depression	Rats, Wistar	0.5% (0.25 g/kg) powder in diet for 34 days/LOAEL	Murakami and Nishiyama, 1986
	DBP	Increased absolute and relative liver weights; peroxisomal proliferation at 2.5% as well as testicular atrophy; LOAEL identified at 0.6%	Rats, F344	0.6, 1.2 and 2.5% (624, 1234 and 2156 mg/kg/day for males; 632, 1261 and 2107 mg/kg/day for females)/21 days	CMA, 1986
	DEP	LD_{50}	Rabbit	1.0 g/kg	Autian, 1973
	DEP	Temporary distress	Rabbit	3.35 g/kg/8 days	Blickensdorfer and Templeton, 1930
	DEP	LD_{50}	Rat	9.16 g/kg/single	Krauskopf, 1973
	DMP	LD_{50}	Mouse	7.2 g/kg	Autian, 1973, Krauskopf, 1973
	DMP	LD_{50}	Rat	2.4 g/kg	Autian, 1973
	DMP	LD_{50}	Guinea pig	2.4 g/kg	Autian, 1973,
	DMP	LD_{50}	Rabbit	4.4 g/kg	Krauskopf, 1973
	DMP	No effect	Mice	1-4 g/kg	Krauskopf, 1973
	DMP	No effect	Dogs	0.7-1.4 g/kg	Krauskopf, 1973
Dermal	DEHP	LD_{50}	Guinea pig	10.0 g/kg	Autian, 1973
	DBP	LD_{50}	Rabbit	20.9 g/kg	Autian, 1973
	DMP	LD_{50}	Rabbit	11.9 g/kg	Autian, 1973
	DEP	LD_{50}	Rabbit	22.35 g/kg/single	Autian, 1973
Inhalation	DBP	Weight loss, increase in gamma globulin	Rat	0.4 mg/m³ for 10 weeks	Dvoskin et al., 1969
i.p. injection	DEHP	LD_{50}	Mouse	14.2 g/kg	Autian, 1973
	DEHP	LD_{50}	Rat	49.2 g/kg	Autian, 1973
	DEHP sonicated into plasma	LD_{50}	Rat	200 mg/kg	Rubin and Chang, 1978
	DEHP sonicated into plasma	LD_{100}	Rat	400 mg/kg	Rubin and Chang, 1978
	DEHP after blood pressure held at shock level 30 minutes	Mortality 2/6	Rat	8-13 mg/kg	Rubin and Chang, 1978
	DEHP	LD_{50}	Mouse, ICR	1.37 mL/kg[a] for 12 weeks	Lawrence et al., 1975
	DEHP	Hepatotoxic effect	Rat, albino	5 mL/kg/day on days 1, 5 and 10 of 22-day test period	Srivastava et al., 1977

TABLE V-1 (cont.)

Exposure/Route	PAE	Critical Effect	Species	Dose/Time	Reference
i.p. injection (cont.)	BBP	LD_{50}	Mouse	3.16 g/kg	Autian, 1973
	DBP	LD_{50}	Mouse	4.0 g/kg	Autian, 1973
	DBP	LD_{50}	Rat	3.19 g/kg	Autian, 1973
	DBP	Growth depression, peritonitis	Mouse, Swiss Webster	0.5 g/kg/day for 6 weeks	Calley et al., 1966
	DEP	LD_{50}	Mouse	2.8 g/kg	Autian, 1973
	DEP	LD_{50}	Rat	5.65 g/kg	Autian, 1973
	DEP	Some temporary distress	Rabbit	2.24 g/kg bw/8 days	Blickensdorfer and Templeton, 1930
	DEP	No effect	Guinea pig	1.68 g/kg/8 days	Blickensdorfer and Templeton, 1930
	DMP	LD_{50}	Mouse	1.40-3.99 g/kg	Autian, 1982
	DMP	LD_{50}	Mouse	1.6-3.6 g/kg	Autian, 1973
	DMP	LD_{50}	Rat	4.02 g/kg	Autian, 1973
i.v. injection	DEP	Respiration stimulated initially and then paralyzed	Dogs	0.28 g/kg/single	Blickensdorfer and Templeton, 1930
	DEHP solubilized in nonionic detergent	LD_{50}	Rat, Wistar male	250-300 mg/kg	Schultz et al., 1975
i.m.	DBP	LD_{50}	Rat	8.0 g/kg	Autian, 1973

*Dose represents the LD_{50} calculated for mice receiving i.p. injections 5 days/week. LD_{50} values were calculated each week until the LD_{50} remained the same for 3 weeks (minimum of 10 weeks of dosing).

PAEs range from 1.0 g/kg bw for DEP to 34 g/kg bw for DEHP (see Table V-1) Generally, the acute oral toxicity of the PAE tends to decrease with increasing molecular weight. For any of the tested PAEs, acute toxicity may also vary with species tested. The oral LD_{50}s for DEHP ranged from 26 g/kg bw in rats to 34 g/kg bw in rabbits (Autian, 1973).

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As will be discussed in more detail in Chapter VII, Mechanisms of Toxicity, the toxic effects of phthalate esters are thought to be caused by monoester metabolites. Acute toxicity studies of various phthalate esters are summarized in Table V-1. Krauskopf (1973) reported information on the levels of various PAEs that do not cause death or adverse effects. The information, however, was largely taken from unpublished data. It is presented here to supplement the available information on LD_{50}s but should be interpreted cautiously.

DEHP. Range finding tests performed by the National Cancer Institute/ National Toxicology Program (NCI/NTP) as part of the DEHP carcinogenesis bioassay provided some information on nonlethal levels of DEHP. In these tests, no fatalities occurred within 14 days following the administration of single oral doses of 0.8-20 g/kg of DEHP to groups of five male and five female rats or single oral doses of 1.25-20 g/kg of DEHP to groups of five male and five female B6C3F1 mice. Doses were administered in corn oil by gavage (NTP, 1982a).

Lawrence et al. (1975) studied the short-term toxicity of a number of PAEs to determine the intraperitoneal LD_{50}s. Groups of 10 male ICR mice were administered a range of daily doses for 5 days/week. An apparent LD_{50} was calculated for each week. This dosing schedule continued until the mice had been injected for at least 10 weeks, and the apparent LD_{50} remained constant for 3 consecutive weeks. After the first week, the LD_{50} was 38.35 mℓ/kg for DEHP. In the second week, the LD_{50} was reduced to 6.40 mℓ/kg. By the end of 12 weeks, the LD_{50} was reduced to 1.37 mℓ/kg for DEHP. Cumulative toxicity factors (the ratio of acute LD_{50}:

chronic LD_{50}) was 27.99 for DEHP, indicating increased toxicity (much lower LD_{50}s) over time. Other PAEs had cumulative toxicity factors ranging from 2.04-4.01, indicating that cumulative toxicity was minimal over the test period. Neither the implication of the high cumulative toxicity factors for DEHP nor the reasons for these results, when compared with the other PAEs, are clear. It is possible that very high doses of DEHP prevent the body from eliminating the compound and metabolites to the same degree as occurs when lower doses are repeatedly administered. It is also not known if oral doses would lead to the same or similar results (Lawrence et al., 1975).

A 14-day range finding study using animals fed diets containing DEHP was conducted as part of the NTP Carcinogenesis Bioassay (1982a). The survival and mean body weight responses are presented in Tables V-2 and V-3 for both rats (F344) and mice (B6C3F1). It can be seen in Table V-2 that rats of both sexes (5/group) exposed to 100,000 ppm (616.50 mg/kg/day in males, 505.25 mg/kg/day in females) DEHP experienced high mortality (40% males, 80% females). Significant changes in body weight relative to controls were seen at 25,000, 50,000 and 100,000 ppm (154.13, 308.25 and 616.50 mg/kg/day) in males (-29%, -94% and -197%, respectively), and at 50,000 and 100,000 ppm (252.63 and 505.25 mg/kg/day) in females (-165% and -171%, respectively). As shown in Table V-3, at 50,000 and 100,000 ppm (63.50 and 127.00 mg/kg/day) DEHP, 20 and 100% mortality, respectively, was observed in male mice. Changes in weight relative to controls were dose dependent, ranging from -69 to -315% (6300-100,000 ppm) in males and -50 to -675% (6300-100,000 ppm) in females. Qualitatively similar responses were seen in female mice both with respect to survival and body weight change.

TABLE V-2

Dosage, Survival and Mean Body Weights of Rats Fed Diets Containing Di-(2-ethylhexyl)phthalate (DEHP) for 14 Days[a]

Dose (ppm) (mg/kg/day)[b]	Survival[c]	Mean Body Weights (g)			Weight Change[d] (%)
		Initial	Final	Gain	
Males					
0 (0)	5/5	122.8	168.4	45.0	
6,300 (38.84)	5/5	123.4	174.0	50.6	+12
12,500 (77.06)	5/5	123.4	175.6	52.2	+16
25,000 (154.13)	5/5	123.4	155.2	31.8	-29
50,000 (308.25)	5/5	123.4	126.2	2.8	-94
100,000 (616.50)	3/5	123.4	79.6	-43.8	-197
Females					
0 (0)	5/5	101.2	116.8	15.6	
6,300 (31.83)	5/5	101.0	133.4	32.4	+108
12,500 (63.16)	5/5	101.1	121.0	20.0	+28
25,000 (126.31)	5/5	101.0	117.6	16.6	+6
50,000 (252.63)	5/5	101.0	90.8	-10.2	-165
100,000 (505.25)	1/5	101.0	90.0	-11.0	-171

[a]Source: NTP, 1982a

[b]Assuming that adult rats consume an amount of food equivalent to 5% of their body weight each day. (Average initial body weight for males = 123.30 g; females = 101.05 g)

[c]Number surviving/number per group

[d]Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

TABLE V-3

Dosage, Survival and Mean Body Weights of Mice Fed Diets Containing Di-(2-ethylhexyl)phthalate (DEHP) for 14 Days[a]

Dose (ppm) (mg/kg/day)[b]	Survival[c]	Mean Body Weights (g)			Weight Change[d] (%)
		Initial	Final	Gain	
Males					
0	5/5	25.4	28.0	2.6	
6,300 (8.00)	5/5	25.4	26.2	0.8	-69
12,500 (15.88)	5/5	25.4	26.0	0.6	-77
25,000 (31.75)	5/5	25.4	23.0	-2.4	-192
50,000 (63.50)	4/5	25.4	20.0	-5.4	-308
100,000 (127.00)	0/5	25.4	19.8	-5.6	-315
Females					
0	5/5	18.6	19.4	0.8	
6,300 (5.86)	5/5	18.6	19.0	0.4	-50
12,500 (11.63)	5/5	18.6	19.8	1.2	+50
25,000 (23.25)	5/5	18.6	19.8	1.2	+50
50,000 (46.50)	1/5	18.6	14.7	-3.9	-588
100,000 (93.00)	0/5	18.6	14.0	-4.6	-675

[a]Source: NTP (1982a)

[b]Assuming that adult mice consume an amount of food equivalent to 5% of their body weight each day. (Average initial body weight for males = 25.4 g; females = 18.6 g)

[c]Number surviving/number per group

[d]Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Rhodes et al. (1986) compared morphologic and biochemical changes and toxic effects after 14 days of DEHP exposures to rats and marmoset monkeys. Groups of 10 adult male and female Wistar albino rats and five male and female 12- to 18-month-old marmosets were administered single daily oral doses of 2000 mg/kg bw DEHP in corn oil for 14 consecutive days. In addition, groups of five 24-month-old male marmosets were administered single daily i.p. injections of 1000 mg/kg bw DEHP in corn oil for 14 consecutive days. Treated rats experienced testicular atrophy, hepatomegaly and a significant reduction ($p<0.05$) in body weight gain. Marmosets body weight was reduced with both treatments; however, changes in organ weight were not detected. Hepatic peroxisomes and peroxisomal enzymes were induced in both male and female rats. Hypotriglyceridemic and hypocholesteremic effects were observed only in male rats. Oral and i.p. administration of DEHP to marmosets did not induce peroxisomes and peroxisomal enzyme activity or the hypolipidemic effects. Rhodes et al. (1986) concluded that the data indicated that the interrelationship of hepatomegaly, peroxisomal induction and hypolipidemic effects is complex and appears to be dose- and species-dependent. Marmosets metabolize DEHP differently than rats, which may explain why marmosets are less sensitive to the effects of peroxisome proliferators.

Short et al. (1987) observed similar results. Male cynomolgus monkeys were administered 100 or 500 mg/kg/day DEHP by gavage for 21 days. On day 22 each monkey received a single dose of ^{14}C-DEHP followed by three daily doses on days 23-25. There were no treatment-related changes in relative liver weight, palmitoyl CoA oxidation, carnitine acetyl-transferase or lactic acid 11- and 12-hydroxylation. In the comparative rat study animals

were fed diets containing 11, 105, 667, 1223 and 2100 mg/kg/day DEHP for 21 days. There was metabolic, biochemical and morphologic evidence of peroxisomal proliferation at doses comparable with those in the monkey. Peroxisomal proliferation was thought to be the result of a relationship between DEHP treatment and the formation of metabolite I [numbered according to the Albro et al. (1973) system. See Chapter III, Figure III-2]. Urinary levels of metabolite I in monkeys were low compared with levels found in the rat. Short et al. (1987) stated that rats do not provide a good basis for predicting results of DEHP exposure in higher primates.

Although not a normal route of environmental exposure, the possibility of exposure to PAEs from medical devices such as blood bags and plastic tubing has prompted studies of injection exposures. DEHP may constitute as much as 40% of the plastic material in blood storage bags and medical tubings (Sjöberg et al., 1985b). The type of vehicle or preparation of DEHP used in administration may influence the pharmacokinetic pattern observed. Due to the limited solubility of DEHP in blood and blood products, the total dose given to animals would be relatively small and, in general, no acute toxicity would be expected (U.S. EPA, 1980). Rubin (1976) has suggested, however, that pulmonary effects may occur when surfactant-solubilized DEHP is administered i.v. This type of pulmonary pathology, characterized by an inflammatory state, has been referred to as "shock lung" or "wet lung" (Rubin, 1975).

In earlier studies, DEHP was mixed by sonication into the collected plasma of donor rats (unspecified strain) at concentrations $\leq$10 mg/mℓ

(Rubin and Chang, 1978). The plasma was then returned to the original packed cell volume, resulting in whole blood DEHP concentrations of $\leq$5 mg/mℓ. Similarly prepared DEHP-free blood was used for treatment of controls. In one set of experiments, 40-80 mℓ of DEHP-treated blood was exchanged with the rats' own blood, resulting in received doses $\leq$400 mg/kg. All nine control rats survived, while in DEHP-treated rats, a dose-related increase in lung edema and in lethality was observed with an LD_{50} of ~200 mg/kg. At 400 mg/kg, all of the six rats tested died. Necropsy revealed severe lung hemorrhage and edema. In the second set of experiments, rats were bled until their blood pressure dropped to 50 mm Hg. This pressure was maintained for 30 minutes, then an equal volume of donor blood containing 1.25 mg/mℓ DEHP was reinfused. All five control rats survived. Lung weights were elevated in the control rats, but the lungs were not grossly hemorrhagic. Two of the six rats receiving 7.7-13.0 mg/kg DEHP died within 90 minutes of the transfusion. In all six rats receiving DEHP, lungs were grossly hemorrhagic. The authors concluded that sensitivity to DEHP was greatly increased in animals whose blood pressure was held at shock levels.

The LD_{50} for male Wistar rats receiving i.v. injections of DEHP solubilized in a nonionic detergent was 250-300 mg/kg (Schultz et al., 1975). The primary effect was a respiratory distress syndrome progressing to death from respiratory failure. The overt signs and morphologic alterations observed with DEHP/detergent treatment were not observed in control animals.

Mangham et al. (1981) studied the oral effects of DEHP on the liver and testes. In this study, groups of six male and six female Wistar rats were administered daily doses of 2500 mg/kg/day DEHP by gastric intubation in a

corn oil vehicle for 7-21 days. DEHP produced pronounced liver enlargement at 7 or 21 days in both sexes of rats. The activity of succinate dehydrogenase was decreased in male rats administered DEHP for 7 or 21 days. No effect on this enzyme occurred in females. Histopathologic changes were not present in the livers of rats treated with DEHP, although ultrastructural studies revealed proliferation of smooth endoplasmic reticulum (SER), an increase in the number of microbodies (peroxisomes) and mitochondrial changes. Effects of DEHP on the liver are summarized in Table V-4. Mangham et al. (1981) also noted a significant decrease ($p<0.001$, student's t-test) in the weight of the testes (relative to body weight) after 7 and 21 days. Treatment also resulted in bilateral tubular atrophy after 21 days.

Mitchell et al. (1985) observed similar results when groups of four male and four female Wistar albino rats were administered nominal doses of 50, 200 or 1000 mg/kg/day DEHP in the diet for 3, 7, 14, 28 days or 9 months. Histopathologic examinations were performed on the major abdominal organs at all time points. The livers of male rats were significantly ($p<0.05$) enlarged at all time points with 1000 mg/kg/day DEHP. With the two lower dose groups liver enlargement was noted only at 14 days and 9 months in male rats; however, it was significant ($p<0.05$) only at the 1000 mg/kg/day dose. There were no significant differences in testes weight when control animals were compared with experimental animals. Further details were not given. Liver cells from male rats showed marked proliferation of peroxisomes after 3 days of treatment with 200 or 1000 mg/kg/day. Treatment with 50 mg/kg/day resulted in increased numbers of peroxisomes after 14 days. Proliferation of the smooth endoplasmic reticulum in both males and females occurred at all doses in a dose-dependent manner. DEHP administration caused an initial

TABLE V-4

Summary of Short-term Effects of DEHP on Weight, Morphology and Biochemical Constituents of Liver[a]

Species/Strain	Route	Dose/ Treatment Duration	Principal Findings	Reference
Rat/CD	diet	0.2, 1 and 2%/17 weeks (100, 500 and 1000 mg/kg/day)[b]	Liver enlargement	Nikoronow et al., 1973
Rat/NR	diet	5000 ppm/7 weeks (270 mg/kg/day)[b]	Liver enlargement	Daniel and Bratt, 1974
Rat	diet	0.35%/3 months (175 mg/kg/day)[b]	Liver enlargement	Gray et al., 1977
Rat/albino Wistar	oral intubation	2000 mg/kg/21 days	Liver enlargement, proliferation of SER, increase in number of microbodies and swelling of mitochondria with shortening of cristae, decreased SDH activity, altered aniline hydroxylase activity, decrease in glucose-6-phosphatase activity	Lake et al., 1975
Rat/Sprague-Dawley	oral intubation	1000 mg/kg/day/14 days	Increase in liver weight, palmitoyl-CoA oxidation, enoyl-CoA hydratase, and carnitine acetyltransferase ($p<0.01$)	Lake et al., 1986
Hamster/Syrian	oral intubation	1000mg/kg/day/14 days	Increase in liver weight, palmitoyl-CoA oxidation, and carnitine acetyl transferase ($p<0.01$)	Lake et al., 1986
Hamster/Chinese	oral intubation	1000 mg/kg/day/14 days	Increase in liver weight, palmitoyl-CoA oxidation, and carnitine acetyl transferase ($p<0.01$)	Lake et al., 1986
Partially hepatectomized rats/ Sprague-Dawley	diet	0.5%/10 weeks (250 mg/kg/day)[b]	Increased liver weight and carnitine acetyltransferase activity, decreased GGT enzyme activity	Deangelo et al., 1986
Ferret/NR	diet	1200 mg/kg/day[c]/ 14 months	Increase in liver weight, enlarged parenchymal cells not distributed uniformly, lysosomal changes and dilation of SER, absence of glycogen	Lake et al., 1976
Monkey/rhesus D	i.v.	74.80 mg/12 months	Liver necrosis, diffuse portal lymphocytic infiltration and evidence of binucleate cells	Jacobson et al., 1977
Monkey/rhesus H		20.52 mg/12 months		
Rat/Wistar	diet	2 and 4%/4 weeks (1000 and 2000 mg/kg/day)[b]	Inhibition of glycolysis and decrease in glycogen content and blood glucose	Sakurai et al., 1978

TABLE V-4 (cont.)

Species/Strain	Route	Dose/ Treatment Duration	Principal Findings	Reference
Rat/Wistar	oral intubation	2500 mg/kg/day/1-21 days	Pronounced liver enlargement at 7 or 21 days in both sexes; succinate dehydrogenase decreased in male rats at 7 or 21 days; proliferation of SER; increase in number of microbodies and mitochondrial changes	Mangham et al., 1981
Rat/Sprague-Dawley	i.v.	0, 5, 50 or 500 mg/kg bw	Increase in liver weights and number of liver peroxisomes; no differences in serum enzymes or BSP clearance values	Sjöberg et al., 1985b
Rat/albino Wistar	diet	50 mg/kg/day/28 days	Decreased glucose-6-phosphatase activity in females. Increased lipid content in liver; LOAEL of 50 mg/kg/day identified based on liver enlargement at 14 days of exposure.	Mitchell et al., 1985
	diet	200 mg/kg/day/28 days	Decreased glucose-6-phosphatase activity in females	
		1000 mg/kg/day/28 days	Decreased glucose-6-phosphatase. Cytochrome P-450 increased after 3 and 7 days, decreased to control levels after 14 days and increased after 28 days; significantly increased ($p<0.05$) liver weights at 3, 7, 14 and 28 days	
Rat/albino	diet	320 mg/day/30 days	Significant increase in liver weight; significantly reduced serum cholesterol levels; significant increase in carnitine acetyltransferase and glycerophosphate activity	Nair and Kurup, 1986
Mice/albino Wistar	diet	60 mg/day/30 days	Significant increase in liver weight and carnitine acetyltransferase and glycerophosphate activity	Nair and Kurup, 1986
Rats/Sprague-Dawley (CD)	oral intubation	10 mg/kg/bw/5 days to 6-10, 14-18, 16-20, 21-25, 42-46, 86-90 day old rats	Increased activities of hepatic peroxisomal enzymes palmitoyl CoA oxidase and carnitine acetyltransferase in all age groups	Dostal et al., 1987a
		100 mg/kg/bw/5 days to 6-10, 14-18, 16-20, 21-25, 42-46, 86-90 day old rats	Significant increases in relative liver weights in all but 1-week-old rats; increased absolute liver weight in all but 1-week-old rats; significantly increased activity of hepatic peroxisomal enzymes palmitoyl CoA oxidase and carnitine acetyltransferase in all age groups	

TABLE V-4 (cont.)

Species/Strain	Route	Dose/ Treatment Duration	Principal Findings	Reference
Rats/Sprague-Dawley (CD)	oral intubation		Significantly decreased body weight gain in rats ages 6-10, 16-20 and 21-25 days old, death in 66-70% of rats ages 14-18 days; significant increases in relative liver weights in all age groups; increased relative kidney weight in 21 day or older rats; significantly increased activity of hepatic peroxisomal enzymes, palmitoyl CoA oxidase and carnitine acetyl transferase in all age groups	Dostal et al., 1987a
		2000 mg/kg bw/5 days to 6-10, 14-18, 16-20, 21-25, 42-46, 86-90 day old rats	Death in rats ages 6-25 days old; significantly decreased body weights in 42-46 and 86-90 day old rats; significantly increased relative kidney weight in 42 day or older rats	
Rats/Wistar	oral intubation	2000 mg/kg/14 days	Hepatomegaly, testicular atrophy and reduced body weight gain, induction of hepatic peroxisomes and peroxisomal enzymes; hypolipidemic effects	Rhoades et al., 1986
Monkeys/marmoset	oral intubation	2000 mg/kg/14 days	No effect on peroxisomal enzyme activity; reduced body weight gain	Rhoades et al., 1986
	i.p.	1000 mg/kg/14 days	No effect on peroxisomal enzyme activity; identified as a LOAEL based on reduced body weight gain.	

[a]Source: Adapted from Seth, 1982

[b]Assuming rats consume 5% of their body weight

[c]Mean daily intake

NR = Not reported

increase in mitosis. DNA synthesis was significantly elevated ($p<0.05$) in all treated males at 3 days. Changes in lipid content and distribution were observed at all dose levels. Loss of glycogen was observed at 1000 mg/kg/day starting at 7 days, and lipofuscin accumulation after 28 days at 200 and 1000 mg/kg/day. Biochemical changes were also noted as summarized in Table V-4.

Lake et al. (1975) examined liver effects after oral administration (gavage) of 2000 mg/kg (236 mg/kg/day) DEHP for periods of 4, 7, 14 and 21 days. Increased relative liver weights and a number of biochemical and ultrastructural changes were noted. Komitowski et al. (1986) also observed ultrastructual changes after a single i.p. dose of DEHP. Six-week-old Syrian golden hamsters were administered either 0, 30, 300 or 3000 mg/kg DEHP. The investigators did not observe gross or histopathologic changes. However, ultrastructural changes such as increased number and size of peroxisomes were demonstrated in the high-dose group. The same type of changes were less pronounced in the middle-dose group. In the low-dose group only variability in peroxisomal size and shape occurred.

Sjöberg et al. (1985b) investigated the effect of DEHP on the liver of young male Sprague-Dawley rats after repeated i.v. infusions. Emulsions of DEHP were administered every other day on six occasions in daily doses of 0, 5, 50 or 500 mg/kg bw DEHP to groups of 6, 6, 6 and 5 40-day-old male Sprague-Dawley rats, respectively. Infusions were administered every other day on six occasions. Cannula were surgically inserted into the jugular veins of the rats 2 or 3 days before administration. DEHP emulsion was infused for 3 hours at a rate of 1.0 mℓ/hour. Blood samples were drawn 7

and 17 minutes after the i.v. injection throughout the experiment. A significant dose-related increase in liver weights ($p<0.0001$) and number of liver peroxisomes ($p<0.0051$) was observed. However, smaller mitochondria occurred in the livers of both control and treated animals, but were more common in the DEHP-treated groups. There were no differences in serum enzymes or BSP clearance values in treated animals when compared with control animals. The kidneys appeared normal. Although the relationship between dose and effect has not been established, the author concluded from the above results that measures be taken to reduce the exposure to DEHP through i.v. transfusion exchange.

Effects of DEHP on lipid and protein metabolism are summarized in Table V-5. Rats receiving 0.5% (250 mg/kg/day assuming rats consume 5% of their body weight) DEHP in a normal protein diet showed accumulation of phospholipids, decrease in cholesterol and triglyceride contents in liver and plasma and a rise in fatty acid levels in plasma (Reddy et al., 1976). The importance of the altered lipid concentrations in the body is not clear at present. Although the effects of PAEs on protein metabolism have not been studied, protein content in the liver has been shown to increase in DEHP-treated rats. The increase in liver protein content has been attributed to a decrease in protein breakdown.

Recent studies indicate that PAEs may cause adverse effects when transported to the developing organism by milk. Groups of seven nursing rat pups were randomly assigned to five dams (at birth). The dams were gavaged with 2000 mg/kg bw DEHP for 21 days. On day 21, three of the pup livers were examined. Parmar et al. (1985) observed a decrease in body weight gain and changes in enzyme activities of aniline hydroxylase, ethylmorphine,

TABLE V-5

Effects of DEHP on Lipid and Protein Metabolism Relating to Hepatotoxicity[a]

Species	Route	Dose/Exposure Length	Principal Findings	Reference
Rat	oral	0.5 or 1.0% in diet/ 10 or 18 days, respectively (250 or 500 mg/kg/day)[b]	Inhibition of lipid biosynthesis	Bell and Nazir, 1976
Rat	oral	2.5 mmol./100 g diet/21 days (490 mg/kg/day)[b]	Inhibition of lipid biosynthesis	Bell et al., 1977
Rat	i.p.	5 mg/kg bw/on days 1, 5 and 10	Decrease in cholesterol content	Srivastava et al., 1978
Rat	oral	1, 2 or 4% in diet/4 weeks (500, 1000 or 2000 mg/kg/day)[b]	Decrease in triglycerides and increase in phospholipids	Sakurai et al., 1978
Rat, mouse	oral	0.5, 2.0 and 4% in diet/1-4 weeks (250, 1000 and 2000 mg/kg/day)[b]	Decrease in serum cholesterol; proliferation of peroxisomes; increase of catalase and carnitine acetyltransferase	Reddy et al., 1976
Rat, mouse	oral	2% w/w /2 weeks (1000 mg/kg/day)[b]	Induction of enzymes of fatty acyl-CoA β-oxidation	Osumi and Hashimoto, 1978
Rat	oral	0.5% in diet/7 days (250 mg/kg/day)[b]	Increase in mitochondrial and microsomal phospholipids; increase in protein content	Yanagita et al., 1979
Rat	oral		Increase in protein content (decrease in protein degradation)	Pillai and Seth, 1978
Rat	oral	1000 mg/kg	Decreased glycogen in liver	Mitchell et al., 1985

[a]Source: adapted from Seth, 1982

[b]Assuming rats consume 5% of their body weight

N-demethylase and arylhydrocarbon hydroxylase, and decreased levels of cytochrome P-450 in 21-day-old rats. Dostal et al. (1987b) observed an increase in hepatic peroxisomal enzymes palmitoyl CoA oxidase and carnitine acetyltransferase in rat dams and their suckling pups exposed to DEHP. Rats were orally administered 5 daily doses of 2 g/kg bw DEHP on days 2-6, 6-10 or 14-18 of lactation. At all three stages of lactation relative liver weight was increased as well as palmitoyl CoA oxidase and carnitine acetyltransferase activity in both treated dams and their suckling pups. Plasma cholesterol and tryglyceride concentrations were decreased by 30-50% in DEHP treated dams at all three stages of lactation. Although mammary gland weights were decreased in treated dams, the investigators attributed these results to decreased food consumption in the DEHP-treated rats.

BBP. Agarwal et al. (1985a) investigated the effects of BBP on the hematopoietic system in a 14-day dietary study in F344 rats fed levels of 0.0, 0.625, 1.25, 2.5 and 5.0% BBP (0.0, 375, 750, 1250 and 1667 mg/kg/day, respectively). At the 0.625% and 1.25% levels, liver and kidney weights were significantly ($p<0.05$) increased. In addition, the incidence of proximal tubular regeneration of the kidney increased in a dose-related manner beginning at the 0.625% dose level. At the 2.5% and 5.0% levels, effects included decreased weight of the testes, epididymus, seminal vesicles and thymus, histologic evidence of atrophy of the testes and accessory sex organs. The authors reported no significant effects on the circulating blood components or blood clotting ability. Effects on the partial thromboplastin time were increased, but not significantly; however, mean values and large variability were observed at the 2.5 and 5.0% BBP levels. Bone marrow cellularity was significantly ($p<0.05$) reduced at 2.5

and 5.0% BBP in a dose-related manner. These authors conclude that prolonged exposure to BBP could alter the development of blood components and lead to a deficit in clotting ability.

Male and female Fischer 344 rats were fed 0, 0.6, 1.2 or 2.5% BBP for 21 days (CMA, 1985). Corresponding dose levels were 0, 639, 1277 and 2450 mg/kg/day for males and 0, 679, 1346 and 2628 mg/kg/day for females, respectively. Relative liver to body weights significantly increased ($p<0.001$ in both males and females except for $p<0.01$ at 0.6% in female rats) in all treatment groups. However, absolute liver weights were significantly increased ($p<0.01$ in males, $p<0.01$ at 1.2% and $p<0.001$ at 2.5% in females) only at the 1.2 and 2.5% dietary levels. Significantly reduced testes weight ($p<0.001$) and testicular atrophy occurred in the 2.5% treatment group. Relative kidney weights were higher in BBP-treated rats; however, the differences were not dose-related. In both males and females, total cholesterol concentrations were lower than the controls, but there was no dose relationship. Treatment with BBP at all dose levels of male rats and at 2.5% of female rats significantly ($p<0.01$ in males at 0.6% and $p<0.001$ at all other dose levels) increased cyanide-insensitive palmitoyl Co-A oxidation. Male rats were more sensitive than females with respect to increases in 11- and 12-hydroxylation of lauric acid.

Lake et al. (1978) administered 160, 480 or 1600 mg/kg/day BBP by gastric intubation for 14 days to six male Sprague-Dawley rats per group. Biochemical or morphologic changes in the liver were not observed at 160 mg/kg/day. Significantly increased activities of ethylmorphine N-demethylase ($p<0.05$) and cytochrome oxidase ($p<0.01$) were observed in the

480 and 1600 mg/kg/day BBP treatment groups. In addition, significant ($p<0.001$) liver enlargement was observed at 1600 mg/kg/day BBP as were increases in microsomal cytochrome P-450 ($p<0.05$) content and cytosolic alcohol dehydrogenase ($p<0.001$). Liver sections from animals at the highest dose revealed ultrastructural changes such as gross dilation of the rough endoplasmic reticulum and increased number of peroxisomes. Administration of 1600 mg/kg/day BBP also produced marked depression of both absolute and relative testes weights as well as severe testicular atrophy. Effects on testes weights were not observed in the animals given 160 or 480 mg/kg/day; however, testicular atrophy was observed in 1/3 of the animals administered 480 mg/kg/day BBP.

A second study was conducted to confirm the testicular effects. Both Sprague-Dawley and Wistar albino rats were gavaged with 480 and 1600 mg/kg/day BBP for 14 days. A significant depression ($p<0.001$) in either the absolute or relative testes weight was observed in both strains of rats at 1600 mg/kg/day. Additionally 1600 mg/kg/day BBP significantly reduced ($p<0.05$) the growth rate and increased the absolute ($p<0.05$) and relative ($p<0.001$) liver weights in both strains of rats. Relative liver weights were also significantly ($p<0.001$) increased in Wistar rats at 480 mg/kg BBP. Histologic examination of the testes revealed testicular atrophy in both strains (1600 mg/kg/day) with the extent of the lesions being more severe in the Sprague-Dawley rats. At 480 mg/kg/day BBP, 1/6 Sprague-Dawley rats showed a degree of testicular atrophy, whereas the Wistar albino strain revealed no histologic changes.

DBP. Calley et al. (1966) found that weight gain retardation and peritonitis occurred in Swiss Webster mice that had received daily i.p. injections of 250 or 500 mg/kg DBP for 6 weeks. Testicular atrophy occurred in the DEHP-treated rats (see Reproductive Section). No clear hematologic differences were found between control and experimental test groups.

In a dietary study DBP was fed to male and female Fischer 344 rats at 0, 0.6, 1.2 and 2.5% for 21 days (CMA, 1986). Corresponding dose levels were 0, 624, 1234 and 2156 mg/kg/day for males and 0, 632, 1261 and 2107 mg/kg/day for females, respectively. Absolute and relative liver weights were significantly increased in both male and female rats at all treatment levels. Male rats fed 2.5% DBP had severe testicular atrophy and significantly lower testes weight. Samples of liver from rats administered the 2.5% level showed moderate peroxisomal proliferation. In addition lauric acid 11- and 12-hydroxylase increased in males given 0.6, 1.2 and 2.5%. Cyanide-insensitive palmitoyl CoA oxidation increased at 1.2 and 2.5% in males and 2.5% in females.

Murakami and Nishiyama (1986) fed male Wistar rats powdered diets containing 0, 0.5 or 5% DBP, MBP, PA or DEHP. Corresponding levels were 250 mg/kg and 2500 mg/kg (assuming 0.05 kg food consumption and a 350 g rat). The relative weights of liver, kidney, testicle and spleen were significantly increased in the 5% DBP group. Ultrastructural examination of liver cells revealed increased numbers of peroxisomes, lysosomes and mitochondria (5% DBP). Only hepatocytes of animals in the 5% dose group were examined. Marked spermatogenic damage and testicular atrophy occurred at 5% DBP. The succinate and pyruvate dehydrogenase activities in liver mitochondria were

significantly decreased at both the 0.5 and 5% DBP levels. The investigators concluded that the adverse effects of DBP at least on the liver may be caused by the direct action of intact DBP entering the liver.

DEP. Blickensdorfer and Templeton (1930) studied the toxic properties of DEP in rabbits, guinea pigs and dogs. Rabbits were administered 2 cc/kg bw (2.24 g/kg) i.p. for 8 successive days. No abnormal conditions or "paralysis" was observed, although during and after the period of administration there was some temporary distress. Similar results occurred when rabbits were fed 3 cc/kg (3.35 g/kg) DEP by stomach tube for 8 successive days. The rabbits appeared normal during feedings and for 2 weeks following the last administration. The authors had not yet developed a satisfactory quantitative method for urine analysis; however, they did estimate quantitatively (methods not reported) that >50% was excreted by the kidneys. In the same experiment, guinea pigs administered 1.5 cc/kg (1.68 g/kg) i.p. for 8 successive days showed no permanent ill effects at any time during or after treatment. The authors did not explain what was meant by no permanent ill effects or the length of observation after treatment. Dogs were administered 0.25 cc/kg (0.28 g/kg) DEP in a physiologic salt solution by injection into the femoral vein. Respiration was first stimulated and then paralyzed. Traces of DEP were detected in urine samples taken after the injection began. The authors stated that "considerable" quantities of DEP (2 cc/kg bw in rabbits) may be taken without causing any damage. However, they also stated that since DEP is rapidly excreted by the kidneys, functional damage to the kidneys may cause sufficient DEP accumulation in the blood leading to "nervous system damage".

DMP. Krauskopf (1973) summarized data on the LD_{50}s for several PAEs, some of which came from unpublished reports. LD_{50}s for DMP in guinea pigs, mice, rabbits and rats were 2.4, 7.2, 4.4 and 6.7-6.9 g/kg, respectively. In one acute oral study, mice and dogs ingested a single dose of 1-4 and 0.7-1.4 g/kg DMP, respectively, without observable effects. Details of the study were not reported (Krauskopf, 1973).

Christian (1985) also summarized LD_{50} results. Rats, mice, rabbits, guinea pigs and chicks were orally administered undiluted DMP. The LD_{50}s were 6.9, 7.2, 4.4, 2.4 and 8.5 mℓ/kg (8.2, 8.6, 5.2, 2.9 and 10.1 g/kg), respectively. Animals were observed for 6 days following treatment. Details of the study were not reported.

Long-Term Toxicity

Long-term toxicity has been evaluated for several phthalate esters. Results of these long-term studies in mammals are discussed as follows and are summarized in Table V-6. The primary target organs of PAE toxicity are the liver and the testes. Other organs and cellular systems have also been shown to exhibit toxic responses following exposure to PAEs. Examples of these include lungs, kidneys and blood platelets (reviewed in Thomas and Thomas, 1984). These complex responses are probably not related to any single active moiety of the PAEs.

DEHP. The oral toxicity of DEHP has been investigated by numerous authors. One of the earliest oral studies of DEHP was reported by Shaffer et al. (1945). In this study, groups of five male albino rats, weighing 120-150 g, were fed dietary levels of 0.375, 0.75, 1.5 and 3.0% DEHP for 90

TABLE V-6

Summary Table of Long-term Toxicity Studies of PAEs in Mammals

Exposure/Route	Critical Effect	Compound	Species	No./Sex	Dose/Time	Reference
Oral	Mortality 75%, pathological changes in digestive tract	DEHP	rat, Wistar	10F 10M	3.40 g/kg/day for 90 days by gavage in olive oil	Nikoronow et al., 1973
	Increase in liver weight	DEHP	rat	10F 10M	0.34 g/kg/day for 90 days	Nikoronow et al., 1973
	Testicular injury (within 2 weeks)	DEHP	rat, unspecified	NS	0.6 g/kg/day for 90 days	Cater et al., 1977
	Testicular injury	DEHP	rat, unspecified	NS	0.12 g/kg/day for 90 days	Cater et al., 1977
	Testicular degeneration, tubular atrophy	DEHP	rat, albino	5M	0.9 and 1.9 g/kg/day for 90 days	Shaffer et al., 1945
	Slight decrease in growth	DEHP	rat	5	0.4 g/kg/day for 90 days	Shaffer et al., 1945
	No effect level	DEHP	rat	5M	0.2 g/kg/day for 90 days	Shaffer et al., 1945
	Body weight slightly decreased in males; NOAEL for testicular atrophy	DEHP	rat, F344	50F, 50M	6000 ppm (322 mg/kg/day in males; 394 mg/kg/day in females) for 24 months	NTP, 1982a; Kluwe et al., 1982a
	Testicular atrophy; pituitary hypertrophy trend toward increase, liver clear cell damage	DEHP	rat, F344	50F, 50M	12,000 ppm (674 mg/kg/day in males; 774 mg/kg/day in females) in diet for 24 months	NTP, 1982a; Kluwe et al., 1982a
	Slight depression of weight gain in females only	DEHP	mouse, B6C3F1	50F, 50M	3000 ppm (674 mg/kg/day in males; 799 mg/kg/day in females) in diet for 24 months	NTP, 1982a; Kluwe et al., 1982a
	No increased mortality when compared to controls but slight decreased weight gain in females only; testicular atrophy and chronic inflammation of the kidney in males	DEHP	mouse, B6C3F1	50F, 50M	6000 ppm (1325 mg/kg/day in males; 1821 mg/kg/day in females) in diet for 24 months	NTP, 1982a; Kluwe et al., 1982a

TABLE V-6 (cont.)

Exposure/Route	Critical Effect	Compound	Species	No./Sex	Dose/Time	Reference
Oral (cont.)	Testicular damage, cessation of spermatogenesis, reduced renal function (females)	DEHP	rat, CD (Sprague-Dawley derived)	15F, 15M	1440 mg/kg/day male; 1414 mg/kg/day female for 17 weeks	Gray et al., 1977
	Growth retardation, testicular damage, cessation of spermatogenesis, increased liver weight	DEHP	rat, CD (Sprague-Dawley derived)	15F, 15M	737 mg/kg/day male; 797 mg/kg/day female for 17 weeks	Gray et al., 1977
	Decreased spermatogenesis, increased liver weight	DEHP	rat, CD (Sprague-Dawley derived)	15F, 15M	143 mg/kg/day male; 154 mg/kg/day female for 17 weeks	Gray et al., 1977
	Mild toxic effect	DEHP	dog, unspecified breed	1M	5 g/kg/day by stomach tube for 14 weeks	Harris et al., 1956
	No effect level	DEHP	dog, unspecified breed	1F	0.1 g/kg/day mixed in diet for 14 weeks	Harris et al., 1956
	Significantly increased liver weights in males only; peroxisomal proliferation	DEHP	rat, Wistar albino	4F, 4M	50 mg/kg/day for 9 months	Mitchell et al., 1985
	Significantly increased liver weights; peroxisomal proliferation; significantly decreased glucose-6-phosphatase activity activity in males only; increased number of lysosomes	DEHP	rats, Wistar albino	4F, 4M	200 mg/kg/day for 9 months	Mitchell et al., 1985
	Significantly reduced body weight; alterations in thyroids; significantly increased liver weights; peroxisomal proliferation; reduction in glucose-6-phosphatase activity; increased number of lysosomes	DEHP	rats, Wistar albino	4F, 4M	1000 mg/kg/day for 9 months	Mitchell et al., 1985
	Increased liver and kidney weights	DEHP	rat, Sherman	32F, 32M	~200 mg/kg/day for up to 24 months	Carpenter et al., 1953
	No adverse effects	DEHP	rat, Sherman	32F, 32M	60 mg/kg/day for up to 24 months	Carpenter et al., 1953
	Increased liver and kidney weight (after 90 days); decreased body weights/LOAEL	DEHP	rat, Wistar	43F, 43M	range of 300-400 mg/kg/day for 24 months	Harris et al., 1956

TABLE V-6 (cont.)

Exposure/Route	Critical Effect	Compound	Species	No./Sex	Dose/Time	Reference
Oral (cont.)	No adverse effects	DEHP	rat, Wistar	43F, 43M	range of 50-80 mg/kg/day for 24 months	Harris et al., 1956
	Mortality (30%), increased liver enlargement, increased kidney weight, decreased body weight	DEHP	rat, Wistar	20F, 20M	0.19 g/kg/day for 12 months	Nikonorow et al., 1973
	No significant adverse effects	DEHP	dog, both sexes, 2 pure blooded cocker spaniels, 2 wire-haired terriers		0.03 mL/kg/day (30 mg/kg/day) first 19 doses and then 0.06 mL/kg/day (60 mg/kg/day) for 240 doses	Carpenter et al., 1953
	Statistically significant increase in relative liver weights in females	DEHP	guinea pigs	23F, 23M	19 mg/kg/day for 1 year	Carpenter et al., 1953
	Statistically significant increase in relative liver weights in females	DEHP	guinea pigs	24F, 24M	64 mg/kg/day for 1 year	Carpenter et al., 1953
	No effect	BBP	rats	NS	0.25% (125 mg/kg/day) and 0.50% (250 mg/kg/day) in diet for 90 days	Krauskopf, 1973
	Increased liver weights; mild decrease in growth	BBP	rats	NS	1.00, 1.50 and 2.00% (500, 750 and 1000 mg/kg/day) in diet for 90 days	Krauskopf, 1973
	No effect	BBP	rats/F-344	15 males/group	0.03, 0.09, 0.28, 0.83% (17, 51, 159, 470 mg/kg/day) for 26 weeks in the diet	NTP, 1985
	Depressed weight gain; decreased mean organ weights in heart, right kidney, lungs, seminal vesicle and right testes; kidneys contained focal cortical areas of infarct-like atrophy; atrophy of seminiferous tubules	BBP	rats/F-344	15 males	2.50% (2875 mg/kg/day) for 26 weeks in the diet	NTP, 1985
	Mortality (50% in first week)	DBP	rat, Sprague-Dawley	10M	1.25% (600 mg/kg/day) in diet for 12 months	Smith, 1953

TABLE V-6 (Cont.)

Exposure/Route	Critical Effect	Compound	Species	No./Sex	Dose/Time	Reference
Oral (cont.)	No adverse effects	DBP	rat, Sprague-Dawley	10M	0.25% (125 mg/kg/day) in diet for 12 months	Smith, 1953
	No effects at 0.5 and 2.5%; decreased growth rate at 5.0%	DEP	rats, albino	15M, 15F/group	0.5% (250 mg/kg/day), 2.5% (1250 mg/kg/day) and 5.0% (2500 mg/kg/day) for 2 years	Food Research Lab., 1955
	No adverse effects	DEP	rats/CD	15M, 15F	150 mg/kg/day for 16 weeks in diet	Brown et al., 1978
	Reduced weight gain in females only	DEP	rats/CD	15M, 15F	770 mg/kg/day in males and 750 mg/kg/day in females for 16 weeks in diet	Brown et al., 1978
	Reduced weight gain in both sexes; increased relative weights of the brain, liver, kidney, stomach, small intestine and full caecum	DEP	rats/CD	15M, 15F	3160 mg/kg/day in males and 3710 mg/kg/day in females for 16 weeks in diet	Brown et al., 1978
	No effects on growth at 2.0% level; effects on growth at 4.0 and 8.0%; chronic nephritis at 8.0%; NOAEL identified at 1000 mg/kg/day	DMP	rats	10F/group	2.0, 4.0 and 8.0% (1000, 2000 and 4000 mg/kg/day) for 2 years	Lehman, 1955
i.p. injection	LD_{50}	DBP	mouse, ICR	10M	0.85 mL/kg[b] (0.89 g/kg)	Lawrence et al., 1975
	LD_{50}	DEP	mouse, ICR	10M	1.39 mL/kg[b] (1.46 g/kg)	Lawrence et al., 1975
	LD_{50}	DMP	mouse, ICR	10M	1.18 mL/kg[b] (1.24 g/kg)	Lawrence et al., 1975
Inhalation	Dose-related increase in gamma globulin	DBP	rat, albino	60M	0.098, 0.256 and 0.98 mg/m³ (0.12, 0.33 and 1.2 mg/kg/day)[a] continuous for 93 days	Men'shikova, 1971

[a]Assuming a default of 50% absorption factor

[b]Dose represents the chronic LD_{50} calculated for mice receiving i.p. injections 5 days/week. LD_{50} values were calculated each week until the LD_{50} remained the same for 3 weeks (minimum of 10 weeks of dosing).

NS = Not specified

days. The author determined approximate daily intakes of 0.2, 0.4, 0.9 and 1.9 g/kg bw/day DEHP, respectively. A fifth group served as a control. At the three highest levels a slight decrease in growth was "somewhat retarded" relative to the controls. Quantitative data was not collected. At 1.5 and 3.0% DEHP, tubular atrophy and degeneration of the testes were observed. No deaths occurred in any of the treated animals and the blood cell counts, hemoglobin concentrations, and differential white cell counts remained normal. The authors concluded that no adverse effects from oral administration would occur at ~0.2 g/kg bw/day or less; however, a slight retardation in growth occurred at 0.4 g/kg bw/day.

Nikonorow et al. (1973) administered DEHP in olive oil by gavage to groups of 10 male and 10 female Wistar rats weighing 90-120 g for 3 months at levels of 340 and 3400 mg/kg/day. The higher dose level resulted in 75% mortality. Pathologic examination of the dead animals revealed congestion of the small intestine and loss of mucosa in the stomach and endometritis. The mean liver weight of animals treated with the lower dose level increased relative to that of the controls. These authors also reported that a daily dietary dose level of 0.36% (180 mg/kg/day assuming rats consume 5% of their body weight) DEHP for ≤12 months resulted in 30% mortality in groups of 20 female and 20 male Wistar rats. Relative to the controls, significant liver enlargement and decreased body weight occurred in rats administered 0.36% DEHP in feed.

Gray et al. (1977) reported the effects of a 17-week dietary intake of 0, 0.2%, 1.0% or 2.0% DEHP on groups of 15 female and 15 male CD (Sprague-Dawley-derived) strain rats. Mean daily intakes of DEHP, calculated from food consumption data, were 143, 737 and 1440 mg/kg bw/day for male rats fed

0.2, 1.0 and 2.0% DEHP and 154, 797 and 1414 mg/kg bw/day for female rats fed 0.2, 1.0 and 2.0% DEHP, respectively. At the two highest dose levels, the rate of body weight gain and food intake were reduced; however, a paired feeding study showed that the effect on body weight gain was not entirely due to decreased food consumption. Renal concentrating and diluting ability were reduced in the females receiving 2.0% DEHP. At 1.0% or 2.0% dose levels, the relative testes weights were significantly ($p<0.001$) decreased and histopathologic examination revealed severe seminiferous tubular atrophy and cessation of spermatogenesis. At 0.2% DEHP, the testis weight was not reduced, but there was histologic evidence of decreased spermatogenesis. Significantly increased relative liver weight ($p<0.001$ at all levels in males; $p<0.05$ at 0.2%, $p<0.01$ at 1.0% and $p<0.001$ at 2.0% in females) occurred at all treatment levels. Absolute weights of most other organs (brain, heart, spleen, kidneys, adrenals) were decreased at the 1.0 and 2.0% levels, but relative weights (organ weight:body weight) were increased. Because effects on the liver and testes were observed at all dietary levels tested in this study, the NOAEL for DEHP in rats is below the lowest doses tested, 143 or 154 mg/kg/day for males and females, respectively. Cater el al. (1977) also found testicular effects at similar dose levels in a 90-day feeding study conducted by BIBRA.

Mitchell et al. (1985) administered DEHP in the diet of Wistar albino rats (4/sex/group) at nominal doses of 50, 200 or 1000 mg/kg/day DEHP for 3, 17, 14, 28 days or 9 months. Effects at earlier time points are described in Table V-4. Histopathologic examinations were performed on the major abdominal organs. By 9 months the body weights of both sexes treated with 1000 mg/kg/day were significantly reduced. Lesions were also seen in the

thyroids of rats treated with 1000 mg/kg/day. Liver weights were significantly ($p<0.05$) increased at all dose levels in males and at the 200 and 1000 mg/kg/day dose levels in females. Histologic examination revealed marked centrilobular eosinophilia and increased number of lipofuscin deposits in the hepatocytes in both male and female rats (1000 mg/kg/day). Proliferation of the smooth endoplasmic reticulum occurred at all doses in a dose-dependent manner. Marked peroxisomal proliferation was apparent after treatment with 200 or 1000 mg/kg/day. The increased numbers of peroxisomes at 50 mg/kg/day were less pronounced, with males exhibiting greater increases than females. Glucose-6-phosphatase activity was reduced in both sexes at all dose levels. However, decreases were significant at the 200 and 1000 mg/kg/day level in males and only at the 1000 mg/kg/day level in females. Loss of this endoplasmic reticulum enzyme activity may be indicative of hepatotoxicity (Mitchell et al., 1985). Increased number of lysosomes were observed in both sexes of animals at 200 and 1000 mg/kg/day DEHP, however the increase was less marked at the 200 mg/kg/day level.

Carpenter et al. (1953) conducted one of the first long-term oral multigeneration toxicity studies on DEHP using Sherman rats, guinea pigs and dogs. In the rat study, 32 males and 32 females constituting the parental (P_1) generation were fed diets containing 0.04%, 0.13% or 0.4% DEHP for 2 years. The daily intakes of DEHP were calculated to be the following: 0.20 g/kg bw/day for the first year and 0.19 g/kg bw/day for the 2-year period at the 0.4% DEHP level; 0.06 g/kg bw/day for both periods at the 0.13% level; and 0.02 g/kg bw/day for both periods at the 0.04% level. In addition, ~80 first filial generation (F_1) rats were fed ~200 mg DEHP/kg/day for 1 year. Numbers of rats per group surviving the 2-year test period were not

specified. However, it was reported that 70.3% mortality occurred in the parental (P_1) controls. This figure was 9.3% higher than mortality among the ~200 and 60 mg/kg/day treated groups and 5% higher than that for the 20 mg/kg/day group. It was unclear how the percentages were calculated. No increases in mortality were associated with DEHP in the diet in either the (P_1) or the progeny (F_1) test groups.

Mean weights of the liver and the kidneys of the 0.4% (P_1) group sacrificed after 365 days and of (F_1) rats (also fed 0.4% DEHP) were significantly ($p<0.05$) increased compared with their respective control groups. The organ weight of rats in the lower dose groups (0.04%, 0.13%) was not significantly affected. Microscopic examination of organ tissues did not reveal significant ($p<0.05$) incidences of organ damage in any of the treated groups. Tumor incidence was similar in controls and treated animals. Although the type and location of tumors were not specified, it was noted that none of the tumors were characterized as being malignant. No effects were observed on hematologic parameters. The authors also concluded there was "no true effect upon fertility."

In another portion of the same study (Carpenter et al., 1953), groups of hybrid guinea pigs (23 or 24/sex/group) were fed diets containing 0, 0.04 and 0.13% DEHP for 1 year. Based on food consumption data, the authors calculated daily intakes for the last 10 months of the study to be 0.019 and 0.064 g/kg bw/day DEHP for the 0.04% and 0.13% DEHP groups, respectively. No effects were observed on mortality, life expectancy, body weight, food consumption, micropathology or neoplasms. Liver and kidney weights of male guinea pigs from the treated groups and kidney weights of treated females were not different from controls. Liver weights were significantly higher

in the female guinea pigs from treated groups than in the control animals. The liver weights in females, as percentage of body weight, were 3.07%, 3.43% and 3.49% for the control, 0.04% and 0.13% groups, respectively. However, combining the data for both sexes removed the significance at the 0.04% dietary level but not at 0.13%. The authors concluded that the effect was not related to DEHP concentrations since the increase in liver weight did not appear to be dose-related. A "no effect" dose (NOEL) of DEHP in guinea pigs (for 1 year) was estimated to be ~0.06 g/kg bw/day.

Carpenter et al. (1953) also studied dogs after 1 year of exposure to DEHP. Four pure-blooded cocker spaniels and four wire-haired terriers were randomly separated by breed and sex into two groups. The dogs in one group served as controls. The second group was administered DEHP in gelatin capsules at 0.03 mL/kg/day, 5 days/week for the first 19 doses and then 0.06 mL/kg/day for 240 doses. The mean weight gain of dogs receiving 0.06 DEHP was 0.78 kg as compared with 0.31 kg in the controls. The two groups were never statistically ($p<0.05$) different in weight during the study. The mean liver and kidney weights were 2.66 and 0.47% of body weight in DEHP-treated dogs and 3.00 and 0.52% of body weight in controls. These figures are statistically ($p<0.05$) similar. There was no gross or microscopic pathology of the lung, heart, liver, stomach, upper and lower intestine, cecum, spleen, adrenal, ovary or testes, bladder or thyroid gland. The authors determined the "no effect" level in dogs at 0.06 mL/kg/day (0.06 g/kg/day).

Harris et al. (1956) conducted a long-term oral toxicity study in which groups of 43 male and 43 female Wistar rats were fed diets containing 0, 0.1% and 0.5% DEHP. Rats were sacrificed periodically, and food consumption

records, body weight, and liver, testes, kidney, lung, brain, stomach, heart and spleen weights were examined. Food consumption of the 0.5% DEHP group was ~75% that of the control group by the end of the first year. At that time the DEHP intake of the 0.1% dietary group ranged between 0.05 and 0.08 g/kg bw/day and that of the 0.5% group between 0.3 and 0.4 g/kg bw/day with the higher amounts consumed during the first 6 months. Histopathologic studies were also conducted on selected tissues and organs. The study was terminated after 24 months. Significant increases in liver and kidney weights were noted at the 0.5% dose level at 3 and 6 months but not at 1 and 2 years. The liver and kidney weights did not differ significantly (analysis not provided) in any of the groups, but the authors pointed out that this may have been due to the small number of rats that remained after these longer periods. During the 2-year test period, 85-95% of the rats died. No unusual organ or tissue pathology was noted. The average body weight of the 0.5% DEHP group was ~50 g less than the 0.1% and control groups at the end of 1 year. Body weight averages of the three groups at 2 years were similar. The authors reported no adverse effect on mortality with increasing percentages of DEHP in the diet. However, they did not report the DEHP consumption of individual survivors. The results of this study, at least for the first year, appear to be consistent with those of Carpenter et al. (1953) in that no effect levels and doses producing liver and kidney enlargement were comparable. However, high mortality in all groups prevent statistical analysis of results reported in the 2-year study by Harris et al. (1956).

Kluwe et al. (1982a) reported on the non-neoplastic effects observed in male and female Fischer 344 rats and B6C3F1 mice during the 2-year NCI/NTP carcinogenesis bioassays on DEHP. Details of the experimental procedures

for this study are given in the section titled "Carcinogenicity" later in this chapter. In male Fisher 344 rats fed diets containing 6000 and 12,000 mg/kg of DEHP and female rats fed 12,000 mg/kg, body weight gain was slightly decreased throughout the latter 78 weeks of the study. This decrease was also found in female mice treated with either 3000 or 6000 mg/kg diet of DEHP but did not occur in male mice treated at these levels. Treated male and female rats consumed slightly less food than did control rats, but food consumption in mice was largely unaffected. Mean daily intake of DEHP calculated from the food consumption data was 322 and 674 mg/kg bw/day for low- and high-dose male rats, 394 and 774 mg/kg bw/day for low- and high-dose female rats, 674 and 1325 mg/kg bw/day for low- and high-dose male mice, and 799 and 1821 mg/kg bw/day for low- and high-dose female mice. No other clinical signs of toxicity were observed in either rats or mice. Survival was not significantly ($p<0.05$) affected in male or female rats or in male mice. In the low-dose female mouse group, however, survival was significantly decreased ($p<0.05$) with most deaths occurring after 75-90 weeks of treatment. The authors felt that these deaths were not due to DEHP because pathologic changes in tissues were not observed microscopically, and deaths were not observed at the higher DEHP dose.

Several nonneoplastic lesions were associated with DEHP treatment. Among male rats receiving 12,000 mg/kg diet (high-dose) of DEHP, seminiferous tubular degeneration and testicular atrophy occurred in 90% of the animals compared with an incidence rate of 2% in controls. The tubules in the affected animals were devoid of spermatocytes and germinal epithelium and only Sertoli cells were found lining the basement membrane. These lesions occurred in only 5% of the male rats receiving 6000 mg DEHP/kg and were not significantly increased. Another effect observed in male rats

fed 12,000 mg/kg DEHP in the diet was hypertrophy (cytoplasmic enlargement) of the cells in the anterior pituitary. This effect occurred in 45% of the animals compared with 2% in controls and none in low-dose males. Cellular hypertrophy of this sort probably occurs as a secondary effect of atrophy of the seminiferous tubule epithelium and may be indicative of anterior pituitary hyperactivity (Kluwe et al., 1982a). A dose-related increase in the number of animals with foci of clear cell changes in the liver was observed among both male and female rats; however, pairwise comparison of controls to low- or high-dose groups did not show significent differences.

In male mice ingesting 6000 mg/kg diet (high-dose), incidence of seminiferous tubular degeneration was increased ($p<0.05$) compared with controls, and the lesion was similar to that in rats. Chronic inflammation of the kidney was significantly ($p<0.05$) increased in the high-dose male mice. Although nonneoplastic effects were observed at the low-dose levels, the carcinogenic effects of DEHP preclude identifying a NOAEL for effects on the liver.

Immature rhesus monkeys (6 months old, <5 pounds) were used in a study of the toxic effects of DEHP solubilized in blood or in blood products stored in PVC blood bags (Kevy et al., 1978). These blood products were transfused bi-weekly into the animals for time periods ranging from 6 months to 1 year. This dosing program attempted to mimic actual transfusion levels expected in selected patients requiring large-volume blood or blood products transfusions. The total concentration of DEHP received by the monkeys ranged from 6.6-30 mg/kg in platelet-rich plasma. Liver function was assessed by hepato-splenic ratio determination, by bromosulfophthalein

(BSP), by kinetic compartmental analysis, and by routine light microscopy of liver tissues. For hepato-splenic ratio determinations the abdomen of each monkey was scanned 45 minutes after a ^{99m}Technetium-labeled sulfur colloid injection. The hepato-splenic ratio was determined by detecting the mean counts as measured over the liver and the spleen. The tests were repeated 3, 6, 12, 17 and 26 months following the beginning of transfusions. The BSP calculations were done by computer analysis of the plasma disappearance curve following a single BSP injection. Measurements were also made of the following serum chemistries: SGPT, SGOT, lactic acid dehydrogenase, bilirubin and alkaline phosphatase. These were made prior to the start of the experiment and at 4-month intervals. The results of this study showed that abnormalities in liver function persisted $\leq$14 months following cessation of transfusion therapy. DEHP was detected in liver tissue $\leq$14 months after the last blood transfusion in an amount equivalent to 0.8% of that infused. Blood chemistry levels remained normal; however, the authors believed the time period between their measurements was too long to allow detection of transient changes. The work of Kevy et al. (1978) is important since it demonstrates DEHP effects through an exposure route applicable to humans.

DEHP was also tested in rhesus monkeys (2 or 3/group) given repeated transfusions (for 1 year) of plasma containing this chemical (Jacobson et al., 1977). Total DEHP doses ranged from 7-33 mg. DEHP was detectable in the liver of these animals for as long as 5 months after the cessation of exposure to DEHP. This treatment did not induce cancer; however, abnormal liver histopathologic effects and function (such as, decreased BSP clearance) were observed. Morphologic changes included hyperplasia and vacuolation of Kupffer cells, foci of parenchymal necrosis and chronic inflammatory cell infiltrate.

Effects on energy and carbohydrate metabolism have been observed after DEHP exposure. Lake et al. (1976, 1977) reported that a 14-month dietary intake of 1200 mg/kg/day DEHP produced the following effects in male albino ferrets: marked liver enlargement; decreased body weight; and decreased activities of succinate dehydrogenase, aniline 4-hydroxylase and glucose-6-phosphatase. Mitchell et al. (1985) reported similar results in rats administered 1000 mg/kg/day DEHP (Table V-7).

BBP. In a final report, NTP (1985) conducted a concomitant toxicity and mating trial study (discussed in the Reproductive Effects, BBP Section) in F344 rats. For the toxicity study, male rats (15/group) were administered concentrations of either 0, 0.03, 0.09, 0.28, 0.83 or 2.50% BBP in the diet for 26 weeks. Using data presented in the report these dietary levels correspond to 0, 17, 51, 159, 470 and 1417 mg/kg/day, respectively. In this study powdered rodent meal was provided in such a way that measured food consumption at the highest dose level could include significant waste and spillage rather than true food intake. For this reason a standard food consumption rate of 5% rat body weight was used in the 2.5% dose conversion. Throughout the study body weight gain was significantly depressed at the 2.5% BBP level when compared with the controls. There were no deaths attributed to BBP toxicity. All the rats given 2.5% BBP had small testes upon gross necropsy at the 26-week terminal kill. Five of 11 had soft testes, and 1/11 had a small prostate and seminal vesicle. In the 0.03, 0.09, 0.28 and 0.83% BBP dose groups there were no grossly observable effects on male reproductive organs. Terminal mean organ weight values significantly decreased ($p<0.05$) for the heart, right kidney, lungs, seminal vesicles and right testes in the 2.5% treatment group, whereas significant increases ($p<0.05$) occurred in the brain at 0.03 and 0.09% BBP (Table V-8).

TABLE V-7

Long-Term Effects of DEHP on Biochemical Constituents Relating to Hepatotoxicity

Species/ Strain	Route	Dose/Time	Principal Findings	Reference
Ferret/NR	diet	1200 mg/kg/day*/ 14 months	Absence of glycogen, decreased SDH activity, aniline 4-hydroxylase and glucose-6-phosphatase	Lake et al., 1976, 1977
Rat/albino Wistar	diet	50 mg/kg/day/ 9 months	Increased number of peroxisomes; laurate acid hydroxylase induction	Mitchell et al., 1985
		200 mg/kg/day/ 9 months	Increased number of peroxisomes; decreased glucose-6-phosphatase activity in males only; laurate acid hydroxylase induction; β-D-galactosidase induction in females	
		1000 mg/kg/day/ 9 months	Increased number of peroxisomes; decreased glucose-6-phosphatase activity in males and females; decreased cytochrome P-450; laurate acid and β-D-galactosidase induction	

*Mean daily intake

NR = Not reported; SDH = succinate dehydrogenase

TABLE V-8

Mean Terminal Organ Weights in Male Rats After 26 Weeks[a,b]

Dose Level (%)	Number of Surviving Animals	Brain	Heart	Right Kidney	Liver	Lung	Seminal Vesicles	Right Testes
Control	12	1.966±0.108	1.182±0.230	1.277±0.394	12.181±1.840	1.570±0.204	1.387±0.364	1.517±0.151
0.03	14	2.067±0.067[c]	1.179±0.109	1.281±0.145	13.017±0.744	1.726±0.200	1.654±0.387	1.581±0.062
0.09	14	2.081±0.044[c]	1.226±0.145	1.365±0.114	13.759±1.428	1.745±0.171[c]	1.798±0.179[c]	1.619±0.108
0.28	14	2.030±0.086	1.139±0.126	1.249±0.137	12.599±1.247	1.648±0.133	1.489±0.426	1.559±0.100
0.83	15	2.033±0.070	1.140±0.141	1.422±0.173	14.261±1.353[c]	1.638±0.164	1.538±0.343	1.594±0.093
2.50	11	1.916±0.132	0.796±0.118[d]	1.021±0.192[d]	11.782±2.511	1.147±0.150[d]	0.979±0.345[d]	0.457±0.088[d]

[a]Source: Adapted from NTP (1985) draft report

[b]Organ weights in grams (mean±standard deviation)

[c]Significantly greater ($p<0.05$) compared with controls: Dunnett's t-test

[d]Significantly less ($p<0.05$) compared with controls: Dunnett's t-test

At 0.83% the effects noted were significantly ($p<0.05$) increased absolute liver weight, increased liver-to-body weight and liver-to-brain weight ratios and increases in mean corpuscular hemoglobin. Hematological effects at 2.5% BBP included decreased red cell mass, which the authors state is indicative of deficient hemoglobin synthesis, reduced values for hemoglobin, total RBC and hematocrit. The kidneys of six animals in the 2.5% group contained focal cortical areas of infarct-like atrophy. In addition, testicular lesions were also observed at the 2.5% dose level. Lesions were characterized by atrophy of seminiferous tubules and aspermia. The 0.03, 0.09, 0.28 and 0.83% treatment groups showed no evidence of abnormal morphology in any other organs. Collectively, the effects associated with feeding BBP at 2.5% included depression of body weight gain, growth retardation, decreased testicular size, suppression of male reproductive capacity and alterations in hematology values.

In an addendum to the NTP (1985) final report, evaluation of the data revealed a significantly reduced total marrow cell count in the 2.5% dose group (NTP, 1986). The change in total cell count was comprised primarily of significant decreases in neutrophil, metamyelocytes, bands, segmenters, lymphocytes and leasophilic rubricytes. The total marrow cell counts, metamyelocyte and leasophilic rubricyte counts were also significantly decreased in the lowest dose group (0.03%). No statistically significant differences were noted in the middle dose groups (0.09, 0.28 or 0.83%) when compared with controls. The addendum states that decreased total marrow cell count in the 0.03 and 2.5% dose groups represent change of uncertain meaning in light of the systemic effects noted in the middle dose groups. Trend analysis by the Terpstra-Jonckheere test revealed significantly

($p<0.05\%$) decreasing trends in all of the previously mentioned parameters as well as an increasing trend for monocytes at 0.03 and 2.5%.

Krauskopf (1973) presented data on BBP from an unpublished long-term rat and dog study done by Monsanto (1972). No effects were observed in rats administered levels of 0.25 (125 mg/kg/day) and 0.50% (250 mg/kg/day) BBP in the diet for 90 days. Liver weights were increased in animals fed diets containing 1.0, 1.5 or 2.0% (500, 750 or 1000 mg/kg/day, respectively) for 90 days, and a mild decrease in growth rate was reported for the 1.50 and 2.00% groups. No other hematologic, histopathologic or urinalysis effects were observed. Dogs were given gelatin capsules containing BBP at doses equivalent to 1.0, 2.0 or 5.0% of the daily diet (10, 20 and 50 g/kg) for 90 days. No deaths occurred, and weight gain was not affected at the 1.0 and 2.0% dose levels. The group fed 5.0% gained less weight initially because they refused to eat; however, the use of capsules restored normal eating. No effects were found at any dose level on hematologic parameters, urinalysis or liver and kidney functions (Krauskopf, 1973).

DBP. Smith (1953) studied the effects of feeding DBP to groups of 10 male 5-week-old Sprague-Dawley rats, weighing 55-65 g. Rats were fed dietary levels of 0, 0.01, 0.05, 0.25 and 1.25% DBP for 1 year. Body weights, food and DBP intakes were measured daily for the first 3 months and weekly for the remainder of the study. The dietary intakes for DBP were 0, 5, 25, 125 and 600 mg/kg/day for 0.01, 0.05, 0.25 and 1.25%, respectively, estimated from data by Smith (1953). Hematologic parameters (hemoglobin, total erythrocyte and total and differential leucocyte counts) were assessed at intervals of 3, 6 and 9 months. Necropsies were performed when rats

showed marked weight loss or signs of severe infection. Animals remaining at the end of 1 year were sacrificed and examined for gross pathologic changes. While it was stated that several organs were sectioned and stained, no histologic evaluation was reported. No adverse effects on growth, survival, gross pathology or hematology were observed in the animals fed diets containing 0.01, 0.05 or 0.25% DBP. However, the number of animals surviving the 1-year period were not reported for the control or three lowest dose groups. In the group fed 1.25% DBP, half of the animals (5/10) died during the first week of the experiment. The remaining animals gained weight proportionate to controls. It was not indicated whether the deaths were thought to be treatment-related. The daily intake of food and plasticizer (mg/kg bw/day) decreased as the rats increased in size. No changes in hematologic parameters or gross pathology were observed at any dose level. Results of this study suggest that DBP has low chronic oral toxicity. However, this study is weakened by the small number of animals used in the study, the lack of animal survival data, animal infections, the few survivors among the high-dose group, and a lack of micropathologic examination.

DEP. In a 2-year study (Food Research Laboratories, Inc., 1955) groups of 30 rats (15 of each sex) were fed either 0.5, 2.5 or 5.0% DEP (250, 1250 or 2500 mg/kg bw/day, respectively, assuming rats consume 5% of their body weight) in the diet. No effects were observed at levels of 0.5 or 2.5%. DEP at the 5.0% dose level resulted in a small but significant decrease in the growth rate of the rats without any effect on food consumption. No information was available on the numbers of rats surviving the 2-year study period. Also as part of this study, 13 young mongrel dogs were

fed DEP in the diet at levels of 0.5, 1.5, 2.0 and 2.5% for 1 year. The average weekly intakes of DEP calculated by the investigators were 0.8, 2.4, 3.5 and 4.4 g/kg/week in order corresponding to increasing dietary level. Accordingly, three dogs were maintained at 0.5%, one each at 1.5 and 2.0%, and three at the 2.5% level. No effects were noted in dogs as a result of DEP exposures; however, histopathologic examinations were performed only on the kidney and liver in all the dogs. In addition, the heart, spleen, pancreas, GI tract, adrenal glands and thyroid glands were histopathologically examined in the three dogs of the 2.5% dosage group.

Brown et al. (1978) also studied the long-term oral toxicity of DEP in rats. Groups of 15 CD strain rats of each sex were given diets containing 0, 0.2, 1.0 or 5.0% DEP for 16 weeks. The authors estimated the mean intakes to be 0, 150, 770 and 3160 mg/kg/day in males and 0, 150, 750 and 3710 mg/kg/day in females, respectively. Water intake, food intake and body weights were measured weekly. Variables monitored in the study included body weight, food consumption, water intake, hematology, urinalysis, serum biochemistries, and gross histopathology. Autopsies and histologic examinations were carried out at the end of 16 weeks. No changes in behavioral patterns or clinical signs of toxicity were observed. Female rats fed diets containing 1% DEP and both sexes fed diets of 5% DEP gained significantly less weight than the controls. Mean food consumption of rats of both sexes given 5% DEP and females given 1% DEP was significantly lower than that of control rats. In order to rule out palatability as the possible cause in decreased weight gain, a paired-feeding study was conducted. Test rats fed 5% DEP consumed more food (total) and gained less weight than controls. Absolute weights of the brain, heart, spleen and kidney were significantly

lower in male and female rats fed 5% DEP. Female rats given 5% DEP showed a statistically significant increase in "full caecum" weight. There were no statistically significant changes in the absolute weights of any organs below the 5% DEP dietary level. Relative (to body) weights of the brain, liver, kidney, stomach, small intestine and full caecum were significantly higher in both sexes at the 5% dietary level when compared with the controls. These changes were attributed to the compound-related effect on growth rate since dose-related changes in gross or microscopic pathology were not observed. No other effects were observed.

DMP. In a study of insect repellants by Lehman (1955), DMP was fed in the diet to 10 female rats per dose group at levels of 2.0, 4.0 and 8.0% (1000, 2000 and 4000 mg/kg/day assuming rats consume 5% of their body weight) for 2 years. Details of the study were brief. No effect on growth was observed at the 2% DMP dietary level. However, at the 4.0 and 8% DMP levels there was a "slight but significant" (analysis not reported) effect on growth. It was not stated if the effect was an increase or decrease. Chronic nephrilis occurred only at the 8.0% DMP level. Mortality rates did not differ in treated rats when compared with the controls.

Reproductive Effects

Studies have shown that several PAEs have adverse effects on reproduction. These effects are summarized in Table V-9.

DEHP. Peters and Cook (1973) administered 4 mL/kg (4 g/kg) of DEHP in saline to pregnant rats (Sprague-Dawley) i.p. on days 3, 6 and 9 of gestation. At this dose level, implantation of embryos was prevented in 4/5

TABLE V-9

Summary of Teratogenicity and Reproductive Effects of Phthalates

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
Oral	Embryonic mortality	DEHP DEHP	mouse mouse	1.0%[a] (10 g/kg) 0.5%[b] (50 g/kg)	gestation days 0-18	Hamano et al., 1977
	Retarded ossification	DEHP	mouse	0.05% (70 mg/kg/day)	gestation days 0-18	Shiota and Nishimura, 1982
	Reduced fetal weight, ossification retardation, skeletal abnormalities, decreased maternal weight	DEHP	mouse	0.2% (410 mg/kg/day)	gestation days 0-18	Shiota and Nishimura, 1982
	No viable fetuses	DEHP	mouse	0.4% (830 mg/kg/day) 1.0% (2200 mg/kg/day)	gestation days 0-18	Shiota and Nishimura, 1982
	Decreased fetal weight, increased fetal resorptions; LOAEL of 340 mg/kg/day	DEHP	rat	340 mg/kg/day 1700 mg/kg/day	21 days 21 days	Nikonorow et al., 1973
	Average body weight of viable fetuses was significantly lower than the controls	DEHP	mouse	49.3 and 98.6 mg/kg	single dose on day 7 of gestation	Tomita et al., 1982a
	Dose producing same mortality rate as that of controls estimated from dose-response curve	DEHP	mouse	~64 mg/kg	single dose on day 7 of gestation	Tomita et al., 1982a
	Fetal LD_{50}; estimated from dose-response curve	DEHP	mouse	592 mg/kg	single dose on day 7 of gestation	Tomita et al., 1982a
	NOAEL for gross abnormalities; estimated from a dose-response curve	DEHP	mouse	~789 mg/kg	single dose on day 7 of gestation	Tomita et al., 1982a
	NOAEL for skeletal abnormalities; estimated from a dose-response curve	DEHP	mouse	~670 mg/kg	single dose on day of gestation	Tomita et al., 1982a
	No effect on fertility or reproductive performance	DEHP	mouse	0.01% (100 mg/kg)	in diet 7 days premating 98 days continuous breeding	NTP, 1984a

TABLE V-9 (cont.)

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
Oral (cont.)	Reduced fertility, decreased number of litters, decreased viable fetuses	DEHP	mouse	0.1% (1 g/kg)	in diet 7 days premating 98 days continuous breeding	NTP, 1984a; Lamb et al., 1987
	Total infertility[c]	DEHP	mouse	0.3% (3 g/kg)	in diet 7 days premating 98 days continuous breeding	NTP, 1984a; Lamb et al., 1987
	Reduced fetal body weight at 1.0, 1.5 and 2.0%; reduced gravid uterine weight at 2.0%; not teratogenic at any dose level; increased number and percentage of resorption, nonlive and affected inplants per litter at 2.0%	DEHP	rats	0, 0.5, 1.0, 1.5 and 2.0% (357, 666, 856 and 1055 mg/kg/day)	dietary levels administered on gestational days 0-20	Tyl et al., 1988
	Reduced gravid uterine weight at 0.10 and 0.15%; increased number and percentage of resorptions, nonlive and affected inplants per litter at 0.10 and 0.15%; number of litters with affected inplants increased at 0.05%; number and percentage of fetuses malformed/litter elevated at 0.05, 0.10 and 0.15%; embryofetal NOEL determined to be 44.07 mg/kg/day	DEHP	mice	0, 0.025, 0.05, 0.10 and 0.15% (44, 91, 191 and 292 mg/kg/day)	dietary levels administered on gestational days 0-17	Tyl et al., 1988
	Significant increase in incidence of resorptions and dead fetuses at 1000 and 2000 mg/kg; dose-related decrease in average weight and increase in incidence of malformed viable fetuses; significant only at 1000 and 2000 mg/kg	DEHP	mouse	0, 250, 500, 1000 and 2000 mg/kg	intubation in olive oil on gestation days 7, 8 and 9	Shiota and Mima, 1985

TABLE V-9 (cont.)

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
Oral (cont.)	Retarded body weight gain in a dose-dependent manner (statistically significant at 5000 and 20,000 ppm; significantly reduced absolute and relative testis weight at 20,000 ppm; no significant changes in weights of seminal vesicles or pituitary; severe atrophy in seminiferous tubules and loss of spermatogenesis, reduced epididymal sperm density and motility at 20,000 ppm; increased occurence of abnormal sperm and decreased zinc content at 20,000 ppm	DEHP	rats	0, 320, 1250, 5000 or 20,000 ppm (16, 62.5, 250 or 1000 mg/kg/day)[d]	male rats in diet for 60 days	Agarwal et al., 1986
	Tubular atrophy in testis	DEHP	hamster	4200 mg/kg/day	intubation in corn oil for 9 days	Gray et al., 1982
	Tubular atrophy in testis; significant increased urinary zinc excretion; significant decreased testicular zinc concentration	DEHP	rat	2800 mg/kg/day	intubation in corn oil for 9 days	Gray et al., 1982
	No effect	BBP	rat	0.03% (17 mg/kg/day)	in diet for 10 weeks	NTP, 1985
	No effect	BBP	rat	0.28% (159 mg/kg/day)	in diet for 10 weeks	NTP, 1985
	No effect	BBP	rat	0.83% (470 mg/kg/day)	in diet for 10 weeks	NTP, 1985
	Significant reduction in body weight gain; significant reduction in mean organ weights of right kidney, liver, lungs, prostate, seminal vesicles and right testes; atrophy of seminiferous tubules and near total absence of mature sperm production	BBP	rat	2.5% (2875 mg/kg/day)	in diet for 10 weeks	NTP, 1985

TABLE V-9 (cont.)

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
Oral (cont.)	Increased liver and kidney weights	BBP	rat	0.625% (375 mg/kg/day) 1.25% (750 mg/kg/day)	14 days in diet	Agarwal et al., 1985a
	Decreased body weight; slight decrease in food consumption; decreased testes, epididymus, seminal vesicle and thymus weights; histological atrophy of testes and accessory sex organs	BBP	rat	2.5% (1250 mg/kg/day) 5.0% (1667 mg/kg/day)	14 days in diet	Agarwal et al., 1985a
	No adverse effects	DBP	rat	120 mg/kg/day	21 days	Nikonorow et al., 1973
	Fetal weight significantly reduced/LOAEL	DBP	rat	600 mg/kg/day	21 days	Nikonorow et al., 1973
	Retarded ossification	DBP	mouse	0.05% (80 mg/kg/day) 0.1% (180 mg/kg/day) 0.2% (370 mg/kg/ay)	gestation days 0-18	Shiota and Nishimura, 1982
	Reduced fetal weight; retarded ossification	DBP	mouse	0.4% (660 mg/kg/day)	gestation days 0-18	Shiota and Nishimura, 1982
	Reduced fetal weight; retarded ossification; decreased maternal weight; neural tube defects	DBP	mouse	1.0% (2100 mg/kg/day)	gestation days 0-18	Shiota and Nishimura, 1982
	Embryonic mortality	DBP	mouse	0.5%[b] (50 g/kg)	gestation days 0-18	Hamano et al., 1977
	Significant depression of testes weight; atrophy of seminiferous tubules; loss of spermatids; reduction in number of primary spermatocytes and spermatogonia	DBP	mouse	2000 mg/kg	intubation in corn oil for 9 days	Gray et al., 1982

TABLE V-9 (cont.)

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
Oral (cont.)	Significant depression of testes weight; atrophy of seminiferous tubules; loss of spermatids; reduction in number of primary spermatocytes and spermatogonia	DBP	guinea pigs	2000 mg/kg	intubation in corn oil for 7 days	Gray et al., 1982
	No histological changes; reduction in testes weight but was not significant	DBP	hamsters	3000 mg/kg	intubation in corn oil for 9 days	Gray et al., 1982
	Decreased number of litters, litter size and fertility as well as decreased mean pup weight at 10 g/kg; NOAEL at 3 g/kg	DBP	CD-1 mice	0.03, 0.3 and 1% (0.3, 3 and 10 g/kg)	for 7 days prior to pairing and for 98 days to breeding pairs and for an additional 21 days	NTP, 1984b
	No effect on maternal weight gain, litter size or average pup weight	DMP	mouse	3500 mg/kg	gestation days 7-15	Plasterer et al., 1985
i.p. injection	Gross or skeletal abnormalities	DEHP	rat	5 mL/kg (5 g/kg), 10 mL/kg (10 g/kg)	days 5, 10 and 15 of gestation	Singh et al., 1972
	Decreased body weight gain for 12 weeks after exposure to 5.2 g/kg/day; testis weight not affected; no reductions in sperm numbers at any dose; no increases in sperm morphology	DEHP	rats	0, 0.52, 2.6, 5.2 g/kg/day	5 days of exposure followed by 12 weeks of observation	Douglas et al., 1986
	Decreased body weight gain at 6.0 g/kg; significant decline in testis weight 8 weeks after exposure to 6.0 g/kg; significant reduction in sperm number per epididymis from 4 weeks after exposure to 6.0 g/kg; no increases in sperm morphology	DEHP	mice	0, 0.6, 3.0, 6.0 g/kg/day	5 days of exposure followed by 12 weeks of observation	

TABLE V-9 (cont.)

Exposure/Route	Critical Effect	Compound	Species	Dose	Time	Reference
i.p. injection (cont.)	Implantation of embryos prevented, adverse effects on parturition (excessive bleeding, incomplete expulsion of fetus, maternal deaths)	DEHP	rat	2 mL/kg (2 g/kg) 4 mL/kg (4 g/kg)	days 3, 6 and 9 of gestation	Peters and Cook, 1973
	No teratogenic effects below the abortifacient dosage of 8000; average weight of viable fetuses and incidence of resorptions and dead fetuses did not differ significantly from the controls	DEHP	mouse	0, 500, 1000, 2000, 4000 and 8000 mg/kg	gestation days 7, 8 and 9	Shiota and Mima, 1985
	Gross or skeletal abnormalities	DBP	rat	0.305, 0.610 and 1.02 mL/kg (0.318, 0.636 and 1.06 g/kg)	days 5, 10 and 15 of gestation	Singh et al., 1972
	Gross or skeletal abnormalities	DEP	rat	0.506, 1.012 and 1.686 mL/kg (0.566, 1.13 and 1.88 g/kg)	days 5, 10 and 15 of gestation	Singh et al., 1972
	Gross or skeletal abnormalities; fetal deaths	DMP	rat	0.338, 0.675 and 1.125 mL/kg (0.40, 0.80 and 1.33 g/kg)	days 5, 10 and 15 of gestation	Singh et al., 1972

[a]Highest concentration used

[b]Lowest concentration used

[c]Subsequent crossover mating of these mice to controls revealed infertility in males and decreased fertility in females

[d]Assuming rats consume 5% of their body weight

rats. At a dose level of 2 mℓ/kg, a similar response occurred in 3/5 rats. Adverse effects on parturition such as excessive bleeding, and incomplete expulsion of fetuses as well as maternal deaths were noted in dams treated with DEHP. DBP and DMP were also tested by these authors; however, the adverse effects were less severe than those observed for the DEHP-treated rats. It was noted that adverse effects prior to gestation day 6 were primarily on implantation while after this time, the effects were primarily on parturition.

In a study by Singh et al. (1975), pregnant Sprague-Dawley rats were injected i.p. on day 5 or 10 of gestation with 5 mℓ/kg (5.6 g/kg) radiolabeled DEHP and 1.0116 mℓ/kg (1.13 g/kg) radiolabeled DEP. Results of this study demonstrated that these PAEs could pass through the placental barrier to the developing fetus. The data indicate that the developmental toxicity of the PAEs could be the result of the direct effect of the compound (or its metabolites) upon developing embryonic tissue.

The teratogenic effects of PAEs following oral administration were studied by Nikonorow et al. (1973). In this study pregnant Wistar rats were administered DEHP orally in olive oil at doses of 0.34 and 1.70 g/kg/day for 21 days following confirmation of conception. Results of this study indicated that fetal weight was significantly reduced at both dose levels of DEHP. No detectable differences were observed in the number of sternum ossification foci, the development of the bones at the base of the skull, paws on the front and hind legs, or rib fusion in fetuses from treated rats when compared with the control animals.

Shiota and Nishimura (1982) studied the effects of DEHP and DBP in mice. DEHP and DBP were administered at dietary levels of 0.05, 0.1, 0.2, 0.4 and 1% by weight to groups of pregnant ICR-ICL mice from days 0-18 of gestation. Average daily doses, calculated from food intake and body weight, were 70, 190, 410, 830 and 2200 mg/kg/day for the 0.05, 0.1, 0.2, 0.4 and 1% dietary levels of DEHP, respectively. Mice were monitored daily for food consumption and weight. On day 18, the mice were sacrificed and uteri were removed. Implantation sites, resorptions and dead fetuses were recorded. Live fetuses were dried of amniotic fluid, weighed, sexed and inspected. Half of the fetuses from each litter were examined for skeletal malformations and the state of ossification. The other half were razor blade sectioned and examined for internal abnormalities. Maternal weight gain was decreased and resorption rate was increased when mice were fed 0.2, 0.4 and 1% DEHP. Intrauterine deaths generally occurred in the early stages of conception. At 0.4 and 1% DEHP, all implanted ova died in utero resulting in no viable fetuses at term. A dose-related decrease in the mean weight of fetuses alive at term was found in the treated groups. Malformed fetuses resulted from treatment with 0.2% DEHP. The major malformations in these fetuses were neural tube defects (exencephaly and myeloschisis), indicating that the PAEs affect neural tube closure in developing embryos. Ossification was retarded in all treated groups except the one given 0.1% DEHP. The authors concluded that delayed ossification was related to the general underdevelopment of the fetuses. Incomplete skull and leg bones also occurred occassionally in the treated groups. Microdissection of the fetuses showed no internal malformations. The authors stated that the maximum nonembryotoxic dose in mice would be at least 70 mg/kg/day for DEHP.

More recently DEHP was evaluated for developmental toxicity in Fischer 344 rats and CD-1 mice (Tyl et al., 1988). Dietary levels of DEHP were administered on gestational days 0-20 to rats at 0, 0.5, 1.0, 1.5 or 2.0% and on gestational days 0-17 to mice at 0, 0.025, 0.05, 0.10 or 0.15%. Corresponding levels in mg/kg/day were 0, 357, 666, 856, 1055 and 0, 44, 91, 191, 292 in rats and mice, respectively. Maternal body weight and fetal body weight were significanty reduced ($p<0.01$) at 1.0, 1.5 and 2.0% DEHP in rats. Gravid uterine weight in rats was also reduced at 2.0% DEHP. In mice, gravid uterine weight was reduced at 0.10 and 0.15% DEHP while maternal relative liver weight was elevated at the same levels. The number and percentage of resorptions, nonlive (dead plus resorbed) and affected (nonlive plus malformed) implants per litter were significantly increased at 2.0% in rats and 0.10 and 0.15% in mice. The number and percentage of fetuses malformed/litter were unaffected in DEHP-treated rats; however, reduced reduced fetal body weight/litter was observed at 1.0, 1.5 and 2.0%. The number and percentage of malformed fetuses/litter were elevated ($p<0.01$) at 0.05, 0.10 and 0.15% DEHP in male and 0.10 and 0.15% DEHP in female mice. Treatment-related malformations consisted of open eye, exophthalmia, exencephaly, short, constricted or no tail, major vessel malformations, fused or branched ribs and fused or misaligned thoracic vertebral centra. Tyl et al. (1988) concluded that DEHP was not teratogenic at any dose tested in Fischer 344 rats. However, treatment did produce maternal and other embryofetal toxicity at 1.0, 1.5 and 2.0%. An embryofetal NOEL in rats was reported as 0.5% (357 mg/kg/day). In mice, doses (0.10 and 0.15%) that produced maternal and embryofetal toxicity also increased incidence of malformations. A dose of 0.05% (91 mg/kg/day) DEHP in mice produced increased incidence of malformations without maternal or embryofetal

toxicity. An embryofetal NOEL in mice was reported as 0.025% (44 mg/kg/day) DEHP.

The teratogenic potential of plasma-soluble extracts of two DEHP-plasticized PVC plastics in Sprague-Dawley rats was investigated by Lewandowski et al. (1980). The extracts were administered intravenously to pregnant rats daily on days 6-15 of gestation. Two groups of rats received extracts from one plastic preparation in doses equivalent to 1.3 and 4.7 mg/kg/day DEHP. Two additional groups received extracts from a second plastic preparation in doses equivalent to 1.4 and 5.3 mg/kg/day DEHP. The high doses were thought to approximate the doses a 60 kg human would receive when undergoing an exchange transfusion of 21-day-old blood. No significant differences between controls and treated groups were found in growth rate and behavior of test animals, fetal weight, number of live and resorbed fetuses or incidence of gross external, skeletal or visceral defects among offspring.

Tomita et al. (1982a) tested the teratogenic and fetotoxic effects of DEHP in mice given single oral doses of 0.05-30 mℓ/kg on day 6, 7, 8, 9 or 10 of gestation. Test animals used were female ddY-Slc(SPF) mice bred to CBA(SPF) mice. A high-dose experiment was performed using dosages of 1.0, 2.5, 5.0, 7.5, 10.0 and 30.0 mℓ/kg (0.986, 2.47, 4.93, 7.40, 9.86 and 29.6 g/kg/day) of DEHP. In this experiment mice were dosed on day 6, 7, 8, 9 or 10 of gestation. A low-dose single administration was also performed using 1/600, 1/300 and 1/30 of the LD_{50} (0.05, 0.1 and 1.0 mℓ/kg of DEHP corresponding to 49.3, 98.6 and 986 mg/kg/day) administered on day 7 of gestation. For each of the experiments, an untreated control group and an ethylurethane-treated control (positive control) group were included. These

experiments showed that number of implantations per pregnant mouse was not significantly different among control and DEHP treated groups; however, the number of early and late embryo deaths varied greatly, depending on day and amount of DEHP administered. Generally, in mice receiving DEHP on days 7 or 8 of gestation, incidence of embryo death was high, whereas incidence of embryo death was low in mice treated on days 6, 9 or 10. The incidence of early embryo death was higher at the higher doses of DEHP, while the incidence of late embryo death was greater at the lower doses. The rate of gross and skeletal malformations was high in mice receiving 2.5 mℓ/kg (2.47 g/kg/day) of DEHP on day 7 of gestation and in mice receiving 7.5 mℓ/kg (7.40 g/kg/day) on day 8 of gestation. No abnormality was observed in the groups receiving 0.05 or 0.1 mℓ/kg on day 7; however, the average body weight of viable fetuses was significantly lower ($p<0.01$) than that of the control group. Treatment-related gross abnormalities included exencephaly, open eyelid, club foot, and bent or no tail, and skeletal malformations such as abnormally shaped thoracic, lumbar, sacral and caudal vertebrae. The authors calculated the fetal LD_{50} to be 592 mg/kg/day assuming that the specific gravity of DEHP is 0.986. The dose level producing a rate of 2% fetal deaths (the mortality rate found in the control group) was ~64 mg/kg/day. The exact values of maximum no-observed-adverse-effect dose for gross and skeletal abnormalities could not be determined due to lack of data but are estimated to be <0.80 mℓ/kg (789 mg/kg/day) and 0.68 mℓ/kg (670 mg/kg/day), respectively, based on their dose-response curves.

Autian (1982) reported results of a study of antifertility effects of DEHP. Male mice were injected subcutaneously with DEHP on days 1, 5 and 10

of the study at dose levels of 1, 2, 5, 10, 15, 20, 40, 60, 80 and 100 mℓ/kg (1, 2, 5, 10, 15, 20, 40, 60, 80 and 100 g/kg). Seven or more mice were used for each dose level. A group of 16 mice receiving injections of saline served as controls. Males were bred to virgin females on day 21 following the first injection. Females were sacrificed on day 12-13 of gestation, and uterine horns and ovaries were exposed surgically to determine the number of corpora lutea, implantations, preimplantation losses, early fetal deaths and viable fetuses. Incidence of pregnancy was decreased at all dose levels compared with controls; however, the statistical significance of this decrease was not evaluated. Males treated with the lowest dose of 1 mℓ/kg per injection produced progeny in 62.5% of the treated group compared with 87.5% in controls. The trend of antifertility appeared to increase as the dose administered to male mice increased. Increased early fetal deaths and preimplantation losses were also noted in the treated groups, generally increasing as the dose administered increased. The author noted, however, that the results of this experiment indicate antifertility effects but cannot be considered definitive under the experimental conditions employed. Autian (1982) also noted that in a parallel study (the details of which were not given), the effects of lower doses of DEHP on testicular structure and function did not show changes in histopathologic organization or macromolecular contents (nucleic acids and protein) of the tissue, suggesting that increased fetal deaths were not a consequence of testicular atrophy. Alterations in the activity of certain mitochondrial and lysosomal enzymes of the testicular tissue were observed after treatment with DEHP, which may account for changes in the functional ability of the reproductive system. PAEs have also been shown to cause testicular atrophy. A more detailed description of these effects is given in the section discussing target organ toxicity.

A recent study of the effects of DEHP on reproduction and fertility was conducted by NTP (1984a). The NTP (1984a) study was subsequently published as Lamb et al. (1987). This study employed a reproductive toxicology testing scheme referred to as "Fertility Assessment by Continuous Breeding". DEHP was administered in the diet at levels of 0, 0.01, 0.1 and 0.3% (0.1, 1.0 and 3.0 g/kg). Male and female CD-1 mice were given continuous dietary exposure to DEHP during a 7-day premating period and a 98-day continuous breeding cohabitation period. A 21-day segregation period with no DEHP exposure followed. The control group consisted of 40 pairs of mice while 20 pairs of mice were tested at each dose level. Results of the study showed that fertility was completely suppressed at the 0.3% DEHP level and was significantly reduced at the 0.1% DEHP level. The fertility index (number of fertile/number cohabited x 100) was 0 and 74% for the 0.3 and 0.1% groups, respectively. The fertility index value for the control and 0.01% groups was 100% in both cases. Among the 0.1% DEHP breeding pairs that were successfully mated, fewer litters were produced, numbers of male and female live pups per litter were decreased, and the proportion of pups born alive per litter was lower when compared with either the control group ($p<0.01$) or the 0.01% group ($p<0.01$). Also, the proportion of live male pups per total live pups per litter and the female live pup weight were increased in the 0.1% DEHP group compared with the control group ($p<0.05$). Live male pup weight adjusted for the total number of pups per litter was significantly lower at the 0.1% level than at the 0 or 0.01% DEHP ($p<0.05$).

Because the continuous breeding test showed that DEHP had significant effects on fertility and reproduction, a second test, the crossover mating trial, was conducted to determine which sex (male and/or female) was

adversely affected. The control males and females from the continuous breeding test were mated with the high-dose (0.3% DEHP) females and males, respectively, from the continuous breeding test. Another group of control males were mated to control females, both from the first test, to serve as the control for this experiment. Results indicated that fertility was significantly reduced ($p<0.01$) in both the 0.3% DEHP male x control female and the control male x 0.3% DEHP female groups when compared with the control male x control female group. Fertility index values for the three groups were 20%, 0% and 90%, respectively. The number of detected matings (determined by the presence of a copulatory plug) did not differ significantly among the three groups. In the 0.3% DEHP male x control female groups, the proportion of pups born alive was significantly lower ($p<0.05$) than in the control male x control female group; however, the number of live pups/litter, sex of pups born alive and live pup weight were not significantly different. Following the crossover mating trial, male and female parental mice from the control and 0.3% DEHP groups were subjected to gross necropsy. Effects on the male reproductive system included significant reductions in percentage of motile sperm and sperm concentration in the cauda epididymus and in weight of the testis, epididymus and prostate in the 0.3% DEHP-treated mice compared with controls. The percentage of morphologically abnormal sperm in the cauda was also significantly increased. Histopathology revealed extensive destruction of the seminiferous tubules in the treated males. Decreased plasma testosterone levels and elevated plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were observed in the treated group, although differences were not significant due to large variation within treatment groups. Among females, no histopathologic effects were observed. The reproductive tract weight in treated

females was significantly decreased, but it is possible that this was an artifact of lack of pregnancy in this group. Significant increases in liver weight were also observed for both males and females in the 0.3% DEHP groups.

A study in which groups of rats were fed diets containing 0.2, 1.0 or 2.0% DEHP for 90 days, corresponding to mean daily intakes of ~150, 750 and 1500 mg/kg bw/day, showed a decrease in the relative testes weight of rats fed 1.0 and 2.0% DEHP (Gray et al., 1977). All three treatment levels produced histologic evidence of testicular injury and "castration" cells in the pituitary. The histopathologic changes in the testes were characterized by a marked reduction in the diameter of seminiferous tubules, presence of a germinal epithelium that consisted only of Sertoli cells, spermatogonia and a few spermatocytes, and a cessation of spermatogenesis. Interstitial tissues and Leydig cells appeared normal. At the 2% dietary level, testicular atrophy occurred within 2 weeks of treatment. A 2-week target organ experiment showed that, whereas DEP had no discernible adverse effects on the testes, DBP produced testicular atrophy, possibly more severe than that produced by DEHP (Gangolli, 1982).

Cater et al. (1977) summarized an unpublished study on DEHP in which an unspecified strain and number of rats were fed various dose levels of the ester for 90 days. At a daily level of 0.2%, DEHP produced testicular injury. When the level of DEHP was increased to 1.0%, testicular injury was noted in 2 weeks. The authors further stated that DEHP and DBP have about the same potency in causing testicular atrophy in rats. It was noted that other esters of phthalic acid were studied; however, no data were presented.

Young rats appear to be more sensitive than older rats to the testicular effects induced by DEHP. Curto (1984) found that 32-day-old male rats given 2000 mg/kg of DEHP for 5 days showed testicular atrophy and reduced zinc, DNA and RNA concentrations in the gonads while 62-day-old rats did not. However, older rats were not completely insensitive to testicular effects of DEHP. Intraperitoneal injection of 100 mg/kg DEHP every other day for 20 days caused reduced zinc gonadal and prostatic concentrations in adult rats.

Curto (1984) also studied the reversibility of the effects in 32-day-old rats. At 1 day post-treatment, testicular atrophy, reduced zinc and RNA concentrations and increased alkaline phosphatase activity were observed. All parameters with the exception of testicular atrophy had returned to normal at 20 days post-treatment.

Results presented by Mushtaq and Datta (1981) in an abstract also indicate that young rats may be more sensitive to the testicular effects of DEHP than older rats. These authors studied the effects of DEHP in young male albino rats ranging in age from 4-12 weeks old. Animals were given 2000 ppm (2000 mg/kg) DEHP daily by oral intubation for 30 days. The weight of the testis was decreased by 60-70% in the 8-week group accompanied by several biochemical changes in the testis. Rats in the older groups showed no decrease in testicular weight and fewer biochemical changes. Histopathologic studies found severe destruction of the seminiferous tubules in 8-week-old rats following DEHP treatment. Similar results were reported by Gray and Gangolli (1986). Oral administration of 2800 mg/kg/day DEHP for 10 days to 4- and 10-week old Sprague-Dawley rats produced histologic changes in the testes along with depression in the weight of the testes, seminal

vesicle and prostate. The effects were less marked in the 10-week-old rats. In 15-week-old rats DEHP had no effect on any of the above organ weights and no histologic abnormalities.

Sjöberg et al. (1986b) investigated the age-dependent response of the rat testes to DEHP. Groups of 25-, 40- and 60-day-old Sprague-Dawley rats were administered dietary dose levels of 1.0 or 1.7 g/kg bw DEHP for 14 days. Body weight gain and testicular weight were reduced in all groups of 25- and 40-day-old rats given 1.7 g/kg DEHP. Testicular damage was more severe in 25-day-old rats administered 1.7 g/kg doses and less severe in 25-day-old rats given 1.0 g/kg and 40-day-old rats given both 1.0 and 1.7 g/kg. No changes were observed in 60-day-old rats at either dose. Similar results were reported after gavage administration of 1.0 g/kg/day DEHP to Sprague-Dawley rats in the same age group (Sjöberg et al., 1985c). Sjöberg et al. (1986b) speculate that the causes for the age-dependent variation in testicular response may be an age-dependent difference in tissue sensitivity or differences in absorption, distribution, metabolism and/or excretion.

Oishi and Hiraga (1983) studied the effects of DEHP on lipid composition of liver, testes and serum in rats fed diets containing 2% DEHP for 9 days. DEHP induced changes in lipid and fatty acid composition, which resembled those caused by a zinc deficiency. In another study Oishi (1984a) studied the lipid composition of serum and testes in DEHP-treated rats. Altered lipid metabolism in the testes is frequently associated with testicular atrophy. Oishi (1984a) found that nonesterified fatty acid increased and cholesterol, triglyceride, phospholipid and zinc decreased in the serum after rats were fed 2% DEHP in the diet. Concentrations of cholesterol and

nonesterified fatty acid increased in the testes, whereas zinc concentrations decreased. Oishi (1984a) concluded that lipid alterations after DEHP administrations were similar to changes caused by a zinc-deficient diet; therefore, testicular atrophy caused by DEHP may be related to zinc concentrations in the testes and hormonal abnormalities.

Oishi (1986) studied the changes in testicular enzyme activity during exposures to DEHP. Changes in testicular cell-specific enzymes appear to be useful biochemical markers of testicular injury. Wistar rats (30 days old) were administered 2 g/kg/day DEHP by gavage daily for 10 days. Testicular weight gain was significantly reduced after 3 days. Zinc concentrations in the testes significantly decreased after 6 and 10 days and decreased in the ventral prostate after 10 days. Concentrations of zinc in the serum and seminal vesicle were not affected. Activities of lactate dehydrogenase isozyme-X (LDH-X), sorbitol dehydrogenase (SDH) and hyaluronidase, which are associated with postmeiotic spermatogenic cells, decreased in treated animals after 10 days. Specific activities of these enzymes increased in controls during the experimental period. By day 10, all seminiferous tubules were shrunken in DEHP-treated rats; primary and secondary spermatocytes and spermatids were absent or showed severe degenerative changes. The specific activities of α-glycerophosphate dehydrogenase (GPDH), β-glucuronidase and γ-glutamyl transpeptidase (γ-GTP), Sertoli cell and spermatogonic specific enzymes also significantly increased ($p<0.05$) after 10 days (Oishi, 1986). Parmar et al. (1986) also observed increases in γ-GTP and LDH-X and decreases in SDH and β-glucuronidase after treatment with DEHP. If biochemical changes are detected prior to histologic changes, they may be useful as markers for tissue damage. The biochemical changes reported in

the Oishi (1986) study occurred after or simultaneously with massive histologic or morphologic changes. The usefulness of this study (Oishi, 1986) is limited since only one dose level was tested and therefore the threshold for biochemical changes cannot be determined.

Oishi (1984b) also investigated the relationship of DEHP-induced testicular atrophy to vitamin A and zinc deficiencies. Zinc deficiencies have been associated with low plasma vitamin A levels, and vitamin A deficiencies have been associated with testicular atrophy and impaired spermatogenesis (Smith et al., 1973; Coward et al., 1966; Mason, 1933). Young male Wistar rats were fed diets containing 2% DEHP for 1 week at which time they were sacrificed. Body weight and testicular weight were decreased while liver weight was increased among DEHP-treated rats. Zinc concentrations were decreased compared with controls in the liver and testis. Vitamin A concentrations were decreased in the liver and increased in the serum and testis of DEHP-treated rats. These results suggest that DEHP-associated testicular atrophy was not due to dietary or intrinsic vitamin A deficiency since DEHP treatment raised testicular and serum vitamin A levels.

Mangham et al. (1981) compared testicular effects of DEHP and dialkyl 79 phthalate (DA79P) in rats. The phthalates were administered orally in corn oil by gavage at a dose level of 2500 mg/kg/day for either 7 or 21 days. DEHP caused a significant reduction of relative (to body) weight of the testes after 7 or 21 days, whereas DA79P reduced the relative weight only after 21 days of treatment. Similarly, DA79P did not produce changes in tubules detectable with a light microscope at 7 days, with the exception of one male where ~20% of the germ cells were missing from the seminiferous

tubules. Among the 7-day DEHP group, 50-80% of the tubules were affected in each animal. After 21 days of treatment, both DEHP and DA79P produced bilateral tubular atrophy and 50-100% of the tubules were affected in all animals. No effects were observed on interstitial cells or Sertoli cells with either compound.

BBP. BBP has also been shown to cause testicular atrophy. In an NTP final report, BBP produced testicular atrophy in rats fed a dietary concentration of 2.5% (NTP, 1985). After 1 day of acclimation male Fischer 344 rats (15 animals/group) were fed diets containing 0, 0.03, 0.28, 0.83 and 2.5% BBP for 10 weeks. Using the data presented in the report, these dietary levels correspond to 0, 17, 159, 470 and 1417 mg/kg/day. In this study powdered rodent meal was provided in such a way that measuered food consumption at the highest dose level could include significant waste and spillage rather than true food intake. For this reason a standard food consumption rate of 5% rat body weight was used in the 2.5% dose conversion. Two untreated females were assigned to each mating trial male after the 10-week pretreatment. Throughout the study body weight gain was significantly depressed at the 2.5% BBP level when compared with the controls. There were no deaths attributed to BBP toxicity. There were no grossly observable abnormalities in the testes at any dose group. In a corresponding 26-week toxicity study on BBP, testicular abnormalities were observed after 26 weeks suggesting that the effects become more pronounced after 10 weeks of exposure (see long-term toxicity section on BBP). Terminal mean organ weight values significantly decreased in the right kidney, liver, lungs, prostate, seminal vesicles and right testes of the 2.5% treatment group, whereas the heart significantly increased at the 2.5%

treatment group. Organ-to-body weight ratios significantly increased in heart, liver, lung and thymus and significantly decreased in the brain and prostate at the 2.5% level. Histopathologic changes were seen at the 2.5% BBP level. After histopathologic examination testicular lesions were characterized by atrophy of seminiferous tubules and a near total absence of mature sperm production. When 10/30 females successfully mated with the 2.5% treatment level males, none of the females were pregnant at necropsy.

Agarwal et al. (1985a) evaluated the effects of BBP on the male reproductive system in a 14-day dietary study using groups of 10 adult male Fischer 344 rats fed BBP at levels of 0.0, 0.625, 1.25, 2.5 and 5.0%. Results of this study showed that the absolute weights of the testis, epididymus, prostate and seminal vesicles were significantly reduced ($p<0.05$) in rats eating the 2.5% and 5.0% BBP diets. The effects were dose-dependent. Since the overall body weight gain was significantly reduced at these two dietary levels, expression of the organ weights relative to body weight reduced the magnitude of the effect, but the decreases in weights of the testis, epididymus and seminal vesicles remained significant ($p<0.05$) compared with controls. Histopathologic examination of these tissues revealed that the decreased weights of these organs were associated with generalized histologic atrophy. The severity of the changes in tissues from the testis, seminal vesicles and prostate were clearly dose-related with degenerative changes found at the 2.5% and 5% levels. In the epididymus, atrophic conditions were predominantly due to the necrosis of the tubular epithelium in the caput (head) portion. Numerous immature spermatogenic cells were found in the lumens of the epididymus. In addition to the effects on the male reproductive organs, relative (to body weight) liver and

kidney weights were increased at all levels of BBP administered. Relative (to body) weight of the thymus was significantly reduced in the 2.5 and 5.0% groups. Plasma levels of testosterone were significantly reduced ($p<0.05$) in the 5.0% group. The testosterone levels were lower than controls at 2.5%, but the decrease was not significant. Plasma FSH was significantly increased ($p<0.05$) in rats fed 2.5% and 5.0% BBP and plasma LH was increased at 2.5%. The LH levels in the 5.0% group could not be determined due to insufficient sample volumes.

DBP. The teratogenic effects of PAEs following oral administration were studied by Nikonorow et al. (1973). In this study female Wistar rats were administered 120 and 600 mg/kg/day DBP in olive oil for ~3 months and mated. Upon confirmation of conception the administration of DBP was discontinued. On day 21 the uteri and fetuses were removed. Results of this study indicated that fetal weight was significantly reduced at 600 mg/kg/day DBP. No detectable differences were observed in the number of sternum ossification foci, the development of the bones at the base of the skull, paws on the front and hind legs, or rib fusion in fetuses from treated rats when compared with the control animals.

Shiota and Nishimura (1982) studied the effects of DBP given to pregnant ICR-ICL mice on days 0 through 18 of gestation. DBP was administered at dietary levels of 0.05, 0.1, 0.2, 0.4 and 1.0% corresponding to 80, 180, 370, 660 and 2100 mg/kg/day. At the 660 mg/kg/day dose level the investigators observed reduced fetal weight in addition to retarded ossification. Diets of 2100 mg/kg/day decreased maternal weight, reduced fetal weight and retarded ossification. Fetuses also experienced neural tube defects at the

2100 mg/kg/day treatment level. The authors concluded that delayed ossification was related to the general underdevelopment of the fetuses. The maximum nonembryotoxic dose as stated by the authors would be 370 mg/kg/day DBP.

Cater et al. (1977) found that DBP induced testicular atrophy in young male Sprague-Dawley rats. In this study, the DBP was dissolved in corn oil and administered by gavage. The doses administered were 500, 1000 and 2000 mg/kg/day while control animals received corn oil in a volume of 5 mℓ/kg. The initial effect was a progressive reduction in weight of the testes. A significant reduction in the relative (to body weight) testes weight occurred within 6 days at 500 mg/kg and within 4 days at 1000 and 2000 mg/kg. By 14 days, the reduction at 2000 mg/kg amounted to 60-70% of the original weight. Since there was also a decrease in body weight, the authors used "relative testes weight" and found that on this basis there was still a significant loss of testes weight. Histopathologic examination of testes tissue demonstrated morphologic damage similar to that produced by DEHP. Further investigations by these authors revealed that the DBP adversely affects zinc metabolism and increases urinary zinc excretion. Similar results were observed by Gray et al. (1982) (see Table V-9).

NTP (1984b) conducted a continuous breeding study in male and female CD-1 mice to determine the reproductive and fertility effects when exposed to DBP. Mice (11 weeks of age) were administered 0, 0.03, 0.3 and 1.0% (0.3, 3.0 and 10.0 g/kg) DBP in the diet for 7 days prior to pairing and for 98 days to breeding pairs and then for an additional 21 days. In the 1.0% dose group, mice experienced decreased average number of litters and litter

size and fertility was 25% lower than the controls. There were no significant differences between the 0, 0.03 and 0.3% dose groups. Only 50% of the pups in the 1.0% dose group were born alive compared with 99% in the 0, 0.03 and 0.3 groups. Of the 50% in the high-dose group (1.0%) a significantly ($p<0.01$) larger portion of the pups were males. Mean pup weight decreased in the highest dose group when adjusted for average litter size. The data indicate that male fetuses may be slightly more resistant to the toxic effects of DBP.

A cross mating trial was also performed by NTP (1984b) in order to determine whether one or both sexes were adversely affected in the continuous breeding study. The crossover mating trial consisted of three combinations of breeding pairs. These were control males x control females, 1% DBP males x control females, and control males x 1% DBP females. Animals were necropsied 26 days after the 7-day crossover mating trial. The proportion of fertile mice was significantly reduced ($p<0.01$) in control male x 1.0% DBP females. In addition the number of live pups/litter, the proportion of pups born alive and the litters per pair were significantly decreased ($p<0.01$) in control males and 1% DBP-treated females. As in the reproductive study, the proportion of live males per litter (males/total) was significantly ($p<0.01$) higher in the 1% treated females and control males. In the 1% DBP-treated male mice there were no significant differences in the percentage of abnormal sperm. No treatment-related gross or histopathologic lesions were noted in the reproductive organs of treated male and female mice. Absolute and relative uterine weight were significantly increased in the 1.0% DBP-exposed group, perhaps reflecting the production of fewer and smaller litters.

An increase in urinary excretion of zinc, which is an essential element for testicular function, was observed following DBP treatment (Gunn and Gould, 1970). In an experiment using ^{65}Zn, treatment with DBP led to a marked increase in urinary excretion of radioactivity and a decrease of ^{65}Zn associated with testicular tissue. The activities of two enzymes containing zinc (alcohol dehydrogenase and carbonic anhydrase) were also decreased.

Foster et al. (1980) tested the testicular effects of a series of di-n-alkyl phthalates ranging from C_1 to C_8 in rats. The PAEs were administered orally at a dose of 7.2 mmole/kg/day (2000 mg/kg/day) for 4 days to young male rats. Results showed that DBP produced testicular injury, whereas both shorter chain compounds (DMP and DEP) and longer chain compounds were inactive. Urinary excretion of zinc and depletion of zinc from the testes were only observed with those compounds producing testicular injury. The reason that these two compounds produce injury is not known at present, but the determining factor does not appear to be related to the intestinal hydrolysis rate, since the monoesters of the inactive compounds were also ineffective in producing gonadal injury. Because these findings are based on short-term (4-days) tests, the compounds shown to be inactive in these tests may in fact cause testicular injury with longer exposure.

Johnson and Gabel (1983) evaluated three PAEs in a study investigating a new procedure using artificial "embryos" from hydra to detect agents causing abnormal development. This is an <u>in vitro</u> teratogen screening protocol wherein a bolus of dissociated hydra cells is monitored for development to normal adult individuals. Test compounds can be added to the medium, and

disruption of development evaluated. In this study, the ratio of the dose level toxic to adults (A) to the dose level affecting development of offspring (D) was reported for mammalian testing, using dose levels from published reports, and for the new hydra procedure. DMP and DBP each had A/D ratios of <3 in mammals and 2 in hydra. DEP had an A/D ratio of 2.5 in mammals and 2.0 in hydra. These ratios indicate that the larger the A/D ratio, the greater the tendency of the compound to cause developmental effects without causing toxicity in adults. For comparison, however, the A/D ratio for thalidomide is about 60, indicating teratogenic effects at concentrations far below those causing maternal toxicity.

DEP. In a study by Singh et al. (1972), Sprague-Dawley rats weighing 200-250 g were injected i.p. with DEP on days 5, 10 and 15 of gestation to ascertain the effect on the fetus. DEP was administered at three dosage levels to groups of five female rats/group. The dosage levels were 1/10, 1/5 and 1/3 the acute LD_{50} of 5.0579 mℓ/kg (5.66 g/kg) DEP corresponding to 0.506, 1.012 and 1.686 mℓ/kg (0.566, 1.13 and 1.88 g/kg), respectively. Animals were sacrificed on day 20 of gestation. No resorptions, dead fetuses or skeletal abnormalities occurred in the untreated control group. Resorptions did not occur at the 1.012 mℓ/kg treatment level. Treatment levels 0.506 and 1.686 mℓ/kg DEP produced 44.4 and 3.6% resorptions, respectively. The authors did not give any reason for this finding. There were no gross abnormalities at any treatment level. Fetuses were significantly ($p<0.01$) smaller than the untreated controls at all treatment levels. The number of skeletal malformations were 26.3, 47.1 and 81.3% for the treatment levels 0.506, 1.012 and 1.686 mℓ/kg DEP, respectively. The skeletal malformations most commonly encountered were elongated

and fused ribs, absence of tail bones, abnormal or incomplete skull bones and incomplete or missing leg bones. The data indicate that incomplete skull bones may be an induced developmental defect in which delayed ossification is secondary to growth and development retardation of the fetus. In another study, Singh et al. (1975) injected rats i.p. on day 5 or 10 of gestation with either 5 mℓ/kg (5.6/kg) radiolabeled DEHP or 1.0116 mℓ/kg (1.13 g/kg) radiolabeled DEP. Results of this study demonstrated that DEP could pass through the placental barrier to the developing fetus. The data indicate that developmental toxicity of the PAEs could be the result of the direct effect of the compound (or its metabolites) upon developing embryonic tissue.

Diethyl phthalate was evaluated to determine the reproductive and fertility effects after oral administration (NTP, 1984c). CD-1 mice (11 weeks of age) were fed diets containing 0, 0.25, 1.25 and 2.5% DEP for 7 days prior to pairing and for 98 days to breeding pairs and then for an additional 21 days. Exposure to DEP did not affect the reproductive performance in mice. The number of litters produced, average litter size, proportion of male pups and live pups and mean pup weight were unaffected. The numbers of live pups/litter, however, did significantly increase at 0.25% DEP.

Since reproductive performance was not adversely affected, a crossover mating trial was performed (NTP, 1984c). The crossover mating trial consisted of controls and litters from high-dose (2.5%) F_0 mice. Mice were weaned at 21 days of age and maintained on the same treatment regime as their high-dose F_0 parents. Mice continuously exposed to DEP via their

mothers experienced lower body weight when compared with controls. There were no statistically significant differences in fertility, proportions of pups born alive, number of live males or females/litter, or live pup weight or sex of pups born alive. On the average, the number of litters were significantly decreased in both treated males x control females and treated females x control males. Sperm assessment of treated F_1 parental mice indicated no significant differences in the percentage of motile or abnormal sperm. Sperm concentration, however, did significantly diminish. In the high-dose males, right testis weight significantly decreased and prostate weight significantly increased when compared with controls. High dose F_1 females exhibited decreased pituitary weights.

DMP. Plasterer et al. (1985) studied the developmental toxicity of DMP in pregnant CD-1 mice. Mice (50/treatment group) were administered 3500 mg/kg DMP by gavage for 8 consecutive days starting on day 7 of gestation. There was no effect on maternal weight gain, litter size or average pup weight. The pups were not examined for malformations. The authors did state, however, that the dose level may have been below the threshold of reproductive effects.

In a teratogenicity study Singh et al. (1972) observed adverse effects on developing rat embryos and/or fetuses after DMP administration. Female Sprague-Dawley rats (200-250 g) were injected i.p. with 1/10, 1/5 and 1/3 the acute LD_{50} of 3.3751 mℓ/kg (4.01 g/kg) DMP on days 5, 10 and 15 of gestation. The dosages corresponding to 0.338, 0.675 and 1.125 mℓ/kg (0.40, 0.80 and 1.33 g/kg) DMP, respectively, were administered to groups of five female rats. Animals were sacrificed on day 20 of gestation. No

resorptions, dead fetuses or skeletal abnormalities occurred in the untreated control group. The 0.675 mℓ/kg DMP treatment level did not produce any resorption sites. However, the 0.338 and 1.125 mℓ/kg DMP treatment group produced 33.3 and 32.1% resorptions. The 0.675 mℓ/kg level did show fetal death and the 1.125 mℓ/kg level showed five fetal deaths. Gross abnormalities of 9.5, 7.5 and 11.1% were observed at 0.338, 0.675 and 1.125 mℓ/kg DMP levels, respectively. Fetuses were significantly ($p<0.01$) smaller than the untreated controls at all treatment levels. The number of skeletal malformations were 25.0, 35.3 and 75.0% for the treatment levels 0.338, 0.675 and 1.125 mℓ/kg DMP, respectively. The skeletal malformations most commonly encountered were elongated and fused ribs, absence of tail bones, abnormal or incomplete skull bones and incomplete or missing leg bones. The authors concluded that incomplete skull bones may be an induced developmental defect in which delayed ossification is secondary to growth and development retardation of the fetus.

Mutagenicity

Thomas and Thomas (1984) and Hopkins (1983) reviewed the mutagenicity and genotoxicity of DEHP, its metabolites and other phthalic acid esters. DEHP and its metabolites, monoethylhexyl phthalate (MEHP) and 2-ethylhexanol, have been tested extensively in Ames assays with *Salmonella typhimurium* with and without metabolic activation. Negative results have been reported by Zeiger et al. (1982), Kirby et al. (1983), Kozumbo et al. (1982), Ruddick et al. (1981), Simmon et al., (1977), Warren et al. (1982), and Yoshikawa et al. (1983). DEHP was also found not to cause reverse mutation in *Escherichia coli* with and without S9 (Tomita et al., 1982b; Yoshikawa et al., 1983). Kozumbo et al. (1982) and Rubin et al. (1979)

reported that DMP and DEP were mutagenic in strain TA100 of S. typhimurium but only in the absence of S9. Seed (1982) reported that DMP, DEP (with and without S9) and DBP (without, but not with, S9), but not DEHP, di-n-octyl, diisodecyl and diisobutyl phthalates, were found to cause mutation to 8-azaguanine resistance in bacterial suspension assays with S. typhimurium; the DEHP metabolite, 2-ethylhexanol, was found to be mutagenic without S9. Tomita et al. (1982b) reported that MEHP, but not DEHP, yielded positive results in rec assays with Bacillus subtilis.

DEHP. The work of Tomita et al. (1982b) indicate that DEHP, while not a direct-acting mutagen, can be metabolized to a mutagenic form, MEHP. MEHP, but not DEHP, was shown to be a direct-acting DNA-damaging agent in the Bacillus subtilis rec assay. In the Salmonella reverse mutation assay MEHP was a direct-acting mutagen for strain TA100 whereas DEHP required addition of S9 to produce this effect. When administered in vitro MEHP was mutagenic at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in V79 cells and produced both chromosomal aberrations and sister chromatid exchanges in these cells, which have little capacity for metabolism of xenobiotic compounds. DEHP or MEHP was also administered to pregnant Syrian hamsters on day 11 of gestation, and the transplacentally exposed fetal cells were cultured. Both DEHP and MEHP induced mutations at the HGPRT locus, chromosomal aberrations and morphologic transformation in the cultured cells.

With two exceptions, in vitro genotoxicity assays have yielded negative results. DEHP failed to cause an increase in chromosomal aberrations in human lymphocytes (Turner et al., 1974), in Chinese hamster fibroblasts (Abe

and Sasaki, 1977; Ishidate and Odashima, 1977), and in CHO cells (Phillips et al., 1982). DEHP did not cause aneuploidy in human fetal lung cells (Stenchever et al., 1976). DEHP and its metabolites (MEHP and 2-ethylhexanol) failed to induce unscheduled DNA synthesis in primary rat hepatocytes (Hodgson et al., 1982). MEHP was reported to cause an increase in chromosomal aberrations and SCE in Chinese hamster V79 embryonic cells (Tomita et al., 1982b) and CHO cells (Phillips et al., 1982).

Chromosomal aberrations were observed in embryonic cells in a study in which Syrian golden hamsters were treated orally with 3.75-15 g/kg DEHP on day 11 of gestation (Tomita et al., 1982b). Putman et al. (1983) failed to observe significant increases in clastogenic changes in bone marrow cells taken from male F344 rats treated by gavage with DEHP (0.5-5 g/kg/day) or MEHP (0.01-0.14 g/kg/day) for 5 days. Positive results were observed in a dominant/lethal study on ICR mice, where DEHP was administered as a single intraperitoneal dose (2/3 LD_{50}) (Singh et al., 1974).

Agarwal et al. (1985b) evaluated the antifertility and mutagenic effects of DEHP in ICR mice. Eight male mice per group were given DEHP by s.c. injection at doses of 0.99, 1.97, 4.93 and 9.86 g/kg on days 1, 5 and 10 of the experiment. Sixteen control animals were given saline by s.c. injection. On day 21, each male was housed with a female for 7 days.

Mutagenicity was evaluated utilizing two indices: preimplantation loss/implants per pregnancy and early fetal deaths/implants per pregnancy. The preimplantation loss mutagenicity index was significantly increased during the early study segment in the 0.99, 1.97 and 9.86 g/kg groups and

for the overall study (weeks 1-8) in the 1.97, 4.93 and 9.86 g/kg dose groups. The early death index was significantly increased for all doses at all study segments.

In experiments with F344 rats, Albro et al. (1982) showed that radio-labeled DEHP and MEHP (but not ethylhexanol) associated strongly with DNA. Covalent binding, however, was not demonstrated.

DEHP was one of 10 chemicals recently examined in an international collaborative study employing a wide range of short-term assays (Ashby et al., 1985). A total of 69 assays were conducted on DEHP, some of which were identical tests performed in different laboratories. Negative results were consistently (though not universally) observed in assays measuring gene mutations and structural chromosome aberrations. Negative results were also reported for unscheduled DNA synthesis and DNA single-strand breakage.

Positive results were observed in four of five cellular transformation assays and in four of six mitotic aneuploidy assays. The mechanism of cell transformation is not clear, but positive results for this endpoint with agents that are not mutagenic is not without precedent. Aneuploidy is believed to be due to missegregation of chromosomes during mitosis, probably as a result of damage to the spindle fiber proteins. Hence, positive results for these endpoints are not inconsistent with the conclusion that DEHP is not mutagenic.

BBP. BBP was negative in *Salmonella typhimurium* when tested with S9 (Rubin et al., 1979; Kozumbo et al., 1982; Zeiger et al., 1982). For more information, see Table V-10.

TABLE V-10

Summary of Genotoxicity Tests of Phthalatic Acid Esters

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
		PROKARYOTES - DNA DAMAGE			
DEHP	Bacillus subtilis (rec assay)	none mouse pancreas S9	negative positive	Tested at 500 μg/disk	Tomita et al., 1982b
		PROKARYOTES - MUTATION			
DEHP	Salmonella typhimurium (reverse mutation, his)	rat liver S9 (Aroclor)	negative	At 1000 μg/plate with and without S9 in strains TA98 and TA100	Rubin et al., 1979
		rat liver S9 (Aroclor)	negative	With and without S9 in strains TA1535, TA1537, TA1538, TA98 and TA100. Dosage unspecified in reference	Simmon et al., 1977
		rat liver S9 (Aroclor)	negative	Tested up to 1000 μg/plate with and without S9 in strains TA98 and TA100	Kozumbo et al., 1982
		rat liver S9 (Aroclor)	negative	Tested up to 10,000 μg/plate in strains TA98, TA100, TA1535 and TA1537, preincubation assay	Zeiger et al., 1982
		rat liver S9 (unspecified)	positive	Tested at 5 mg/plate in strain TA100	Tomita et al., 1982b
		rat liver S9 (Kanaclor 400)	negative	Tested with and without S9 in strains TA98 and TA100 at 0.1 mL/test tube	Yoshikawa et al., 1983
DEHP	Salmonella typhimurium (forward mutation, $8AG^R$)	rat liver (unspecified)	negative	Tested with and without S9 dose not specified	Seed, 1982
DEHP	Escherichia coli (reverse mutation, his)	rat liver S9 (Kanaclor 400)	negative	Tested with and without S9 in strains WP2 uvr A^+ and WP2 uvr A^- at 0.1 mL/test tube	Yoshikawa et al., 1983

TABLE V-10 (cont.)

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
		PROKARYOTES - MUTATION (cont.)			
BBP	Salmonella typhimurium (reverse mutation, his)	rat liver S9 (Aroclor)	negative	At 1000 µg/plate with and without activation in strains TA98 and TA100	Rubin et al., 1979
		rat liver S9 (Aroclor)	negative	Tested up to 1000 µg/plate with and without S9 in strains TA98 and TA100	Kozumbo et al., 1982
BBP	Salmonella typhimurium (reverse mutation, his)	rat and hamster liver S9 (Aroclor)	negative	Tested up to 11,550 µg/plate in strains TA98, TA100, TA1535 and TA1537 in preincubation assay	Zeiger et al., 1982
DBP	Salmonella typhimurium (forward mutation, $8AG^R$)	rat liver S9	positive, weakly mutagenic	Mutagenicity observed at 0.09-0.18 mM. Tested with and without S9	Seed, 1982
DBP	Salmonella typhimurium (reverse mutation, his)	rat liver S9 (Aroclor)	negative	At 1000 µg/plate with and without activation in strains TA98 and TA100	Rubin et al., 1979
		rat liver S9 (Aroclor)	negative	Tested up to 1000 µg/plate with and without S9 in strains TA98 and TA100	Kozumbo et al., 1982
		rat and hamster liver S9 (Aroclor)	negative	Tested up to 10,000 µg/plate in strains TA98, TA100, TA1535 and TA1537 in preincubation assay	Zeiger et al., 1982
DEHP	Salmonella typhimurium (reverse mutation his)	rat liver S9	negative	Tested with and without S9 in strains TA98 or TA100 at 100, 200, 500, 750, 1000 and 2000 µg/plate	Agarwal et al., 1985c
DEP	Salmonella typhimurium (reverse mutation his)	rat liver S9	positive	Tested without S9 in strains TA100 and TA1535 at 100, 200, 500, 750, 1000 and 2000 µg/plate	Agarwal et al., 1985c
		rat liver S9	negative	Tested with and without S9 in strain TA98 at 100, 200, 500, 750, 1000 and 2000 µg/plate	Agarwal et al., 1985c
DBP	Salmonella typhimurium (reverse mutation his)	rat liver S9	weakly positive	Tested without S9 in strains TA100 at 200, 500, 750, 1000 and 2000 µg/plate	Agarwal et al., 1985c

TABLE V-10 (cont.)

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
		PROKARYOTES - MUTATION (cont.)			
DMP	Salmonella typhimurium (reverse mutation his)	rat liver S9	weakly positive	Tested without S9 in strain TA1535 and TA100 at 100, 200, 500, 750, 1000 and 2000 µg/plate	Agarwal et al., 1985c
DEP	Salmonella typhimurium (reverse mutation, his)	rat liver S9	negative	At 1000 µg/plate in strains TA98 and TA100	Rubin et al., 1979
		none	positive and negative	At 1000 µg/plate without activation in strain TA100; negative for TA98	Rubin et al., 1979
		none	positive and negative	At 500 and 1000 µg/plate in strain TA100; negative in TA98	Kozumbo et al., 1982
		rat liver S9 (Aroclor)	negative	At 1000 µg/plate in strain TA100	Kozumbo et al., 1982
		host-mediated assay	negative	Urine tested in strain TA100	Kozumbo et al., 1982
		rat liver S9 (Aroclor)	negative	Tested up to 10,000 µg/plate in strains TA98, TA100, TA1535 and TA1537 in preincubation assay	Zeiger et al., 1982
DEP	Salmonella typhimurium (forward mutation, 8AG^R)	rat liver S9 (unspecified)	positive, weakly mutagenic	Mutagenicity observed at 2.2-3.3 mM. Tested with and without S9	Seed, 1982
DMP	Salmonella typhimurium (reverse mutation, his)	rat liver S9 (Aroclor)	negative	Tested at 1000 µg/plate in strains TA98 and TA100	Rubin et al., 1979
		none	positive and negative	At 1000 µg/plate in strain TA100; negative for TA98	Rubin et al., 1979
		rat liver S9 (Aroclor)	negative	Tested up to 6666 µg/plate in strains TA98, TA100, TA1535 and TA1537, preincubation assay	Zeiger et al., 1982
		none	positive and negative	At 500 and 1000 µg/plate in strain TA100; negative for TA98	Kozumbo et al., 1982

TABLE V-10 (cont.)

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
		PROKARYOTES - MUTATION (cont.)			
DMP	Salmonella typhimurium (reverse mutation, his)	rat liver S9 (Aroclor)	negative	At 1000 µg/plate with S9 in strain TA100	Kozumbo et al., 1982
		host-mediated assay	negative	Urine tested in strain TA100	Kozumbo et al., 1982
	Salmonella typhimurium (forward mutation, 8AG^R)	rat liver S9 (unspecified)	positive, weakly mutagenic	Mutagenicity observed at 5-10 mM. Tested with and without S9	Seed, 1982
		FUNGI - MUTATION			
DBP	Saccharomyces cerevisiae (reverse mutation, multiple loci)	mouse S9 (unspecified)	negative	Tested up to 100 µg/mL	Shahin and von Borstel, 1977
		MAMMALIAN CELLS - MUTATION			
DEHP	Syrian hamster embryo cells (forward mutation, 8AG^R, 6TG^R)	none	positive	3.75-1.5 g/kg administered transplacentally. Dosage resulting in mutations unspecified	Tomita et al., 1982b
		CHROMOSOME EFFECTS			
DEHP	Chinese hamster fibroblast (CHL) cells (chromosomal aberrations)	none	negative	Tested at 4.1 mg/mL	Ishidate and Odashima, 1977
	Chinese hamster ovary (CHO) cells (chromosomal aberrations)	none	negative	Tested up to 2.0 mM	Phillips et al., 1982
	Syrian hamster embryo cells (chromosomal aberrations)	none	positive	At 7500 and 15,000 mg/kg applied transpacentally	Tomita et al., 1982b
	Human leukocytes (chromosomal aberrations)	none	negative	Tested up to 60 µg/mL	Stenchever et al., 1976
	Human leukocytes (chromosomal aberrations)	none	negative	Tested at 6.0 µg/mL	Stenchever et al., 1976

TABLE V-10 (cont.)

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
			CHROMOSOME EFFECTS (cont.)		
	Human leukocytes (chromosomal aberrations)	none	negative	Tested at 0.25 µg/mL	Tsuchiya and Hattori, 1977
DBP	Chinese hamster fibroblast (CHL) (chromosomal aberrations)	none	weakly positive	Tested at 1.1 mg/mL	Ishidate and Odashima, 1977
	Human leukocytes (chromosomal aberrations)	none	negative	Tested at 0.25 µg/mL	Tsuchiya and Hattori, 1977
DEP	Chinese hamster fibroblast cells (chromosomal aberrations)	none	negative	Tested at 11.3 mg/mL	Ishidate and Odashima, 1977
	Human leukocytes (chromosomal aberrations)	none	negative	Tested at 0.25 µg/mL	Tsuchiya and Hattori, 1977
DMP	Human leukocytes (chromosomal aberrations)	none	negative	Tested at 0.25 µg/mL	Tsuchiya and Hattori, 1977
			CELL TRANSFORMATION		
DEHP	Syrian hamster embryo cells (morphological transformation)	none	positive	At 7500 and 15,000 mg/kg, transplacental application	Tomita et al., 1982b
			MAMMALIAN IN VIVO TESTS		
DEHP	Mice - Harlan ICR strain (incidence of pregnancies, implantations per pregnancy, early fetal death)		positive	At 12.8, 19.2 and 25.6 mL/kg bw, i.p. reduced incidence of pregnancies, increase in fetal deaths, reduced number of implants	Singh et al., 1974
	Mouse (dominant lethal mutations)		positive	Male mice injected s.c. with doses ranging from 1.0-100 mL/kg bw. Antifertility effect at 1 mL/kg and increased with dose. Mutagenic index increased with dose.	Autian, 1982

TABLE V-10 (cont.)

Compound	Organism (assay)	Exogenous Activation System*	Reported Results	Comments	Reference
			MAMMALIAN IN VIVO TESTS (cont.)		
DEHP	Mouse (dominant lethal mutations)		positive	Male mice injected s.c. with 0.99, 1.97, 4.93 and 9.86 g/kg. Statistically significant increase in mutagenicity index in terms of preimplantation losses, early fetal deaths and all spermatogenic stages	Agarwal et al., 1985b
DMP	Mouse (dominant lethal mutations)		negative	Treated i.p. with 1250 µg/kg bw and at 1250 mg/kg bw applied to skin	Yurchenko and Gleiberman, 1980
	Rat (mutations in regenerating hepatocytes)		positive	Unspecified dose applied to skin	Yurchenko, 1977

*S9 refers to the postmitochondrial fraction (supernatant from 9000 xg centrifugation) of homogenates of organs. Inducing treatment of animals used for S9 preparations is in parentheses.

DBP. Kozumbo et al. (1982) found the ortho diester, DBP, to produce a dose-related mutagenic response in a modified version of the reverse mutation plate incorporation assay in Salmonella (Ames test). This activity, which was observed only in strain TA100, a detector of both base pair and frameshift mutagens, was eliminated upon addition of S9. In addition, DBP showed some evidence of clastogenic activity in Chinese hamster fibroblasts (Ishidate and Odashima, 1977) (see Table V-10).

DEP. DEP is a direct-acting mutagen for Salmonella typhimurium (Rubin et al., 1979). Seed (1982) found DEP weakly mutagenic in a forward mutation assay in Salmonella typhimurium (see Table V-10).

DMP. Extracted urine of rats administered 2 g/kg DMP i.p. was found not to be mutagenic (Kozumbo et al., 1982). In vitro assays by these authors showed that S9-associated esterases hydrolyzed DMP to the monoester, which has not been shown to be mutagenic in the Ames assay, and to methanol thereby eliminating its mutagenic capacity. An abstract by Yurchenko and Gleiberman (1980) indicates that DMP is not positive in a mouse dominant lethal test. Rubin et al. (1979) and Kozumbo et al. (1982) found DMP to be a direct-acting mutagen for Salmonella typhimurium. In a forward mutation assay DMP was weakly mutagenic in Salmonella typhimurium conducted in liquid suspension (Seed, 1982). For more information see Table V-10.

Carcinogenicity

The most conclusive information on the carcinogenicity of PAEs was obtained from bioassays performed by the NTP. Wilbourn and Montesano (1982) reviewed the results from carcinogenicity testing of PAEs conducted prior to

the NTP bioassays and concluded that all the studies were limited with respect to study design or reporting, making the results inconclusive.

DEHP. Carcinogenicity studies have been conducted by the NTP for several PAEs including BBP and DEHP (Kluwe et al., 1982a,b; Huff and Kluwe, 1984). The tested PAEs discussed in this document are DEHP and BBP. Essentially the same protocols were used for each compound. The compounds (administered in the diet) were tested for 2 years in both Fischer 344 rats and B6C3F1 mice using an untreated control group, a low-dose group and a high-dose group. The high-dose used in testing was the estimated maximum tolerated dose (MTD) determined by preceeding 90-day subchronic feeding studies prior to the chronic exposure bioassay. The low-dose was one-half of the estimated MTD. For each dose group, 50 animals of each sex and species were tested. Animals that died during the study and animals sacrificed at the end of the study were subjected to a gross necropsy and a complete micropathologic examination. Statistical comparisons of incidences of animals with systemic pathology, especially tumors at specific anatomical sites and of survival and body weight gain, were made using both pairwise comparisons (Fisher's exact test) and trend tests (Cochran Armitage trend test).

DEHP was administered in the diet for 103 weeks at levels of 0, 6000 and 12,000 ppm for male and female F344 rats and 0, 3000 and 6000 ppm for male and female B6C3F1 mice (NTP, 1982a). In this study procedure rodent meal was provided in such a way that measured food consumption actually represented significant spillage and waste rather than true food intake. For this reason a standard food consumption rate of 13% of mouse and 5% of

rat body weight was used in the dose conversion. Corresponding dose levels are 300 and 600 mg/kg/day for rats and 390 and 780 mg/kg/day for mice (low and high dose, respectively). No clinical signs of toxicity were observed in either rats or mice. Survival was not significantly decreased in any group except the female mice receiving the low-dose level. The increased number of deaths in this group were not attributed to DEHP administration, since pathologic changes in tissues were not observed and excessive deaths did not occur at the higher dose. The nonneoplastic lesions observed in this study were discussed previously under chronic toxicity.

Specific incidences of neoplastic lesions for the various treatment groups are presented in Table V-11. The major neoplastic effect observed among animals treated with DEHP was development of liver tumors. The incidence of animals with hepatocellular carcinomas was significantly increased among female rats fed DEHP at either 300 or 600 mg/kg bw when compared with controls. Male rats experienced a significant increase of the incidence of hepatocellular carcinomas and neoplastic nodules only in the 600 mg/kg bw DEHP group. Significant dose-related trends for increased numbers of animals bearing hepatocellular carcinomas and for increased numbers of animals bearing either hepatocellular carcinomas or neoplastic nodules were found in both male and female groups. Among male rats, incidences of animals with pituitary tumors, thyroid C-cell tumors or testicular interstitial-cell tumors were all significantly reduced among treated groups by both pairwise comparison and trend tests (see Table V-11). Comparison of occurrence of non-neoplastic effects in rats with occurrence of neoplastic effects showed that the presence of testicular interstitial cell tumors was negatively correlated ($p<0.005$) with either the presence of pituitary

TABLE V-11

Incidences of Animals with Neoplastic Lesions in the NTP Carcinogenicity Bioassay of DEHP[a]

Species	Sex	Neoplastic Lesions	Incidence[b]		
			Control	Low-Dose	High-Dose
Rat	M	Liver, hepatocellular carcinoma[c]	1/50 (2%)	1/49 (2%)	5/49 (10%)
		Liver, neoplastic nodule	2/50 (4%)	5/49 (10%)	7/49 (14%)
		Liver, hepatocellular carcinoma or neoplastic nodule[d]	3/50 (6%)	6/49 (12%)	12/49 (24%)[e]
		Pituitary carcinoma[f]	4/46 (9%)	1/43 (2%)	0/49 (0%)
		Pituitary carcinoma or adenoma[f]	8/46 (17%)	6/43 (14%)	1/49 (2%)[g]
		Thyroid, C-cell carcinoma[f]	4/48 (8%)	1/47 (2%)	0/46 (0%)
		Thyroid, C-cell carcinoma or adenoma[f]	5/48 (10%)	2/47 (4%)	0/46 (0%)[g]
		Testis, interstitial cell tumor[h]	47/49 (96%)	42/44 (95%)	11/48 (23%)[i]
Rat	F	Liver, hepatocellular carcinoma[d]	0/50 (0%)	2/49 (4%)	8/50 (16%)[j]
		Liver, neoplastic nodule[c]	0/50 (0%)	4/49 (8%)	5/50 (10%)[j]
		Liver, hepatocellular carcinoma or neoplastic nodule[d]	0/50 (0%)	6/49 (12%)[e]	13/50 (26%)[j]
Mouse	M	Liver, hepatocellular carcinoma[c]	9/50 (18%)	14/48 (29%)	19/50 (38%)[e]
		Liver, hepatocellular adenoma	6/50 (12%)	11/48 (23%)	10/50 (20%)
		Liver, hepatocellular carcinoma or adenoma[d]	14/50 (28%)	25/48 (52%)[e]	29/50 (58%)[j]
Mouse	F	Liver, hepatocellular carcinoma[d]	0/50 (0%)	7/50 (14%)[e]	17/50 (34%)[j]
		Liver, hepatocellular adenoma	1/50 (2%)	5/50 (10%)	1/50 (2%)
		Liver, hepatocellular carcinoma or adenoma[d]	1/50 (2%)	12/50 (24%)[e]	18/50 (36%)[j]

[a]Source: Kluwe et al., 1982a,b; Huff and Kluwe, 1984. Fischer 344 rats and B6C3F1 mice were fed diets containing 0(control), 3000 (lower dose, mice), 6000 (higher dose, mice; lower dose, rats) or 12,000 ppm (higher dose, rats) of DEHP for ~2 years.

[b]Incidences are expressed as the number of animals exhibiting the lesion over the number of animals examined microscopically; percentages are given in parentheses.

[c]Significant dose-related increased trend ($p<0.05$)

[d]Significant dose-related increased trend ($p<0.01$)

[e]Significantly greater than controls ($p<0.05$)

[f]Significant dose-related decreased trend ($p<0.05$)

[g]Significantly less than controls ($p<0.05$)

[h]Significant dose-related decreased trend ($p<0.0001$)

[i]Significantly less than controls ($p<0.0005$)

[j]Significantly greater than controls ($p<0.01$)

hypertrophy or the presence of testicular degeneration. In addition to the tumors presented in Table V-11, myelomonocytic leukemia, mammary fibroadenoma, clitoral gland carcinoma and uterine endometrial stromal polyps were observed in one or more rats, but their incidences in treated animals did not differ significantly from those found in controls.

The number of mice bearing hepatocellular carcinomas was significantly increased in both male and female groups receiving the high dose (780 mg/kg bw) of DEHP and in female mice receiving the low dose (390 mg/kg bw) (see Table V-11). Trend tests showed significant dose-related effects for both sexes. Metastases of the hepatocellular tumors to the lungs were found in 12 male and 8 female DEHP treated mice bearing hepatocellular carcinomas. Pulmonary metastases were not found in any of the control mice with liver tumors. Incidences of mice with hepatocellular adenomas did not differ significantly from controls; however, the incidences of mice with either hepatocellular carcinoma or adenoma was significantly increased at both dose levels in both sexes, and significant dose-related trends were present. Lymphomas, hemangiomas, mammary gland adenocarcinomas and alveolar or bronchiolar carcinomas or adenomas were also found in one or more treated mice, but incidences did not differ significantly from those observed in controls. In conclusion, the DEHP feeding studies in rats and mice indicate that statistically significant increases in hepatocellular carcinomas, neoplastic nodules and adenomas occurred. These tumors were found in both species and both sexes. There were metastases of hepatocellular tumors to the lungs of treated mice.

A summary of the interpretation of the bioassay results by NTP is shown in Table V-12. The only compound for which there was clear evidence of carcinogenicity was DEHP. In both cases, the effect observed was an increased incidence of liver carcinomas in mice and rats (Huff and Kluwe, 1984).

Northrup et al. (1982) criticized the conclusions drawn from the NTP DEHP study (Kluwe et al., 1982a,b; Huff and Kluwe, 1984) claiming that the results could not be interpreted as showing a carcinogenic effect. Northrup cited as one problem that the designated maximum tolerated dose (MTD) had been exceeded (based on differences in body weight gain) in both rats and mice at several of the treated groups. Another criticism of the study was that there was a significantly lower incidence of tumor bearing animals among female mice used in the control groups. When all the control groups (both rats and mice) were pooled, the incidence of total primary tumors associated with DEHP treatment was no different for all control groups except among male rats, which showed a decrease in total number of tumors. Finally, Northrup et al. (1982) claimed that critical data on food consumption, nutritional status, clinical signs, clinical pathology and intestinal microorganisms were lacking. Northrup et al. (1982) also felt that the incidence of liver tumors could have been influenced by the altered intestinal flora induced by DEHP. The authors postulated that the effects of DEHP were attributable to epigenetic mechanisms of carcinogenicity such as chronic tissue injury, nutritional deficiency, hormonal imbalance or promotional activity, since evidence of direct genotoxic effects were lacking. Also, Northrup et al. (1982) felt that because DEHP is metabolized differently in rats than in humans, effects in these rodents cannot be extrapolated to indicate human risk.

TABLE V-12

Summary of the Carcinogenic Effects of DEHP on the NTP Bioassays and Interpretation of These Findings[a]

Test Chemical	Species	Sex	Neoplasms	Interpretation[b]
DEHP	rats	M	Liver neoplastic nodules/carcinomas	Some evidence
	rats	F	Liver carcinomas	Clear evidence
	mice	M&F	Liver carcinomas	Clear evidence

[a]Source: Huff and Kluwe, 1984

[b]Evidence of Carcinogenicity-- Five categories of interpretative conclusions have been adopted for use in the NTP Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice) one category is selected to describe the findings. This category refers to the strength of the experimental evidence and not to either potency or mechanism (Huff and Kluwe, 1984).

On the other hand, Kluwe et al. (1983) defended the conclusions reached in the NTP study on DEHP (Kluwe et al., 1982a) by noting that the MTD was estimated based on prechronic oral studies, and that the MTD was not technically exceeded since survival of animals was not adversely affected. In response to the other criticisms, it was noted that the liver tumors were increased regardless of which set of historical control data were used; the DEHP bioassay was conducted using state-of-the-art procedures for animal carcinogenicity testing, and that the results of the bioassay were approved by independent peer review panels. Kluwe et al. (1983) also noted that the metabolic difference between rodents and humans, cited by Northrup et al. (1982), would not be expected to affect the response to the hepatocarcinogenic effects of DEHP observed in rodents. The authors noted that the International Agency for Research on Cancer (IARC, 1982) working group reviewed the study and concluded that there was "sufficient evidence for carcinogenicity of DEHP in mice and rats". There is some evidence suggesting that peroxisome proliferation, which occurs in both mice and rats at the dose levels used in the NTP bioassay, is involved in a secondary mechanism of cancer induction (Reddy et al., 1986). Peroxisomal proliferation is discussed in detail in Chapter VII, Mechanisms of Toxicity.

Similar results were reported in cynomolgus monkeys (Short et al., 1987). In this study no treatment-related evidence of hepatic peroxisomal proliferation was found in monkeys exposed to levels $\leq$500 mg/kg/day DEHP. Exposure to similar levels (100, 1000, 6000, 12000 and 25000 ppm) of DEHP in rats produced hepatic peroxisomal proliferation. It is difficult to compare exposure levels since monkeys were administered bolus doses and rats were administered feed. For further detail see the metabolism section of Chapter III.

Ward et al. (1983) studied the patterns of promotion of hepatocellular neoplasia by DEHP and phenobarbitol (PB) following initiation by i.p. diethylnitrosamine (DEN). B6C3F1 mice were given a single i.p. injection of 80 mg/kg of DEN at 4 weeks of age followed by oral administration of PB or DEHP beginning 2 weeks after DEN injection and continuing for $\leq$6 months. DEHP was administered in the diet at concentrations of 3000, 6000 or 12,000 ppm, and PB was given in drinking water at 500 ppm. Few foci of hyperplasia were found in the liver at 2, 4 or 6 months in animals exposed only to DEN, PB or DEHP, while numerous foci and hepatocellular neoplasms were found in mice treated with DEHP or PB after initiation with DEN. The pattern of response of DEHP differed from that of PB. In DEHP-exposed mice, the numbers of foci did not increase between 4 and 6 months as they did for PB, but the foci did increase in mean diameter and volume as the study progressed. Foci and tumors appeared earlier in the higher dose group of DEHP and, although the number of foci per unit volume of liver was similar for all DEHP dose groups, the volumes of the foci were dose-related. The type of hepatocytes found in the foci and neoplasms differed for PB and DEHP; those for PB were predominantly eosinophilic hepatocytes while those in DEHP-treated mice were predominantly basophilic and were more malignant in appearance. After 6 months exposure, the neoplasms in the high-dose DEHP and DEN mice were significantly larger ($p<0.02$) than those for PB and DEN, although histochemistry revealed similarities in the lesions. DEHP did not exhibit initiating action when given once orally followed by PB for 6 months in drinking water.

Ward et al. (1986) found that DEHP did not cause tumor promotion in female F344/NCr rat livers. Rats were initially injected with 282 mg/kg DEN

and then fed diets containing 12,000 ppm DEHP or placed on drinking water containing 500 ppm PB. Animals were sacrificed after 14 days of exposure. DEHP failed to increase the number or size of focal hepatocellular proliferative lesions (FHPL). The FHPL were morphologically similar between DEN and DEN-DEHP treated rats. Based on the above studies (Ward et al., 1983, 1986) the investigators suggest that liver cell replication is not a requirement for tumor promotion and that the hepatomegaly induced by DEHP appears to be a consequence of increased size of parenchymal cells.

Garvey et al. (1987) found that a single oral dose of 10 g/kg or 12 weeks of feeding 1.2% DEHP did not serve to initiate carcinogenesis in female F344 rats. Promoting agents were 2-acetylaminofluorene, for the single dose, and carbon tetrachloride and PB for the 12-week study. In addition Williams et al. (1987) demonstrated that DEHP had no initiating or enhancing effect on male rat carcinogenesis when DEHP was given alone for 24 weeks or for 7 weeks followed by the PB. The absence of enhanced development of foci in DEHP-treated rats is also indicative of a lack of promoting activity (Williams et al., 1987).

BBP. BBP was fed for ~2 years to both male and female rats and male and female mice at concentrations of 0, 6000 and 12,000 ppm (0, 780 and 1560 mg/kg/day, respectively) (Kluwe et al., 1982b; NTP, 1982b). Body weight gains were decreased in male and female mice and in female rats ingesting BBP; however, survival among these groups was not affected. Excessive mortality occurred among male rats treated with 6000 and 12,000 ppm BBP due to apparent internal hemorrhaging, after ~3 months of exposure. Due to the high mortality the study of male rats was terminated early, precluding

evaluation of the animals for tumorigenic responses. Incidences of tumors at specific anatomical sites in BBP-treated male or female mice did not differ significantly from controls. However, the incidence of mononuclear cell leukemia was greater among the high-dose female rats than among controls. The incidences of leukemia in female rats are presented in Table V-13. Although the increase in leukemia was statistically significant, the biologic relevance of this finding was questioned due to considerable variation in the background incidence of mononuclear cell leukemia in Fisher 344 rats. The conclusions reached by the peer review group of this study indicated that BBP "was probably carcinogenic in F344 female rats". A summary of the interpretive conclusions drawn from the NTP carcinogenesis testing of BBP is shown in Table V-14. BBP was not carcinogenic in mice of either sex. In reports of a 26-week subchronic study, NTP (1985, 1986) revealed significantly reduced total bone marrow cell counts at the 0.03 and 2.5% dose groups, but not at the 0.09, 0.28 or 0.83% dose groups when compared with controls. This change was comprised primarily of decreases in neutrophil metamyelocytes, bands, segmenters, lymphocytes, and basophilic rubricytes.

The NTP is currently repeating the rat portion of the cacner bioassay for BBP. Testing began in June, 1989 (NTP, 1991). When information from this study becomes available, the weight-of-evidence for the carcinogenicity of BBP will be re-evaluated.

Using the results of the NTP carcinogenisis bioassays, Kluwe (1986) compared the carcinogenic effects of DEHP and BBP and related compounds to determine the structure-activity relationships. Among the PAEs shown to be

TABLE V-13

Incidences of Female Rats with Tumors of the Hematopoietic System in the NTP Carcinogenicity Bioasssay of BBP[a,b]

Hematopoietic System Tumor	Incidence		
	Control	Low-Dose	High-Dose
Myelomonocytic leukemia	7/49	7/49	18/50[c]
Lymphoma	0/49	0/49	1/50
Myelomonocytic leukemia or lymphoma	7/49	7/49	19/50[c]

[a]Source: Kluwe at al., 1982b; NTP, 1982b

[b]Female Fischer 344 rats were fed diets containing 0 (control), 6000 (low-dose), 12,000 ppm (high-dose) of BBP for ~2 years. The ratios of female rats bearing tumors of the hematopoietic system to the total number of female rats examined microscopically are depicted.

[c]Significantly greater than controls, $p<0.05$

TABLE V-14

Summary of the Carcinogenic Effects of BBP in the NTP Bioassays and Interpretation of These Findings[a]

Test Chemical	Species	Sex	Neoplasms	Interpretation[b]
BBP	rats	M	--	Inadequate study
	rats	F	Leukemia	Some evidence
	mice	M&F	--	No evidence

[a]Source: Huff and Kluwe, 1984

[b]Evidence of Carcinogenicity-- Five categories of interpretative conclusions have been adopted for use in the NTP Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice) one category is selected to describe the findings. This category refers to the strength of the experimental evidence and not to either potency or mechanism (Huff and Kluwe, 1984).

potentially carcinogenic, the target sites of carcinogenic action varied. For example, DEHP induced hepatocellular carcinoma, while BBP was associated with effects of the hematopoietic system. It was concluded, therefore, that the carcinogenicity of PAEs may not be due to the acitivity of the phthalate moiety but rather determined by the moiety attached to the phthalate, or to a metabolic byproduct. Support for such an argument is given by studies of compounds containing the 2-ethylhexyl moiety. Comparison of results obtained for DEHP, DEHA and two other compounds [di(2-ethylhexyl)phosphate and 2-ethylhexylsulfate] containing the 2-ethylhexyl moiety revealed that all four compounds possessed some hepatocarcinogenic activity in female mice. The related compounds will not be discussed in this document. Thus, these results may indicate that compounds containing the 2-ethylhexyl group may have a propensity for causing hepatocarcinogenicity in female mice.

DBP. Data regarding the carcinogenicity of DBP could not be located in the available literature.

DEP. Data regarding the carcinogenicity of DEP could not be located in the available literature.

DMP. Data regarding the carcinogenicity of DMP could not be located in the available literature.

Summary

The acute toxicity of PAEs tends to be inversely related to the molecular weight of the compound. Signs of long-term toxicity include decreased body weight gain and increased liver, and in some cases, kidney weights. Target organs of PAEs, particularly DEHP, are the testes, liver and kidney.

The hepatotoxic effects of PAEs have been studied by numerous investigators in a variety of species. Seth (1982) reviewed the hepatic effects of PAEs and described both the morphologic and biochemical alterations attributable to PAE exposure. Most investigators have used DEHP as the representative PAE in testing. Generally, enlargement of the liver has been observed following oral or i.p. administration of PAEs. Examination of tissue from enlarged mouse, rat, hamster and monkey livers has revealed changes in morphology and biochemical constituents. Oral administration of DEHP for 21 days was reported to cause dilation of smooth and rough endoplasmic reticulum, mitochondrial swelling and increase in microbodies in rat liver (Lake et al., 1975). Oral administration of DEHP produced enlargement of cells, dilation of smooth endoplasmic reticulum and changes in hepatic lysosomes of ferrets (Lake et al., 1977) and decreased glucose-6-phosphatase in female rats (Mitchell et al., 1985). Information on the effects of phthalates in primates is limited to a study in which rhesus monkeys were given plasma-solubilized DEHP intravenously (Jacobson et al., 1977). The effects observed included liver necrosis, inflammatory cell infiltration and subtle changes in the clearance time of sulfobromophthalein.

Reproductive and developmental effects have been reported for several PAEs. Studies with DEHP have shown that this compound affects the fertility and reproductive performance in both male and female mice. In males the effects were associated with degenerative changes in the reproductive system and adverse effects on sperm. Testicular atrophy has been shown to occur following exposure to DEHP, BBP and DBP. Long-term oral exposure to DEHP at dietary levels as low as 150 mg/kg/day has induced testicular atrophy in rats.

Testicular injury induced by PAEs appears to be species specific to some extent. The rat, mouse, guinea pig and ferret were susceptible to testicular injury from DEHP and DBP while the hamster appeared to be resistant to the gonadal effects of these compounds and the corresponding monoesters at the dose levels and durations tested (Gangolli, 1982).

Studies on the embryotoxicity of PAEs seem consistent with other data obtained using different toxicologic endpoints (Tyl, 1988; NTP 1984a,b, 1985; Mitchell et al., 1985; Dostal et al., 1987a), that is, there is a range of toxicities that varies as a function of the PAE being tested and, in general, high concentrations of this chemical are required to produce a teratogenic response. Most studies used the mouse or the rat as the test subject and in those situations wherein a teratogenic response occurred, the target was generally the skeletal system (Shiota and Nishimura, 1982; Tomita et al., 1982a; Singh et al., 1972). Based on the high doses used and the differences in PAE metabolism between man and these test species, it is difficult at this time to define clearly the risk for the human population.

PAEs are generally regarded as nonmutagenic although mutagenic responses have been shown for some PAEs in some tests. DEHP is apparently metabolized to a nongenotoxic form in intact animals but not by tissue preparations.

DEHP and BBP have been tested for carcinogenicity in 2-year NCI/NTP carcinogenicity bioassays. DEHP was found to induce hepatocellular carcinomas in both rats and mice. Increased mononuclear cell leukemia was observed in female rats exposed to BBP. Data regarding the carcinogenicity of DBP, DEP and DMP could not be located in the available literature.

VI. HEALTH EFFECTS IN HUMANS

Introduction

Although PAEs are considered to have a low order of toxicity, much concern has been generated by the discovery that PAEs, such as DEHP, may leach from the plastic tubing and plastic bags used for blood storage (Jaeger and Rubin, 1970, 1972, 1973; Peck et al., 1979). Studies on the effects of PAEs in humans have largely provided information about the pharmacokinetics of the compounds. Associations between exposure to PAEs and toxic effects in humans have been limited by the inability to discern doses and responses in light of the ubiquity of phthalates in the environment. DEHP has been detected in both transfused and nontransfused patients (Wallin et al., 1974; Rubin and Nair, 1973; Jaeger and Rubin, 1972). In addition children may be exposed to DEHP in products such as pacifiers, teethers, squeeze toys, plastic baby pants and vinyl fabrics covering playpen pads. A report by the Consumer Products Safety Commission estimated possible increased cancer risk to children exposed to the above products (CPSC, 1983). The widespread presence of PAEs in air, water, food and stored blood indicates that humans are subject to environmental and industrial exposures to PAEs.

Clinical and Case Studies

DEHP. One of the earliest studies involving an assessment of PAEs in humans was performed by Shaffer et al. (1945). Two adult males were administered single oral doses of 5 or 10 g of DEHP in order to estimate its content in urine. In each case 4.5% of the dose was recovered from the urine in 24 hours. These experiments comprise the only controlled ingestion of PAEs cited in the literature. The subject who received the larger dose experienced mild gastric disturbances and moderate catharsis. No other effects were reported at either dose.

Shaffer et al. (1945) also examined the effects of DEHP after dermal exposures to the plasticizer. Undiluted DEHP was applied to the backs of 23 human subjects as patch tests. The compound was left in contact for 7 days and then reapplied on the same spots after 10 days. These exposures did not result in any type of erythema or other effects, suggesting the irritating and sensitizing potentials of DEHP are minimal.

Jacobson et al. (1974) examined the effects of DEHP on tissue cultures of human diploid fibroblasts established from skin biopsies. DEHP was solubilized in sera collected and stored in polyvinyl chloride (PVC) blood packs under standard blood bank conditions (4°C). Tissue culture medium containing 15% of the plastic stored serum was used for incubation of cells. The degree of growth inhibition of the human diploid fibroblasts increased with DEHP concentration. At incubation concentrations of 0.10 mM and 0.18 mM DEHP, cell growth was inhibited by 20% and 50%, respectively. These DEHP levels were comparable with concentrations detected in whole blood stored in PVC blood packs at 4°C for 14 and 26 days, respectively. A 70% inhibition of cell growth was observed when 0.70 mM DEHP was used, which was the concentration detected in platelet concentrations stored at 22°C for 48 hours.

Chromosomal effects of DEHP (Stenchever et al., 1976) were investigated on human leukocytes from the blood of two male and two female healthy donors in their early twenties and on fetal lung cells established from a 16-week fetus delivered by hysterotomy. DEHP was solubilized in Polysorbate 80 (Tween) (1:3, vol:vol) and dispersed in fetal calf serum by sonication. Aliquots were diluted to final concentrations of 0.06, 0.6, 6.0 and 60.0 µg DEHP/mℓ of blood for the leukocyte incubations. Incubations with

DEHP were for 4 hours at 37°C. Phytohemaglutinin was then added for 30 minutes to initiate cell division and cells were cultured for 72 hours. Mitosis was inhibited by addition of democolcine 2 hours prior to harvesting. Metaphase spreads were scored blindly for chromosome abnormalities on Giemsa-stained slides prepared from these cultures. Pooled data from the four donors showed no statistical differences in chromosome breaks, gaps or abnormal forms at any of the incubation concentrations when compared with control cultures. Fetal lung cells were incubated with 6.0 μg DEHP (in Polysorbate 80)/mℓ medium for 5 days. No significant difference in aneuploidy between study and control cultures was seen.

Ishikawa et al. (1983) determined that platelet function decreased as DEHP concentrations increased in PVC blood storage bags. Platelets demonstrated a decrease in ADP-induced aggregation after at least 2 hours of exposure to 100, 300 or 500 μg DEHP/mℓ. Maximum aggregation gradually decreased with incubation time, depending on the DEHP dose. Platelets renewed with fresh plasma showed a restoration of aggregability. The degree of restoration was decreased with increasing DEHP dose.

The effects of DEHP on cultures of the human diploid cell strain, WI-38, were investigated by Jones et al. (1975). Cultures treated with 51, 69 and 160 μM DEHP (soluble concentrations in incubation media) showed a statistically significant decrease in cell protein and longer generation times when compared with control cultures. As indicated by figures these decreases were dose dependent. Cells treated with 160 μM DEHP were no longer viable at day 9 and exhibited decreased cell density on day 6 of treatment. The dose, which caused 50% growth inhibition (ID_{50}), was calculated to be 70 μM. These toxic effects were greater in replicating cell

populations than in those treated after reaching confluency. Cells grown in 160 μM for 3 days and subsequently subcultured into control medium showed only 60% of control growth after 5 days in control medium.

BBP. Malette and von Haam (1952) investigated the dermal effects of various phthalates. A 100% solution of BBP had moderately irritating and slightly sensitizing effects when applied to white rabbits (methodology not specified). Patch tests were performed on 15-30 human subjects and sensitization tests 2 weeks after primary irritation tests. A 10% solution (vehicle not defined), of BBP was applied to the human subjects. A light reaction (not described) was seen in 12% of those tested. The irritative effect was classified as moderate and no sensitizing effect was reported.

DBP. Atmospheric exposures to DBP were studied by Men'shikova (1971). A human olfactory threshold was found to range from 0.26-1.47 mg/m³. Abnormal encephalographic responses were noted in three subjects at atmospheric DBP levels of 0.12 and 0.15 mg/m³. At 0.093 mg DBP/m³, conditioned reflexes were not observed. A maximum atmospheric concentration of 0.1 mg DBP/m³ was recommended.

A single case of accidental ingestion of DBP by a 23-year-old adult male has been reported (Lefaux, 1968). The individual mistakenly ingested a spoonful (~10 g) of DBP instead of a laxative. The individual was hospitalized the next day with complaints of nausea and vertigo. The subject exhibited signs of keratitis and toxic nephritis (excess albumen and red and

white corpuscles in the urine). Unspecified treatment initiated immediately allowed the subject to leave the hospital after 2 weeks without any after-effects.

Epidemiologic Studies

Milkov et al. (1973) performed a cross-sectional investigation of workers exposed to phthalate plasticizers in the manufacture of artificial leather and PVC-based films. The phthalates in use included predominantly DBP and higher alkyl phthalates (DAP-789), but periodically DEHP and BBP. Some formulations contained small amounts of the sebacates [dibutyl sebacate (DBS) and dioctyl sebacate (DOS)] or adipates [dibutyl adipate (DBA) and diocytl adipate (DOA)]. Tricresyl phosphate (TCP) was a component of the incombustible materials produced in 10-20% of machines assigned to various workers. The presence of these other agents without any attempt to account for confounding is a major criticism of this study.

The study population consisted of 147 persons, 87 women and 60 men. The majority (75%) of the population was <40 years of age (mean and range not given). Exposure duration was divided into three categories: 0.5-5.0 years for 54, 6-10 years for 28 and 10-19 years for 65 workers, respectively (mean and range not specified). Job categories included: 60 primers, 28 calender and mill operators, 35 mixing apparatus and paint millers, and 24 winders and final product inspectors. A control population was not identified.

Ambient exposure levels to vapors or aerosols of the plasticizers (mixed esters) in the working zone of the primers ranged from 10-66 mg/m^3.

Similar results were reported for the work station of the mill and calender operators. The plasticizer level in the mixture preparation section was found to be 1.7-40 mg/m³. Other contaminants (vinyl chloride, carbon monoxide and hydrochloric acid) around the calenders and rollers were either below their maximum allowable concentrations or not detected.

The test procedures included algesimetry, olfactometry, audiometry, vibration sensitivity and vestibular function by the caloric method with cold water (60 mℓ at 19°C for 20 seconds). Clinical and biochemical blood studies (sedimentation rate and bilirubin level) were also performed.

The most frequently cited complaint was of pain in the upper and lower extremities accompanied by numbness and spasms, reported in 51.7% of the subjects with a length of service 6-10 years and in 81.6% in those with >10 years. Polyneuritis was found in 47 persons, 32 with an autonomic-sensory and 15 with a mixed form. The incidence of polyneuritis increased with length of service. In 3.4% of the cases, organic disease of a nonoccupational character was noted in the nervous system. An elevation in the threshold for sensitivity to pain was noted in 66.7% of the subjects, and sensitivity to vibration was lowered to some extent in 33.8%. A marked depression in vibration sensitivity was seen only in those subjects also manifesting a significant depression of pain sensitivity. Of 81 subjects undergoing vestibular receptor investigations, 78% were found to have a depression of the vestibulosomatic reactions (absence or lowering of excitability). This depression began with the first years of this occupational contact, often in the absence of any health status complaints. The majority of subjects showed an elevated threshold of excitability when tested by

olfactometry, especially for thymol (82.1%) but also for camphor and tar (50%) and less for rosemary (33.4%). This elevation increased with duration of service. Audiometry did not reveal any pathology in auditory sensitivity. Blood studies revealed a tendency to slight lowering of the number of platelets and leukocytes, hemoglobin level and blood color index. A slight reticulocytosis and a tendency to acceleration of the erythrocyte sedimentation rate among the female subjects (statistically significant, but p value not stated) was also noted.

Thiess et al. (1978a) performed a morbidity study on 101 workers (97 males, 4 females) employed in a DEHP production plant. The age range of the workers was from 22-60 years (no mean was given but the majority were between 35 and 55 years of age). Duration of exposure was between 4 months and 35 years, with an average of 12 years. In 1966 the plant changed from a batch process to a continuous process production so that exposure to workers was reduced to only the processes of removing samples in control passages and during decantation. The investigators concluded that the negative results reported may be attributed to lower exposures after the processing change in 1966. Samples of current exposure concentrations in the work areas in question ranged between 0.0006 and 0.01 ppm (detection limit not stated). A clinical and occupational history was taken and the clinical examination included vital statistics, EKG, lung X-ray, and a complete urinary status with uric acid and creatinine clearance. The blood analyses included a differential count and sedimentation rate, and thymol, total protein, SGOT, SGPT, γ-GT, LDH, alkaline phosphatase, cholesterol and triglycerides determinations.

All the results of the examinations were compared with those obtained from two in-house control groups with possible exposure to styrene and dimethylcarbamic acid chloride (DMCC). No significant differences between the study group and control groups were found. Even when the study group was divided by age and time of exposure (greater and less than 12 years to presumably account for the change in production process), no significant differences were seen. Workers with duration of exposure >20 years (n=6), with an average exposure of 26.3 years, were given a neurologic examination that included: tests of the cerebral nerves; reflexes of the arms, legs and abdominal skin; and sensitivity to depth, pain and vibration. No neurologic disease or toxic nerve damage was indicated. Analyses of absenteeism, accident rate and of a questionnaire regarding premature births, miscarriages and malformations were also negative. Although the study was comprehensive in scope, it lacked exposure data prior to the conversion to a continuous process production so that a definitive conclusion can not be ascertained. As such, however, it represents only one of two epidemiologic studies reported to date on subjects with DEHP or a specific phthalate exposure.

Thiess et al. (1978b) also reported a mortality study on these DEHP production workers. The study was a prospective cohort survey of 221 workers compared with the general population. The study considered data prior to 1976. The population was derived from 28 workers who had worked prior to 1955, 85 workers who had started between 1940-1965, 135 workers who started after 1965 and 109 workers employed at the time of the study. Selection criteria for inclusion in the study population were not provided. The average observation period was 11.5 years. Half of the expected deaths were observed in the exposed population. Eight cases of death were due to

cancer. Thiess et al. (1978b) reported one case of bladder papilloma, which was significantly different from that expected. However, this was attributed to a single case and was not considered to represent an increased health risk. Analysis of natural death cases, after minimal observation of 5-10 years, on workers exposed to durations of 5, 10, 15 or >15 years, did not reveal an increase in mortality with exposure duration.

Thiess and Fleig (1979) also performed chromosomal analysis on blood lymphocytes from a subset of this same study population. Lymphocytes were cultured from 10 exposed production workers according to a modified method of Moorhead et al. (1960). The workers' duration of exposure ranged from 10-34 years (mean = 22.1 years). Lymphocytes from 20 age-matched workers served as controls. It was not mentioned whether these controls were also exposed to styrene and DMCC as mentioned previously. One hundred metaphases were scored for abnormalities on lymphocytes from each worker. The specific structural abnormalities were not defined, but were categorized with and without gaps. Neither category appeared to be different from the controls although statistical analyses were not stated.

In addition to individuals who are occupationally exposed, research indicates that persons who undergo blood transfusions or hemodialysis may receive extensive amounts of PAEs (over background intakes) as a result of the leaching of compounds, such as lipid soluble DEHP, from plastic containers or catheter tubing (Marcel and Noel, 1970; Jaeger and Rubin, 1970, 1972, 1973).

Hillman et al. (1975) studied DEHP levels in neonatal heart and GI tissues. The study tissues were obtained from three infants who previously had umbilical catheters in place but had never received blood products and from 14 infants who previously had umbilical catheters and varying quantities of blood products. Control tissues were obtained from eight stillborn infants, two liveborn infants who had died without administration of any blood products or insertion of catheters, and three older subjects who had not received blood products. The maximal amount of DEHP that could have leached into the blood was determined to be 13.9±1.1 mg of DEHP/5 cm of catheter, based upon the mean ± S.E. of the extraction of four No. 5 French catheters. The maximal amount contributed by blood products was estimated at 4 μg/mℓ, based upon a reference to Marcel (1973). The potential dosage thus ranged from 0.04 mg in infants receiving only 10 mℓ of blood to 1.4 mg in those receiving double exchange (460 mℓ). The minimum detection limit for DEHP was ~0.02 μg/g of tissue under the conditions used. No correlation could be made between hours of catheterization and DEHP levels. In general, the DEHP levels in heart tissue reflected the combined dosage of the numbers of catheters and amount of blood products in infants who died in <24 hours. In infants who lived longer, levels were generally lower and less correlated with dosage, suggesting that some biotransformation and clearance of DEHP was taking place. The mean levels of DEHP for heart residue and pressed extract of the study tissues, 1.27±0.42 and 0.66±0.22 μg/g, respectively, were significantly higher than the corresponding control levels, <0.07±0.03 and <0.07±0.04 μg/g. Three infants who died of necrotizing enterocolitis and who previously had arterial umbilical catheters in place and removed, had gut residue levels of 0.47, 0.63 and 0.16 μg/g. These levels were significantly higher ($p<0.05$) than those in GI tissues from infants without this disease. These higher DEHP levels

may represent increased uptake or decreased metabolism by a dying bowel. A direct causative link could not be determined between exposure to DEHP and the development of necrotizing enterocolitis. However, the study demonstrated that DEHP accumulated in the tissues of critically ill infants. Components of the catheters, including DEHP, should be further investigated as potential vascular or GI toxins, according to the authors of this investigation.

Another group that could be at high risk for the development of toxic responses to exposure to PAEs may be individuals who undergo hemodialysis. Gibson et al. (1976) studied blood samples from nine patients requiring maintenance hemodialysis both before, during and after the hemodialysis process to quantify the levels of DEHP received by these individuals from blood transfusion bags and/or plastic hemodialysis tubing. Hemodialysis was performed using recirculating single-pass machines and coils. Samples for DEHP analyses were obtained at 15 and 30 minutes, 1, 2, 3, 4 and 5 hours, and immediately after dialysis unless dialysis was terminated earlier. The metabolic fate and toxicity of these DEHP levels were not determined. Estimates of the total amount of DEHP delivered to a patient during hemodialysis ranged from 1.5-150 mg for dialyses that lasted from 15 minutes to 5 hours.

Neergaard et al. (1971) reported that exposures to DEHP may have been associated with the development of abnormal liver function tests in three patients (two men, aged 25 and 40 years, and one 25-year-old woman), following the use of a new set of PVC blood tubings in hemodialysis. IR-analyses of DEHP in salt solution perfusates through this blood tubing set ranged from 10-20 mg/ℓ. UV-determinations using the perfusate directly gave a

somewhat higher range, 20-50 mg/ℓ. DEHP could not be washed out by perfusion of three other commercially available blood tubing. Over a 5 month period dialysis machines with this tubing were used 93 times. Of these dialyses, 75 were performed upon the three patients who developed symptoms. Symptoms presented by the three patients after 10-15 dialyses (estimated dose of DEHP not given) included malaise, fever, abdominal pains, nausea, abnormal serum enzyme levels (LDH and SGOT), increased serum bilirubin, and in one case, jaundice. Liver biopsies in one patient revealed changes in accordance with so-called nonspecific reactive hepatitis; and in another patient, a histologic picture compatible with a diagnosis of viral hepatitis. Upon removal from the new dialysis machines to dialysis systems in which DEHP was not detected, the conditions of the three patients improved. A patient who was returned to the new dialysis machine developed a more severe relapse of the symptomology until she was removed to a different dialysis system. Evidence as to the exact etiology of illness associated with the use of the new dialysis machines could not be determined.

In a recent study (Pollack et al., 1985b), circulating concentrations of DEHP and its desterified phthalic acid products, mono(α-ethylhexyl) phthalate (MEHP) and phthalic acid, were quantitated (HPLC/UV monitor) in 11 patients. These patients were undergoing maintenance hemodialysis for treatment of renal failure. The patients underwent hemodialysis 3 times/ week, 4 hours/session, and had been receiving treatment ranging from 1 week to 12 years. The mean estimate of DEHP extracted during a single dialysis session for the 11 patients was 105 mg (range 23.8-360 mg). Serum cholesterol, triglycerides and AAG concentrations were measured to determine their

influence on the extraction of DEHP into blood. Circulating levels of DEHP and MEHP (1.91±2.11 μ/mℓ and 1.33±0.58 μg/mℓ, respectively) during dialysis did not correlate with the length of time the patients had been undergoing dialysis. This, together with the observation that blood concentrations of DEHP during interdialysis were similar to those in nondialyzed patients indicate that these compounds are effectively removed from the circulation between dialysis sessions. This could represent metabolic transformation or sequestration of lipophilic phthalate esters into fatty tissues. There was a strong correlation, however, between phthalic acid concentrations (5.22±3.94 μg/mℓ) and the length in years of previous dialysis treatments ($r=+0.920$, $p<0.001$). There also was no apparent relationship between the concentrations of DEHP and either of its metabolites, indicating the need for future metabolic fate and pharmacokinetic investigations. Of the biochemical factors examined, the sum of the serum cholesterol and triglyceride concentrations correlated most closely with the leachability of DEHP, although the association was weak ($r=+0.565$, $p\sim0.1$). Thus, although hemodialysis patients are exposed to circulating DEHP, the consequence of long-term systemic exposures to the ester and its metabolites remains to be elucidated.

High Risk Subpopulations

Although toxic effects of PAE exposure have not been conclusively demonstrated, individuals who receive exposures above background or environmental levels, such as those requiring hemodialysis or blood transfusions, may be at higher risk for the development of adverse reactions to these compounds. Parenteral administration of PAEs to these individuals may prove to be more toxic because these patients are critically ill or subject to differences in their ability to absorb, metabolize and excrete the compounds.

Summary

Dispite widespread occurrence of PAEs, information concerning the effects of human exposure is limited. In one study of acute exposure, administration of 5 or 10 g DEHP to two adult males did not result in toxic effects other than mild GI disturbances. Accidental ingestion of 10 g DBP, however, proved more toxic and caused nausea, vertigo, keratitis and toxic nephritis. Dermal application of BBP on humans did result in irritative but not sensitizing effects; however, the application of DEHP did not result in either effect. Studies on human tissue and cell cultures have demonstrated inhibition of cellular growth and decreases in platelet function. However, chromosomal effects did not occur in human leukocytes and fetal lung cells.

In epidemiologic studies the results have been largely confounded by exposure to multiple chemicals and lack of quantitative information on levels and duration of exposure. One group of investigators conducted a morbidity study on 101 workers employed in a DEHP production plant. Clinical examination and blood analyses revealed no significant differences between the study group and control groups. No neurologic disease or toxic nerve damage was indicated. Although the study was comprehensive in scope it lacked exposure data prior to a process conversion in the plant. In a cross-sectional investigation of 147 persons exposed to a combination of phthalate plasticizers, the incidence of polyneuritis increased with length of service. Hematologic studies revealed a lowering of the number of platelets and leukocytes, hemoglobin level and blood color index. Exposure to multiple agents make it difficult to interpret these results. Finally in a prospective cohort study of 221 workers exposed to DEHP, half of the expected deaths were observed in the exposed population. However, selection criteria for inclusion in the study population were not provided.

Parenteral administration of PAEs may involve the greatest risk for toxic effects, especially in individuals requiring blood transfusions or hemodialysis. Despite the fact that PAEs may leach into the contents of plastic blood bags or plastic tubes, reports of hepatitis in hemodialysis patients and necrotizing enterocolitis in infants given blood transfusions or umbilical catheters could not be conclusively attributed to PAE exposure.

VII. MECHANISMS OF TOXICITY

Introduction

The relationship between the toxicokinetics and toxic effects of PAEs (and their metabolites) has not been fully elucidated because of the relatively expedient clearance and minimal tissue accumulation of these compounds. However, investigation of the mechanisms of phthalate toxicity has been promoted in part by interest in their carcinogenic potential and their widespread use and environmental disposition.

Interactions

Concern over the ability of PAEs to alter biologic responses to pharmacologic agents and xenobiotics has stimulated research into the possibility that the biotransformation of these chemicals may be modified by the acid esters. DEHP and DBP have been found to interact with the toxicity of other compounds in a synergistic or antagonistic manner. Carbon tetrachloride was found to act synergistically with DEHP by producing extensive necrosis of parenchymal cells in rat liver (Seth et al., 1979). DEHP significantly ($p<0.05$) increased barbiturate-induced sleeping time in male mice (Rubin and Jaeger, 1973). A synergistic effect was noted when DEHP or DBP (applied prior to the application of organophosphate insecticides) increased the mortality of female house flies. When applied simultaneously, DEHP or DBP reduced the toxicity of organophosphate insecticides to house flies (Al-Badry and Knowles, 1980). Antagonism was noted between the effects of DBP and zinc-induced testicular atrophy (Cater et al., 1977). Methylenedioxyphenol compounds and paraoxon inhibited DEHP hydrolysis by rainbow trout liver *in vitro* (Melancon and Lech, 1979). Foster et al. (1980) did not find antagonistic effects between testicular zinc levels and the two phthalates DEP and DMP. In rats initiated with

diethylnitrosamine, the administration of a choline supplemented diet containing DEHP did not result in increased hepatic preneoplastic foci. The administration of a choline deficient diet containing DEHP inhibited the appearance of preneoplastic foci. Choline deficiency has been shown to be a promoter of hepatic preneoplastic foci but the mechanism of DEHP's anti-promoting effect is unclear, according to the investigators (Deangelo and Garrett, 1983). There were no synergistic or antagonistic interactions found for BBP in the available literature.

The interaction between ethanol and DEHP has been studied by Agarwal et al. (1982b). After a single oral or i.p. dose of DEHP to mice, the ethanol-induced sleeping time was increased while hepatic alcohol dehydrogenase activity was inhibited. Repeated administration, however, produced effects that differed with the route of administration. When DEHP was given in repeated oral doses, the ethanol-induced sleeping time was decreased, but increases in the activities of both alcohol and aldehyde dehydrogenases were observed. Repeated i.p. doses, however, resulted in an increase in the sleeping time with decreased alcohol dehydrogenase activity. The authors concluded that DEHP effected changes in the pharmacologic response to ethanol by altering the activities of alcohol dehydrogenase and aldehyde dehydrogenase. In vitro assays with mouse liver preparations revealed that MEHP inhibited alcohol dehydrogenase activity. Furthermore, both MEHP and DEHP inhibited to a statistically significant ($p<0.05$) degree the activities of both high and low K_m aldehyde dehydrogenase activities.

Enzyme Inducing Properties

Most studies of the mechanisms of toxicity have researched the effects of PAEs on enzyme systems and metabolites. Pollack and Shen (1984) used

antipyrine metabolism as a model for metabolic clearance of drugs. Antipyrine's metabolism was increased in normal and renal failure rats (Sprague-Dawley rats in which renal failure was induced by a two-step nephrectomy) after treatment with DEHP. The plasma clearance was increased and elimination half-life of antipyrine decreased upon DEHP administration. An increase in the liver weight and cytochrome P-450 content was also noted as evidence of induction of hepatic microsomal enzymes by DEHP. Renal failure rats appeared to undergo a more marked increase in antipyrine clearance than did control animals after DEHP treatment.

Changes in hepatic enzyme activities are associated with liver enlargement and occur in animals exposed to PAEs (Seth, 1982). One change that has been observed consistently following oral or i.p. administration of DEHP is a decrease in hepatic succinate dehydrogenase (SDH) activity occurring specifically in the periportal zones (Seth, 1982).

One target site for PAE effects on the liver is mitochondria. Results of _in vitro_ studies have indicated that several PAEs produce inhibition of mitochondrial respiration. It has been suggested that PAEs are electron and energy transport inhibitors, and that they can cause uncoupling of oxidative phosphorylation (Seth, 1982). Since DEHP inhibited the activities of succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase) in rat heart, lung, kidney and gonads as well as the liver, suppression of energy-linked reactions may be a generalized effect of DEHP. The enzymatic alterations may not be related to the physical presence of DEHP since effects were present several days after final treatment, by which time the plasticizer would have been excreted from the body.

DEHP has also been shown to affect carbohydrate metabolism. Decreased levels of glycogen were reported in the livers of mice, rats and ferrets receiving DEHP (Seth, 1982). Marked depression of glucose and glycogen levels was found in the livers of rats fed diets containing 2 or 4% DEHP (Sakurai et al., 1978). Glucogenesis and glycogenolysis are also inhibited by DEHP. However, no quantitative conclusion on the inhibition of the reaction could be reached in this experiment.

Agarwal et al. (1982a) also examined the effect of DEHP administration (oral, i.p.) upon hepatic enzymes, lipid peroxidation and hepatic sulfhydryl content in rats. The authors concluded that the PAEs interfered with biotransformation mechanisms of hepatic microsomal drug-metabolizing enzymes. After a single oral or i.p. treatment of DEHP was administered to rats, the activity of aminopyrine-N-demethylase and aniline hydroxylase was inhibited. When DEHP was given in repeated doses, the results showed increases in these enzymes with oral administration but decreases with i.p. injection. The activity of benzo[a]pyrene hydroxylase and concentrations of cytochrome P-450 were also increased in rats that were treated orally with DEHP. The differences in the effects from oral and i.p. administrations may be attributable to variations in the physical state and metabolism of DEHP after introduction of the compound into the intestine and the peritoneal cavity.

Studies by Seth et al. (1981) also indicated that the activities of the liver aminopyrine-N-demethylase and aniline hydroxylase were inhibited by i.p. administration of DMP, DEHP and DBP to rats. Hepatic tyrosine aminotransferase activity was unchanged after a single administration but was increased when the PAEs were given daily for 7 days. The authors concluded

that these results are supportive of previous observations that PAEs prolong barbiturate sleeping time by interference with the metabolic disposition of these pharmacologic agents.

Walseth et al. (1982) demonstrated contrasting results of PAE treatment on rat liver and lung. DBP administered i.p. resulted in significant increases in hepatic cytochrome P-450 but reduced lung concentration by 30%. DMP and DEHP were less effective in this regard. DBP treatment also altered the enzymatic pathways of benzo[a]pyrene (B[a]P) metabolism in liver microsomes while all PAEs tested decreased pulmonary metabolism of B[a]P. These authors did not detect a relationship between carbon chain length of PAEs and effects of microsomal enzyme activities.

While DEHP is associated with increases in the activity of hepatic microsomal enzymes, Khawaja and Dallner (1982) determined that liver protein synthesis in vivo was decreased after administration of the compound in the diet of rats. Although liver weight and protein content increased, the capacity for treated livers to synthesize protein was reduced. The accumulation of protein was explained as a result of reduced degradation or decreased export of liver proteins.

There were no available data concerning the enzyme inducing properties of BBP and DEP.

Cellular Effects

Ekwall et al. (1982) assayed 29 plasticizers including DMP, DEP, DBP, DEHP, BBP and three other PAEs, for cytotoxicity of HeLa cells. Cytotoxicity was measured by pH changes of the medium using phenol red as the

indicator and by microscopic inspection of the cultures (the MIT-24 system). A comparison of the results of this in vitro cytotoxicity test to other cytotoxicity tests demonstrated that as the chain lengths of PAEs increase, lipophilicity increases. A comparison of these in vitro cytotoxicity test results with in vivo test results in mice suggest that a basal cytotoxic action to mouse tissues is responsible for the lethal action of plasticizers to mice.

The cytotoxic mechanisms of PAEs may be better elucidated by studies of subcellular distribution and activity as opposed to assays of tissue distribution (Bell, 1982). Bell (1982) discussed a series of experiments conducted in rats, rabbits and pigs that were directed at the investigation of PAE effects on lipid metabolism. In studies of rats and rabbits that were fed DEHP, the diester impeded cholesterol synthesis by inhibition of 3-hydroxy-3-methylglutaryl CoA reductase, which catalyzes the second step of cholesterol synthesis. The effect was neither sex nor species-specific. Similar inhibition of cholesterol synthesis was found to occur in the adrenal glands and testes. Such impairment of cholesterol synthesis in these tissues was thought possibly to account for fetal abnormalities found in the offspring of phthalate-treated dams, and testicular atrophy in other animals. Plasma and liver cholesterol levels were decreased in rats fed either DBP or DEHP. Inhibition of cholesterol synthesis by these esters may have been the underlying cause for this effect. Experiments with in vitro tissue slices of rats fed DEHP demonstrated that de novo fatty acid synthesis and esterification are inhibited in certain tissues after PAE administration. Phospholipid synthesis may also be selectively affected (Bell, 1982).

Upon further investigation Bell and Buthala (1983) discovered that DEHP inhibits microsomal acylCoA:cholesterol acryltransferase (ACAT) in rats that received this compound in the diet. The biosynthesis of cholesterol from ^{14}C-mevalonate was also inhibited in treated animals indicating that other microsomal enzymes are influenced by DEHP administration. The post-mevalonate segment of the biosynthetic pathway requires the involvement of numerous microsomal enzymes, while cholesterol esterification is largely associated with ACAT.

Bell (1982) also described experiments performed on the effects of DEHP on mitochondrial function. Administration of DEHP to rats (50, 250 and 500 mg/kg/day in the diet assuming rats consume 5% of their body weight), rabbits (490 mg/kg/day in diet assuming rabbits consume 4.9% of their body weight) and pigs (1.6 mg/kg/day) resulted in increased production of palmitic acid by liver mitochondria accompanied by an enhancement of ^{14}C-palmitoyl CoA oxidation. Studies on heart mitochondria demonstrated that DEHP directly added to an isolated suspension will inhibit adenine nucleotide translocase. Thus, exchange of extramitochondrial ADP for intramitochondrial ATP is impeded. Inhibition of translocase was not observed in the mitochondria isolated from rats fed DEHP compared with controls. It was felt that a level of DEHP insufficient to affect the enzyme had accumulated in the heart during the 10-day feeding period. The author concluded that inhibition of heart adenine nucleotide translocase may be related to reports of myocardial cell death and decreases in spontaneous heart rate observed in rat hearts after DEHP perfusion (Rubin and Jaeger, 1973; DeHaan, 1971; Petersen et al., 1972-1975; Aronson et al., 1978). Bell (1982) noted that the biochemical transformations observed in these experiments indicated that the effects of PAEs may result from

alterations of membrane fluidity. The lipophilic properties of the PAEs may, therefore, change the membrane environment sufficiently to modify enzyme responses.

Melnick and Schiller (1982) studied the effects of DMP, DBP and DEHP on liver mitochondria isolated from rats. Active transport of potassium ions (K^+), respiration rates and succinate cytochrome c reductase activities were monitored. DBP was the most effective energy uncoupler as measured by interference with K^+ uptake induced by three energy sources. It also led to a nearly total loss of respiratory control. DMP was less effective in this regard; MEHP, but not the parent DEHP, was an effective uncoupler of energy-linked reactions. The authors indicated that PAEs may affect mitochondrial function by changing the inner membrane permeability and inhibiting succinate dehydrogenase activity.

Several studies evaluating the hepatotoxic effects of PAEs have been performed. Lists of effects upon hepatocytes and enzymatic processes can be found in Tables VII-1 and VII-2. PAEs cause liver hypertrophy by increasing the number of hepatocytes (Ganning et al., 1983). PAEs may change the structure and function of the liver by inducing peroxisomes, greater numbers of mitochondria and higher levels of enzymes (Ganning et al., 1984). It was noted that the capacity to increase mitochondria synthesis is a unique characteristic of DEHP. Mitochondria, by contrast to other intracellular membranes, are seldom increased (Ganning et al., 1984).

The induction pattern of DEHP in the liver has unique properties. Ganning et al. (1981) reported that after treatment of rats with DEHP, hepatic changes included increases in the number of peroxisomes, in the

TABLE VII-1

Cellular Changes in Rat Hepatocytes Induced by DEHP Administration[a]

Organelle	Change[b]
Peroxisomes	
Protein and phospholipid	Increased several-fold
Beta-oxidation enzymes of fatty acids	Increased 2- to 6-fold
Carnitine-acetyl transferase	Doubled
Catalase	Decreased 30-40%
Urate oxidase	Decreased 30-40%
Mitochondria	
Protein and phospholipid	Increased 2- to 3-fold
Beta-oxidation of fatty acids	Doubled
Carnitine-acetyl transferase	Increased 10- to 30-fold
Carnitine-octanoyl transferase	Tripled
Carnitine-palmitoyl transferase	Increased 3- to 4-fold
Dehydrogenase and respiratory	No or moderate change
Respiratory control and oxidative phosphorylation	No change
Microsomes	
Protein and phospholipid	Slight increase (10%)
NADPH-cytochrome c reductase	Increased 40-60%
Cytochrome P-450	Increased 40-60%
Other electron transport enzymes, hydroxylases, phosphatases	No change or moderate increase
Homogenate	
Sterol and squalene synthesis	Decreased 75%
CoA and carnitine	Increased 5- to 6-fold
Acetyl-CoA and acetyl-carnitine	Increased 4-fold
Long chain-acyl CoA and -acyl carnitine	Increased 50%

[a]Source: Ganning et al., 1984

[b]Specific activities or amounts on protein basis compared with the control

TABLE VII-2

Synthesis and Breakdown of Protein and Lipid in DEHP-Treated Rats[a]

	Amount or Activity	Synthesis	Breakdown Control → Treated Half-time in Days
Protein			
Peroxisomes			
Catalase	Decrease	Decrease	1.9 → 5.0
Beta-oxidation enzymes	Increase	Increase	2-3 → 5.5-6.5
Mitochondria			
Membrane	Increase	Increase	6 → 25
Beta-oxidation enzymes	Increase	Increase	Decrease
Microsomal membranes	Unchanged	Increase	3.5 → 5.5
Total cytoplasmic proteins	Unchanged	Increase	2.5 → 5
Lipid			
Microsomal phospholipids	Unchanged	Increase	
Blood cholesterol			
Total	Unchanged		
HDL[b]	Decrease		
LDL[c]	Increase		
VLDL[d]	Unchanged		

[a]Source: Ganning et al., 1984

[b]HDL, high-density lipoprotein

[c]LDL, low-density lipoprotein

[d]VLDL, very low-density lipoprotein

oxidation of CoA-linked fatty acids, in microsomal NADPH-cytochrome c reductase and cytochrome P-450 levels, in the number of mitochondria and in the activity of carnitine-acetyl transferase. Induction of the transferase was attributed to an increase in peroxisomal ß-oxidation. Ganning et al. (1983) demonstrated that although peroxisomal and mitochondrial membranes were increased, the endoplasmic reticulum was not changed in amount or appearance.

Increases in the activity of enzymes in rat hepatic cytosol have been found upon application of various peroxisome proliferators including DEHP, DAP and 2,4,5-triphenoxyacetic acid among others (Katoh et al., 1984). Administration of DEHP resulted in the induction of catalase and two long-chain acyl-CoA hydrolases. An increase in peroxisomal ß-oxidation was also signaled by a marked increase in palmitoyl-CoA oxidation after ingestion of DEHP in the diet.

Primary rat hepatocyte cultures were used to ascertain effects of various alkyl phthalate esters on peroxisomal enzyme activities (Gray et al., 1983). The authors concluded that straight-chain phthalates produce few effects upon rat hepatic peroxisomes. The 2-ethylhexyl ester, e.g., MEHP, increased carnitine acetyltransferase activity and palmitoyl CoA oxidation, and produced increased numbers of peroxisomes.

The effects of different PAEs upon liver cells have been compared with those of clofibrate, another peroxisome proliferator (Lake et al., 1984b). Lake et al. (1984a) had previously determined that DEHP is a potent inducer of rat hepatic peroxisomal enzyme activities. In the more recent study (Lake et al., 1984b), rats were orally administered DEHP, di-n-octyl phthalate (DOP), mono-n-octyl phthalate (MOP) or clofibrate for 14 days.

This resulted in liver enlargement. Liver sections from DEHP and clofibrate treated animals showed an increased number of peroxisomes. Both DEHP and clofibrate stimulated the activities of peroxisomal marker enzymes, increased microsomal cytochrome P-450 content and stimulated microsomal lauric acid hydroxylation activity. The compounds, DOP and MOP, did not produce such effects. The branched chain ester DEHP was thus determined to exert effects that differed markedly from the straight chain analogue, DOP and its metabolite MOP. In addition, DEHP was shown to induce forms of cytochrome P-450 similar to those induced by clofibrate. Okita and Chance (1984) also demonstrated that DEHP, like clofibrate, increased microsomal laurate hydroxylation activities. Potent induction of the cytochrome P-450 mediated fatty acid ω-hydroxylation reaction occurred in rats that were fed a diet containing DEHP.

There may be some species variation in the biochemical actions of PAEs. Lake et al. (1984a) compared DEHP, MEHP and clofibrate-induced hepatic peroxisome proliferation in two species, rats and hamsters. It was discovered that DEHP was much less effective as a peroxisome proliferator in hamsters than in rats. Similar results occurred when clofibrate was utilized. Although all three compounds caused some increase in liver weight and hepatic peroxisome numbers, the response was more marked in rats. For each of the three treatments, dose dependent increases in the peroxisomal marker, cyanide-insensitive palmitoyl-CoA, and in carnitine acetyltransferase were noted in the rats. Only small changes in these parameters were found in the hamsters. The species variation in the effects of DEHP may have been attributable to differences in peroxisome proliferation or in the metabolism of DEHP.

The mechanism of carcinogenicity for DEHP is not well understood; however, it has been suggested that DEHP may fall into the peroxisome-proliferator class of hepatocarcinogens (Warren et al., 1982). Peroxisomal proliferating effects and hepatomegaly do not seem to be related to differences in the sensitivity of suckling rats to toxic effects caused by exposure to DEHP (Dostal et al., 1987a). Changes in relative liver weight and hepatic peroxisomal enzyme activities were similar in age groups showing markedly different changes in body weight and survival rates. Similar increases in activities of both palmitoyl CoA oxidase and carnitine acetyltransferase were noted between suckling and adult rats indicating that suckling rats are equally if not more sensitive to the peroxisomal proliferating effects of DEHP (Dostal et al., 1987a) (see Table V-4). The induction of perioxisomes and peroxisomal enzyme activity as well as hypolipidemic effects was not detected in marmoset monkeys exposed either orally (2000 mg/kg/day) or i.p. (1000 mg/kg/day) to DEHP (Rhodes et al., 1986). Also, there was no increase in cyanide-insensitive acyl oxidase, the peroxisome marker enzyme. The marmoset appears to be less sensitive to the peroxisomal proliferating effects of DEHP. Rhodes et al. (1986) concludes that if marmosets reflect more accurately the response in man, then low levels of DEHP may not be of toxicologic significance with regard to hepatocellular carcinoma.

Possible mechanisms for the hepatocarcinogenic effects of phthlates and other peroxisome proliferators have included the generation of free radicals from increased hydrogen perioxide (H_2O_2) production and decreased catalase activity, and that peroxisome inducing chemicals and/or their metabolites may act as promoters (Ganning et al., 1984). The production of the enzyme catalase by peroxisomes catalyzes the breakdown of hydrogen

peroxide to water. Hydrogen peroxide itself or the hydroxyl ion, that is formed from hydrogen peroxide, causes damage to DNA and chromosomes (Turnbull and Rodricks, 1985). PAEs, such as DEHP, exhibit hypolipidemic activities common to several peroxisome proliferators that include liver enlargement that is not accompanied by frank histologic liver damage, proliferation of smooth endoplasmic reticulum and an increase in the number of hepatic peroxisomes (Cohen and Grasso, 1981). Warren et al. (1982) hypothesized that if DEHP acts similarly to other peroxisome proliferators, the compound may initiate neoplastic transformations of hepatic parenchymal cells by increasing intracellular reactive oxygen species, which could cause DNA damage. Peroxisome proliferators modify peroxisomal enzyme profiles such that fatty acid ß-oxidation, H_2O_2, peroxidized lipofusin and peroxisomal uricase levels are increased and increased catalase activity is smaller with respect to the increased peroxisome volume. That is, catalase activity is increased, but to a much lesser extent than the activities of H_2O_2 generating oxidases.

Turnbull and Rodricks (1985) proposed a possible mechanism of carcinogenicity for DEHP based on a peroxisome proliferation hypothesis (Figure VII-1). As discussed in Chapter III, the initial step in metabolism of orally administered DEHP is hydrolysis to yeild MEHP. MEHP then undergoes ω- and ω-1-oxidation. One of the ω-oxidation products may then undergo ß-oxidation, which is important since in this step of DEHP metabolism hydrogen peroxide is generated. Excess intracellular levels of hydrogen peroxide may be detrimental to the cell. In addition, hydrogen peroxide can react with DNA, causing alteration and liberation of DNA bases and sugar-phosphate backbone breakage (Turnbull and Rodricks, 1985).

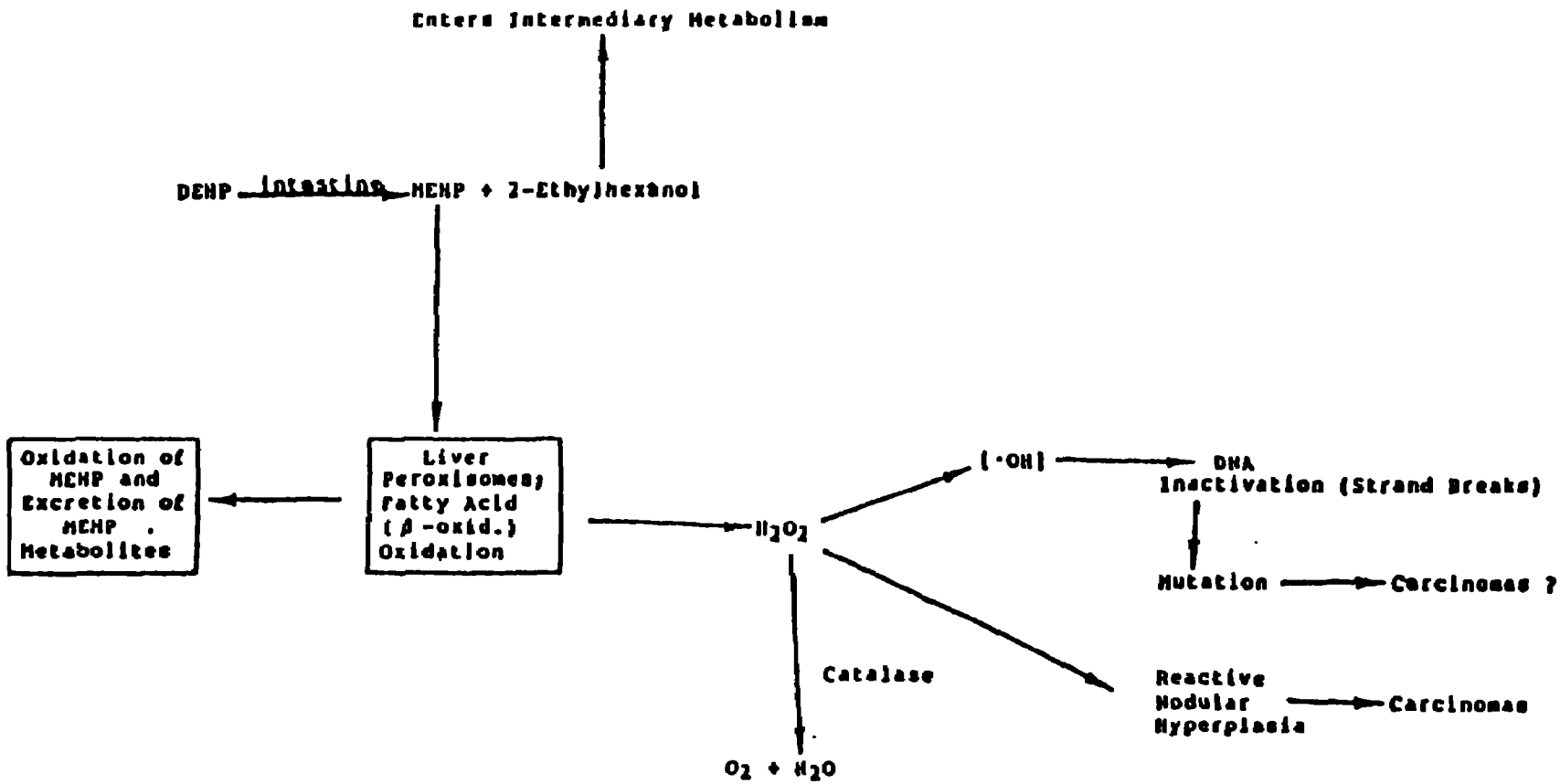

FIGURE VII-1

Schematic of the peroxisome proliferation hypothesis. At higher doses of DEHP, it is proposed that excess H_2O_2 or other oxygen species are produced in excessive amounts because catalase production does not increase as rapidly as peroxide production.

Source: Turnbull and Rodricks, 1985

In a more recent report Rodricks and Turnbull (1987) compared and summarized the differences between peroxisomes found in various mammalian species. The most extensive studies on proliferation of peroxisomes and induction of peroxisomal enzymes have been in male rats. Species differ in their morphologic characteristics of peroxisomes. Humans, as well as other species lack the enzyme uric acid oxidase (uricase) since the central crystalloid core is absent from the peroxisome. When comparing peroxisomal data, there seem to be only slight differences between species; however, there are even some differences within a species relating to age and gender. Of the species and sexes tested, male rats are the most sensitive to chemically induced peroxisomal proliferation. Quantitative measurement of the species differences is not available. However, the authors speculate that it may be due to differences in absorption, metabolism or inherent differences in hepatic susceptibility (Rodricks and Turnbull, 1987).

The possible DNA-binding activity of DEHP has been investigated by Albro et al. (1983a). Ethylhexyl-labeled DEHP, but not ring-labeled DEHP, was found to be associated with the DNA from the livers of rats. The authors determined that the radioactivity was not a result of absorption, intercalation, attachment to RNA or histones, an impurity in the labeled DNA, or artifactual binding from the sample preparation. The source of the ^{14}C may have been carbonyl phosphate, which is a precursor for urea and pyrimidine bases. von Däniken et al. (1984) concluded that DEHP did not bind covalently to hepatic DNA in rats and mice exposed to the labeled PAEs through dietary administration. Radioactivity associated with the DNA was attributed to the biosynthetic incorporation of radiolabeled breakdown products, such as 2-ethylhexanol.

Mechanisms of Reproductive Toxicity

Gonadal toxicity in rats has been linked to the adverse effects of phthalates upon testicular zinc concentrations. Upon administration of DBP or DEHP urinary excretion of zinc was enhanced and the testicular zinc content decreased (Cater et al., 1977; Foster et al., 1980; Thomas et al. 1982). Cater et al. (1977) concluded that after oral administration, DBP is metabolized by nonspecific esterases in the GI tract to the monobutyl phthalate (MBP) prior to absorption into the bloodstream. The monoester or another metabolite of DBP may act as a chelating agent by removing the zinc from the testes. Testicular zinc deficiency is, therefore, the possible causative factor leading to testicular atrophy. Zinc depletions have been noted in both the testes and prostate glands of rodents following oral, s.c. and i.p. PAE exposures. It has been hypothesized that the testicular effects of orally administered diesters are mediated by the monoesters and alcohols produced during diester hydrolysis in the GI tract (Gray and Beamand, 1984). Thomas et al. (1982) provided s.c. and i.p. injection data that demonstrated the action of DEHP upon the depletion of endogenous gonadal zinc was not a function of the interference of the intestinal absorption of the divalent zinc ion.

Further investigations of the mechanism of testicular injury indicated that the testicular injury induced by DBP does not appear to result from the accumulation of metabolites or the formation of covalent adducts in testicular tissue (Gangolli, 1982). Oral administration of ^{14}C-DBP (lactation of ^{14}C not stated) did not show evidence of accumulation of radioactivity in the gonads. Also, testicular atrophy did not appear to be mediated by an interference in androgen synthesis or the availability of gonadotropins.

The DBP-induced testicular injury was not reversed by treatment with testosterone or pregnant mare serum (Gangolli, 1982).

Summary

Research into the mechanisms of PAE toxicity in animal tissues has indicated that the PAEs may interfere with the normal enzymatic or metabolic processes. Investigators have found that PAEs exert their toxic effects by modifying the physical state of membrane-lipids and, therefore, change membrane fluidity. The mechanisms by which these alterations occur has not been clearly delineated. In the liver, phthalates alter the structure and metabolism as characterized by increases in the number of peroxisomes, mitrochondria and enzymes of fatty acid oxidation. Studies primarily on DEHP indicate that lipid and protein metabolism are inhibited. These effects on carbohydrate metabolism are also associated with depressions in the energy coupling systems of the liver, including the mitochondria. Inhibition of cholesterol synthesis in various organs occurs when phthalates inhibit an enzyme necessary for the conversion of acetate to cholesterol. Although PAEs may become associated with hepatic DNA, the data indicate that this does not occur through covalent binding, but rather as a result of the biosynthetic incorporation of PAE metabolites into the genetic material. The gonadal toxicity of PAEs has been related to an effect of these compounds in decreasing endogenous testicular zinc. Researchers have investigated several possible mechanisms of PAE toxicity; however, there is no conclusive evidence on any one mechanism.

VIII. QUANTIFICATION OF TOXICOLOGIC EFFECTS

Introduction

The quantification of toxicologic effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = ____ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicologic effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the

U.S. EPA (1991) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{\text{(RfD) x (Body weight in kg)}}{\text{Drinking Water Volume in } \ell\text{/day}} = ____ \text{ mg/}\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{\text{(NOAEL or LOAEL) x (bw)}}{\text{(UF) x (___ } \ell\text{/day)}} = ____ \text{ mg/}\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicologic evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these

estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biologic mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is

uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

PAEs appear to be ubiquitous based on their detection in a variety of environmental media including air, water, food and soil. They have also been detected in human tissues. Despite widespread occurrence of PAEs, information concerning the effects of human exposure to these compounds is limited. The available studies on human effects are not suitable for use in risk assessment because of the small numbers of subjects studied and the lack of quantitative information on levels and duration of exposure. However, the human studies and case reports provide supplemental information for comparison with health effects reported in animal studies.

Information on the acute oral toxicity of PAEs in humans is limited to the effects observed in three individuals exposed to DEHP. No effects were observed in one adult male subject administered an oral dose of 5 g of

DEHP. A second adult male subject given an oral dose of 10 g of DEHP experienced mild gastric disturbances and moderate catharsis (Shaffer et al., 1945). Accidental ingestion of 10 g of DBP by a young adult male produced nausea, vertigo and signs of keratitis and toxic nephritis (Lefaux, 1968). A single prospective cohort study was identified in the literature. Thiess et al. (1978b) reported that among 221 workers exposed to DEHP, only half of the expected deaths were observed in the exposed population. Although the analysis was not conclusive, no increased risk of adverse health effects was attributed to exposure to DBP in this group of workers. In vitro studies of human tissue and cell cultures revealed that PAEs inhibited cellular growth and decreased platelet function but did not produce chromosomal damage in human leukocytes or fetal lung cells. The greatest risk for toxic effects from PAE exposure appears to be among individuals receiving blood transfusions or hemodialysis due to extraction of PAEs from plastic blood bags or plastic tubing used in these treatments. However, reports of hepatitis in hemodialysis patients and necrotizing enterocolitis in infants given blood transfusion could not be attributed definitively to PAE exposure.

Species differences occur with respect to metabolism of PAEs. Several species of animals have been determined to excrete glucuronide conjugates of MEHP (the major metabolite of DEHP) upon exposure to DEHP with the exception of rats (Tanaka et al., 1975; Williams and Blanchfield, 1975; Albro et al., 1982). The role that glucuronide conjugation may play in the sensitivity between species to toxic endpoints is not known; therefore, studies with rats will still be considered with other test species for quantification of toxicologic effects.

There is no common toxic effect that PAEs as a group of compounds have been shown to produce. Testicular atrophy and hepatic involvement are two effects observed for several, though not all, PAEs. There is evidence that some effects of PAEs, such as the suspected carcinogenic effects of DEHP, are related to the moiety attached to the phthalate group rather than the phthalate group itself (Kluwe, 1986). Thus, different PAEs may have different patterns of toxicity. For example, DEHP induces hepatocarcinomas in rats and mice, whereas BBP appears to affect the hematopoietic system and may induce leukemia in female rats. Because a single toxicologic pattern has not been identified for PAEs, the HAs, DWELs and cancer risk levels are calculated for individual PAEs rather than for the group of compounds generically.

Studies Considered for Noncarcinogenic Quantifications -- DEHP. DEHP has been studied more extensively than any other PAE, in part because it is the most widely used plasticizer. Enlarged liver and testicular atrophy are the two most commonly observed effects of DEHP in rats. Mangham et al. (1981) conducted a short-term test to examine the testicular and hepatic effects of DEHP. DEHP was administered orally by gavage to Wistar rats at a dose level of 2500 mg/kg/day for 7 and 21 days. After 7 or 21 daily doses, weight of the testes was decreased, and histopathologic changes were found in 50-80% of the seminiferous tubules of each male rat. Treatment for 7 or 21 days produced marked liver enlargement in rats of both sexes and decreased activity of succinate dehydrogenase in males. Also, body weight gain was significantly decreased in males. The testicular effects observed are consistent with results of an earlier study by Gray et al. (1977) in which testicular atrophy occurred within the first 2 weeks of treatment at a

dietary level of 2% DEHP (~1440 mg/kg/day). Lake et al. (1975) reported increased liver weights in Wistar rats administered 2000 mg/kg DEHP [236 mg/kg/day assuming 0.013 kg/day intake (Lehman, 1959)] for periods of 4, 7, 14 and 21 days. The investigator did not examine any reproductive organs.

In a recent study, Mitchell et al. (1985) observed similar results when groups of male and female Wistar albino rats were administered diets containing 50, 200 and 1000 mg/kg/day DEHP for 3, 7, 14 and 28 days. There was a total of 90 treated rats and 60 control rats. Histopathologic examinations were performed on the major abdominal organs at all time points. The livers of male rats were significantly enlarged 3 days after treatment with 1000 mg/kg/day DEHP. After 14 days significant liver enlargement was noted at the 50 and 200 mg/kg/day doses in male rats. There were no significant differences in testes weight when control animals were compared with experimental animals. Further details were not given. Liver cells from male rats showed marked proliferation of peroxisomes after 3 days treatment with 200 or 1000 mg/kg/day. Treatment with 50 mg/kg/day showed increased numbers of peroxisomes after 14 days. Female rats, however, showed only increased number of peroxisomes after 14 days treatment with 1000 mg/kg/day. Proliferation of the smooth endoplasmic reticulum in both males and females occurred at all doses in a dose-dependent manner. Biochemical changes such as effects on DNA, catalase activity and laurate hydroxylase activity were also noted in all dose groups.

Subchronic oral studies have been conducted with DEHP on rats (Shaffer et al., 1945; Harris et al., 1956; Nikonorow et al., 1973; Gray et al., 1977; Mitchell et al., 1985; Cater et al., 1977), mice (NTP, 1984a), and

dogs (Harris et al., 1956). The study in which adverse effects were observed at the lowest level of exposure is that of Mitchell et al. (1985). Mitchell et al. (1985) fed male and female Wistar albino rats diets containing 50, 200 and 1000 mg/kg/day DEHP for 9 months. Necropsy of the thoracic, abdominal and other regions was carried out. The livers were subjected to extensive histologic, electron microscopic and biochemical examination. Significant liver enlargement was observed in male rats at all dose levels. In addition, body weights of both male and female rats were significantly reduced. Electron microscopy revealed an increase in peroxisomal proliferation at all dose levels and an increase in number of lysosomes at 200 and 1000 mg/kg/day.

In the study by Gray et al. (1977), male and female CD rats were fed dietary levels of 0, 0.2, 1 and 2% DEHP in the diet for 17 weeks. Daily doses calculated from food consumption data corresponded to 143, 737 and 1440 mg/kg/day for males, respectively, and 154, 797 and 1414 mg/kg/day for females, respectively. Body weight, food consumption, clinical signs of toxicity, serum biochemistry, urinalysis and hematology were monitored. Gross and microscopic pathologic examinations were performed on all rats at the end of the study. Effects were observed at all levels of exposure. Significantly increased absolute and relative liver weights were observed in all exposed groups. Both males and females fed either 1 or 2% DEHP had a significantly reduced packed cell volume compared with controls. At the 0.2% level, liver weight was increased in both sexes and spermatogenesis was decreased in males.

Carpenter et al. (1953) conducted chronic toxicity testing in rats, guinea pigs and dogs. Groups of 32 male and 32 female Sherman rats were fed

dietary levels of 0.04, 0.13 and 0.4% DEHP. Mean daily intakes were 20, 60 and 200 mg/kg/day. During the first year, male and female rats were housed together until the females became pregnant. Parental rats were maintained on their respective diets for 2 years. Offspring of the rats fed 0.4% DEHP were maintained at this dietary level for 1 year. After 1 year, parental rats were reduced in number to a maximum of 8/sex/group. No effects were observed at the 0.04 or 0.13% levels. At the 0.4% level, decreased body weight and increased liver and kidney weights were observed at the end of the first year, but no significant effects on fertility were observed. While this study provides information on a chronic NOEL, the low survival among controls, which experienced 70.3% mortality from causes such as lung infections, postpartum complications, peritonitis, abdominal abscess and intestinal intussusception over the 2-year period, should be noted.

Guinea pigs (23 male and 23 females/group) were also fed DEHP in the diet at levels of 0.04 and 0.13% for 1 year (Carpenter et al., 1953). This experiment showed no effects at the 0.04% level (~19 mg/kg/day) and increased liver weight at 0.13% (64 mg/kg/day). These results appear to indicate that the guinea pig was slightly more sensitive to the effects of DEHP in this study. A group of four dogs given capsules 5 times/week containing 0.03 mℓ/kg/day for 19 doses, then 0.06 mℓ/kg/day for 240 doses showed no significant effects (Carpenter et al., 1953).

Harris et al. (1956) reported similar results in a 2-year rat study. Groups of 43 male and 43 female rats were fed diets containing 0.1 or 0.5% DEHP. No compound-related effects on mortality were observed; however, survival over the 2-year study period was very low with 85-95% mortality.

No effects were observed at the low dietary level (~50-80 mg/kg/day), which is consistent with the findings of Carpenter et al. (1953). At the higher level, food consumption decreased after the first year. Increased liver and kidney weights were observed in high-dose rats sacrificed at 3 and 6 months. The daily intake at the higher dose, calculated for the first 6 months, was ~300-400 mg/kg/day. Testicular atrophy was not reported by Carpenter et al. (1953) or Harris et al. (1956).

The oral NOAEL for reproductive effects of DEHP appears to be near the NOAEL for chronic toxic effects. Tomita et al. (1982a) reported that a single oral dose of 0.05 mℓ/kg administered by gavage to mice on day 7 of gestation was associated with a decrease in body weight of viable fetuses; however, no abnormal fetuses were observed. Since the density of DEHP is 0.985 g/mℓ, the 0.05 mℓ/kg dose is equivalent to 49 mg/kg. Using the dose-response curve for resorptions and deaths, the authors calculated the NOEL for fetal lethality to be 64 mg/kg. Shiota and Nishimura (1982) administered DEHP in the diets of ICR-ICL mice on days 0-18 of gestation. At the 0.05% level (70 mg/kg/day), the only effect observed was retarded ossification. This effect was thought to be related to general and under-development of the fetuses rather than teratogenic activity, since no internal anomolies were observed. At a dietary level of 0.1% (190 mg/kg/day) the number of resorptions and dead fetuses were increased, although the statistical significance of this increase was marginal (p=0.05). At 0.25% (410 mg/kg/day), an increased number of malformations were observed in addition to increased resorptions and dead fetuses, decreased maternal and fetal weights, and retarded ossification. At 0.4% and 1.0% (830 and 2200 mg/kg/day), all fetuses were dead or resorbed.

More recently DEHP was evaluated for developmental toxicity in Fischer 344 rats and CD-1 mice (Tyl et al., 1988). Dietary levels of DEHP were administered on gestational days 0-20 to rats at 0, 0.5, 1.0, 1.5 or 2.0% and on gestational days 0-17 to mice at 0, 0.025, 0.05, 0.10 or 0.15%. Corresponding levels in mg/kg/day were 0, 356, 666, 856, 1054 and 0, 44, 91, 190, 292 in rats and mice, respectively. Tyl et al. (1988) concluded that DEHP was not teratogenic at any dose tested in Fischer 344 rats. However, treatment did produce maternal and other embryofetal toxicity at 1.0, 1.5 and 2.0%. An embryofetal NOEL in rats was determined to be 0.5% (356 mg/kg/day). In mice, doses (0.10 and 0.15%) that produced maternal and embryofetal toxicity also increased incidence of malformations. A dose of 0.05% (91 mg/kg/day) DEHP produced increased incidence of malformations without maternal or embryofetal toxicity. An embryofetal NOEL in mice was determined to be 0.025% (44 mg/kg/day) DEHP.

A study by NTP (1984a) tested CD-1 mice using a newly developed testing scheme designated "Fertility Assessment by Continuous Breeding". Results of this study were similar to those of Shiota and Nishimura (1982); however, the NTP study focused on fertility effects rather than teratogenicity. In the first phase of this test, groups of 20 male and 20 female mice were fed diets containing 0.01, 0.1 and 0.3% DEHP for 7 days premating and for 98 days of continuous mating, after which they were maintained for 21 days with no treatment. Daily intakes of DEHP were not calculated by the authors. However, if one assumes the same daily intake rate as that calculated for the low-dose CD-1 mice in the carcinogenicity bioassay by Kluwe et al. (1982a) of 735 mg/kg/day (averaged for males and females) for a 0.3% dietary level, daily DEHP intakes for the lower dietary levels of 0.01 and 0.1%

would be ~24 and 243 mg/kg/day, respectively. It should be noted that these calculations do not account for differences in food consumption due to pregnancy, age of mice or any additional differences between the two studies. At the 0.3% level, complete suppression of fertility was observed. At the 0.1% level, fertility was decreased and various reproductive parameters were significantly decreased. These parameters included number of litters per pair, and number of live pups per litter, proportion of pups born alive, number of male pups born alive, live pup weight of females and adjusted live pup weight of males. A second phase of this study used the mice from the continuous breeding phase. In this phase control males were mated to the 0.3%-treated females and control females were mated to 0.3%-treated males. In addition, control males were bred to control females to serve as the control group for the second phase. Results of this phase of testing revealed that the decreased fertility was attributable to effects of DEHP in both males and females.

Quantification of Noncarcinogenic Effects -- DEHP.

Assessment of Acute Exposure Data and Derivation of 1-day HA -- Liver enlargement, testicular atrophy in males, depressed weight gain and death have all been observed after oral administration of single doses of DEHP to rats. LD_{50}s for DEHP have been measured in a variety of species and range from 26 g/kg (rats) to 34 g/kg (rabbits). In rats, neonates and sucklings are more sensitive to the weight gain and lethal effects of DEHP than are adults. Dostal et al. (1987a) administered five successive doses (gavage in corn oil) of 0, 10, 100, 1000 or 2000 mg/kg/day to six groups (9-10 pups/group) of rats, 6-86 days old. For neonates and sucklings, doses of 2000 mg/kg/day were lethal and 1000 mg/kg/day caused depressed weight gain

in all groups and increased mortality in sucklings 14 days old. Adults (86 days old) were less sensitive to these effects, with no increase in mortality at any dose and effects on weight observed only at the 2000 mg/kg/day dose level. At 100 mg/kg/day, sucklings and adults exhibited increased liver-to-body weight ratios.

Effects of acute oral exposure to DEHP on the liver have been studied by Mitchell et al. (1985) and Mangham et al. (1981). Mitchell et al. (1985) administered DEHP in the diet of rats (4/sex/group) at nominal doses of 50, 200 and 1000 mg/kg/day. Histopathologic, biochemical cytogenetic analyses were conducted on days 3, 7, 14 and 28, and at 9 months of dosing. Indications of hepatotoxicity (increased liver weight, decreased hepatic glucose-6-phosphatase activity) were first observed in the 50 mg/kg/day dose males at 14 days of treatment. Mangham et al. (1981) observed decreased testicular weight, microscopic changes in the seminiferous tubules, enlarged liver, decreased activity of succinate dehydrogenase and decreased body weight after seven doses of 2500 mg/kg/day.

In addition, DEHP can cause reproductive and developmental toxicity. Tyl et al. (1988) observed fetotoxicity in mice and rats at doses of 91 and 666 mg/kg/day, respectively. Mice were dosed on days 0-17 of gestation while rats were dosed on days 0-20. NOAELs for reproductive and developmental effects of 44 mg/kg/day (mice) or 357 mg/kg/day (rats) were identified. These observations are supported by the work of Tomita et al. (1982a). Mice were administered single doses of 50 µℓ DEHP/kg (~49 mg/kg) or 100 µℓ DEHP/kg (~99 mg/kg) to pregnant dams at day 7 of gestation and decreased fetal weights at birth were observed.

For exposure of 5 days or less, developmental effects in mice and liver enlargement in rats are the most sensitive endpoints of toxicity. From the studies described previously, it is not possible to determine whether a dose of 44 mg/kg/day, the NOAEL for developmental effects, would cause liver effects in rats, observed at 100 but not 10 mg/kg/day. Therefore, the NOAEL for liver enlargement of 10 mg/kg/day from Dostal et al. (1987a) was selected as the basis for the 1-day HA for DEHP, derived as follows:

$$\text{1-day HA} = \frac{10\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell/\text{day}} = 1\ \text{mg}/\ell$$

where:

10 mg/kg/day =	NOAEL based on lack of liver enlargement (Dostal et al., 1987a)
10 kg =	assumed weight of a child
100 =	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study
1 ℓ/day =	assumed water consumption by a child

<u>Assessment of Short-Term Exposure Data and Derivation of 10-day HA</u> -- Effects on the liver appear to be the most sensitive endpoint of toxicity for 10-day exposure. Mitchell et al. (1985) observed liver effects in rats after 14 days at doses as low as 50 mg/kg/day, a dose similar to the NOAEL for developmental toxicity in mice (44 mg/kg/day). A NOAEL for the observed liver effects (decreased glucose-6-phosphatase activity, increase in liver weight, increase in hepatocyte lipid content) was not identified. This number is consistent with the 1-day HA if it were adjusted for a 10-day exposure period. However, the Mitchell et al. (1985) study examined other organs in addition to the liver and provides a better estimate of a 10-day

exposure. The 10-day HA is also protective of developmental toxicity identified at 91.07 mg/kg/day in mice and 666.39 mg/kg/day in rats by Tyl et al. (1988). Thus, the Mitchell et al. study is chosen to derive the 10-day HA as follows:

$$\text{10-day HA} = \frac{50\ \text{mg/kg/day} \times 10\ \text{kg}}{1000 \times 1\ \ell\text{/day}} = 0.5\ \text{mg/}\ell$$

where:

50 mg/kg/day	=	LOAEL based on liver enlargement (Mitchell et al., 1985)
10 kg	=	assumed weight of a child
1000	=	uncertainty factor, according to U.S. EPA and OOW/NAS guidelines for use with a LOAEL from an animal study
1 ℓ/day	=	assumed water consumption by a child

<u>Derivation of Longer-term HA</u> -- Subchronic oral studies have been conducted with DEHP, however none of the studies identify a NOAEL. Mitchell et al. (1985) observed significant liver enlargement in male rats administered 50, 200 or 1000 mg/kg/day for 9 months. No clear progression of hepatotoxic effects was observed from 3-, 7-, 14- or 28-day time points. This is supported by the subchronic study by Gray et al. (1977) where liver weights were increased in both sexes of rats and spermatogenesis was decreased in males (143 mg/kg/day dose level in males; 154 mg/kg/day dose level in females).

Deriving the longer-term HA based on the LOAEL of 50 mg/kg/day is protective of the reproductive (NTP, 1984a) and developmental toxicity (Tyl et al., 1988) observed in mice at doses of 243 and 91 mg/kg/day, respectively. The reproductive study by NTP (1984a) showed no effects on

fertility in mice fed 0.01% (24 mg/kg/day) in the diet for 7 days premating and 98 days continuous breeding. The next highest dietary level of 0.1% (240 mg/kg/day) significantly reduced fertility. Tyl et al. (1988) observed fetotoxicity in mice and rats at 91 and 666 mg/kg/day. NOAELs for reproductive and developmental effects of 44 (mice) or 357 (rats) mg/kg/day were identified.

Therefore, based on a LOAEL for hepatotoxicity in rats the longer-term HA values are calculated as follows:

$$\text{Longer-term HA (child)} = \frac{50\ \text{mg/kg/day} \times 10\ \text{kg}}{1000 \times 1\ \ell/\text{day}} = 0.5\ \text{mg}/\ell$$

where:

50 mg/kg/day = LOAEL based on liver enlargement (Mitchell et al., 1985)

10 kg = assumed weight of a child

1000 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a LOAEL from an animal study

1 ℓ/day = assumed water consumption by a child

$$\text{Longer-term HA (adult)} = \frac{50\ \text{mg/kg/day} \times 70\ \text{kg}}{1000 \times 2\ \ell/\text{day}} = 1.75\ \text{mg}/\ell \quad \text{(rounded to 2 mg}/\ell\text{)}$$

where:

50 mg/kg/day = LOAEL based on liver enlargement (Mitchell et al., 1985)

70 kg = assumed weight of an adult

1000 = uncertainty factor, according to U.S EPA and ODW/NAS guidelines for use with a LOAEL from an animal study

2 ℓ/day = assumed water consumption by an adult

Assessment of Long-Term Exposure Data and Derivation of a DWEL -- No data were available on the effects of chronic human exposure to DEHP. Carpenter et al. (1953) reported no effects in rats exposed for 2 years to dietary levels of 0.04% and 0.13%, equivalent to ~20 and 60 mg/kg/day, respectively. There was low survival of both control and treated rats during the second year. However, results with other species and studies support these results. Carpenter et al. (1953) also reported that no effects were observed in guinea pigs exposed to 0.04% (~19 mg/kg/day) in the diet for 1 year; however, increased liver weights were reported. Increased liver weight was also observed in guinea pigs exposed to 0.13% DEHP (64 mg/kg/day). No histologic effects on liver tissue were observed. The suggested LOAEL is ~19 mg/kg/day. A study by Harris et al. (1956) showed no effects in rats fed 0.1% DEHP (50-80 mg/kg/day) for 2 years. Again, low survival in all groups places limitations on interpretation of the results of this study. A recent reproductive study showed no effects on fertility in mice fed 0.01% DEHP in the diet for 7 days premating and 98 days continuous breeding (NTP, 1984a). Assuming food consumption was similar to that reported by Kluwe et al. (1982a) for mice, daily intake was calculated to be 24 mg/kg/day. The next highest dietary level of 0.1% significantly reduced fertility. The approximate daily intake for this dietary level was calculated to be 240 mg/kg/day. Also, Shiota and Nishimura (1982) reported only reduced ossification in mouse fetuses born to dams fed 70 mg/kg/day on days 0-18 of gestation. Tyl et al. (1988) determined an embryofetal NOEL to be 356.74 mg/kg/day in Fischer 344 rats and 44.07 mg/kg/day in CD-1 mice. In light of these results and the fact that no NOAEL was identified, lower than the lowest LOAEL observed, ~19 mg/kg/day reported by Carpenter et al. (1953) was selected for use in calculating the DWEL (U.S. EPA, 1991).

Using this LOAEL, the DWEL would be derived as follows:

$$RfD = \frac{19\ mg/kg/day}{1000} = 0.019\ mg/kg/day$$

(rounded to 0.02 mg/kg/day)

where:

19 mg/kg/day = LOAEL derived from oral exposure to guinea pigs (Carpenter et al., 1953)

1000 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a LOAEL from a subchronic animal study

$$DWEL = \frac{0.02\ mg/kg/day \times 70\ kg}{2\ \ell/day} = 0.7\ mg/\ell$$

where:

0.02 mg/kg/day = RfD

70 kg = assumed weight of an adult

2 ℓ/day = assumed water consumption by an adult

The 1-day HA, 10-day HA, longer-term HA and DWEL values calculated for DEHP and the effect levels used in the derivations are summarized in Table VIII-1.

Studies Considered for Noncarcinogenic Quantification -- BBP. Toxicity of BBP is limited to a few studies. The most commonly observed effects are liver and kidney enlargement and testicular atrophy. Agarwal et al. (1985a) conducted a 14-day dietary study of male Fischer 344 rats fed levels of 0.625, 1.25, 2.5 and 5.0% BBP. At the 0.625% and 1.25% levels,

TABLE VIII-1

Summary of Data Used to Derive HA and DWEL Values for DEHP, BBP, DEP, DMP and DBP

Compound	Criteria Level	Dose (mg/kg/day)	Study Duration	Species/ Effect Level	Value of HA or DWEL		Reference
					Adult (mg/ℓ)	Child (mg/ℓ)	
DEHP	1-day HA	10	5 days	rat/NOAEL		1	Dostal et al., 1987a
	10-day HA	50	14 days	rat/LOAEL		0.5	Mitchell et al., 1985
	Longer-term HA	50	9 months	rat/LOAEL	2	0.5	Mitchell et al., 1985
	DWEL	19	1 year	guinea pig/ LOAEL	0.7		Carpenter et al., 1953
BBP	1-day HA*	160	14 days	rat/NOAEL		20	Lake et al., 1978
	10-day HA	160	14 days	rat/NOAEL		20	Lake et al., 1978
	Longer-term HA	159	26 weeks	rat/NOAEL	60	20	NTP, 1985
	DWEL	159	26 weeks	rat/NOAEL	7		NTP, 1985
DBP	1-day HA	500	14 days	rat/NOAEL		50	Cater et al., 1977
	10-day HA	125	1 year	rat/NOAEL		10	Smith, 1953

TABLE VIII-1 (cont.)

Compound	Criteria Level	Dose (mg/kg/day)	Study Duration	Species/ Effect Level	Value of HA or DWEL		Reference
					Adult (mg/ℓ)	Child (mg/ℓ)	
DBP	Longer-term HA	125	1 year	rat/NOAEL	40	10	Smith, 1953
	DWEL	125	1 year	rat/NOAEL	4		Smith, 1953
DEP	1-day HA					ND	
	10-day HA					ND	
	Longer-term HA	750	16 weeks	rat/NOAEL	300	80	Brown et al., 1978
	DWEL	750	16 weeks	rat/NOAEL	30		Brown et al., 1978
DMP	1-day HA					ND	
	10-day HA					ND	
	Longer-term				ND	ND	
	DWEL				ND		

*Adopted from the 10-day HA

ND = Not determined (inadequate data for derivation)

liver and kidney weights were significantly increased. In addition, the incidence of proximal tubular regeneration of the kidney increased in a dose-related manner beginning at the 0.625% dose level. At the 2.5% and 5.0% levels, effects included decreased body weight, slightly decreased food consumption, decreased weights of testes, epididymus, seminal vesicles and thymus, histologic atrophy of testes and accessory sex organs, and decreased bone marrow cellularity. Daily intakes of BBP were not calcu- lated. However, daily food consumption and body weight were estimated from the figures presented by Agarwal et al. (1985a). At the lowest dietary level of 0.625%, rats weighing ~250 g consumed 15 g of food per day or 60 g food/kg bw/day. The daily intake of BBP was calculated to be 375 mg/kg bw/day. Since body weights and food consumption were not affected at 1.25%, the same food consumption and body weight were used and the daily intake was calculated to 750 mg/kg bw/day. Food consumption and body weight were decreased at 2.5 and 5.0%, respectively. Again, approximating from the figures in Agarwal et al. (1985a), 200 g rats at the 2.5% level consuming 10 g food/day and 150 g rats at the 5.0% level consuming 5 g food/day, the daily intake was 1250 and 1667 mg/kg bw/day, respectively.

Lake et al. (1978) administered 160, 480 or 1600 mg/kg/day BBP by gastric intubation for 14 days to six male Sprague-Dawley rats per group. Biochemical or morphologic changes in the liver were not observed at 160 mg/kg/day. Activities of ethylmorphine N-demethylase and cytochrome oxidase were significantly increased at the 480 and 1600 mg/kg/day BBP. Significant liver enlargement was observed at 1600 mg/kg/day in addition to ultrastructural changes, such as gross dilation of the rough endoplasmic

reticulum and increased number of peroxisomes. Effects on testes weights were not observed in the 160 or 480 mg/kg/day animals; however, 1600 mg/kg/day BBP produced marked depression of both absolute and relative testes weights as well as severe testicular atrophy. Testicular atrophy was observed in 1/3 animals administered 480 mg/kg/day.

A second study was conducted to confirm the testicular effects. Both Sprague-Dawley and Wistar Albino rats were treated with 480 and 1600 mg/kg/day BBP for 14 days. A significant depression in either absolute or relative liver and testes weight was observed in both strains of rats at 1600 mg/kg/day BBP. Histologic examination revealed testicular atrophy in both strains (1600 mg/kg/day) with the extent of the lesions being more severe in the Sprague-Dawley strain. At 480 mg/kg/day BBP, 1/6 had testicular atrophy, whereas the Wistar albino strain revealed no histologic changes.

There are few oral long-term BBP studies. In a final report, NTP (1985) conducted toxicity and mating trial studies in F344 rats. The toxicity portion was conducted as a dose range-finding study to establish a no effect level and the dose response curve for BBP. Rats were administered concentrations of either 0, 0.03, 0.09, 0.28, 0.83 or 2.50% (0, 17, 51, 159, 470 and 1417 mg/kg/day) BBP in the diet for 26 weeks. Powdered BBP was mixed in to standard rodent meal diet. Because of the manner in which the BBP was administered considerable waste and spillage was found especially at the highest dose level. Therefore, the dose conversion for the highest was based on a 5% food consumption rate/mg rat body weight. There were 15 male animals in each dose group, starting at 6 weeks of age. Throughout the

study, body weight gain was significantly depressed at the 2.5% BBP level when compared with the controls. There were no deaths attributed to BBP toxicity. All the rats given 2.5% BBP had small testes upon gross necropsy at the 26-week termination. Five of 11 had soft testes, and 1/11 had a small prostate and seminal vesicle. At 0.83%, significantly ($p<0.05$) increased absolute liver weight, liver-to-body weight, liver-to-brain weight ratios and increases in mean corpuscular hemoglobin were noted. In the 0.03, 0.09, 0.28 and 0.83% BBP dose groups there were no grossly observable effects on male reproductive organs. The kidneys of six animals in the 2.5% group contained focal cortical areas of infarct-like atrophy. In addition, testicular lesions were also observed at the 2.5% dose level. Lesions were characterized by atrophy of seminiferous tubules and aspermia. The other treatment groups showed no evidence of abnormal morphology in any other organs.

Histopathologic changes were also seen at the 2.5% BBP level after 10 weeks of exposure in the mating trial portion of this study. After histopathologic examination, testicular lesions were characterized by atrophy of seminiferous tubules and a near total absence of mature sperm production. When 10/30 females successfully mated with the 2.5% treatment level males, none were pregnant at necropsy. The investigators concluded that the data suggest a depression in male reproductive organ weights by either a direct or indirect toxic effect after 2.5% BBP administration. BBP at 0.83% in the diet did not result in any treatment-related effects.

The only other information on the subchronic effects of BBP is taken from an unpublished study by Monsanto (1972). Rats fed diets containing BBP

at levels of 0.25 (125 mg/kg/day) and 0.50% (250 mg/kg/day) for 90 days showed no toxic effects. A dietary level of 1.0% (500 mg/kg/day) BBP resulted in increased liver weight. Levels of 1.5 (750 mg/kg/day) and 2.0% (1000 mg/kg/day) BBP were associated with increased liver weight and a decrease in growth rate. No effects were observed in dogs administered BBP in capsules at levels equal to 1.0, 2.0 and 5.0% of the diet. No further details of this study were available for review.

Quantification of Noncarcinogenic Effects -- BBP.

Assessment of Acute Exposure Data and Derivation of the 1-day HA -- No information was available on the effects of BBP in humans. The only studies available on acute oral toxicity in animals used lethality as the toxic end-point or were inadequate for deriving a 1-day HA. Therefore, lack of sufficient data preclude the derivation of a 1-day HA for BBP. It is recommended that the 10-day HA of 20 mg/ℓ be adopted as a conservative estimate for the 1-day HA.

Assessment of Short-Term Exposure Data and Derivation of a 10-day HA -- Information presented in a 14-day study was used to approximate the 10-day HA values. Agarwal et al. (1985a) administered BBP to male F344 rats in the diet for 14 consecutive days at dose levels of 0.625, 1.25, 2.5 and 5.0%. Effects observed beginning at the 0.625% level were significantly increased liver and kidney weights. Dose-related histopathologic changes (proximal tubular regeneration) were also noted in the kidney beginning at the 0.625% level. Using approximations of food consumptions and body weight obtained from figures presented in this study, the daily intake at 0.625% level was calculated to be 375 mg/kg/day.

In male Sprague-Dawley rats administered 160, 480 or 1600 mg/kg/day BBP for 14 days by gastric intubation, biochemical or morphologic changes in the liver as well as effects on testes weights were not observed in the 160 mg/kg/day dose group (Lake et al., 1978). However, at 480 mg/kg/day activities of ethyl morphine N-demethylase and cytochrome oxidase were significantly increased and testicular atrophy was observed in 1/3 Sprague-Dawley rats in the first portion of this experiment. In the second portion, the 480 mg/kg/day dose induced testicular atrophy in 1/6 Sprague-Dawley rats, whereas the Wistar albino strain revealed no such effects (Lake et al., 1978).

When comparing the two studies Lake et al. (1978) identifies a NOAEL of 160 mg/kg/day. It is questionable whether 480 mg/kg/day represents a NOAEL; however, Agarwal et al. (1985a) observed significant increases in liver and kidney weights and kidney pathology at 375 mg/kg/day, which represents a LOAEL. It is therefore recommended that the NOAEL of 160 mg/kg/day identified in the Lake et al., (1978) study be used in deriving the 10-day HA. Although the method of treatment was gavage in the study by Lake et al. (1978) and diet in the study by Agarwal et al. (1985a), treatment-related effects across similar dose ranges, including liver effects in both studies in two sensitive strains of rats, support use of 160 mg/kg/day as NOAEL in rats given BBP orally for 14 days.

The 10-day HA is calculated as follows:

$$\text{10-day HA} = \frac{160\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell\text{/day}} = 16\ \text{mg/}\ell\ \text{(rounded to 20 mg/}\ell\text{)}$$

where:

160 mg/kg/day	=	NOAEL based on the absence of liver and testicular effects from animal data (Lake et al., 1978)
10 kg	=	assumed weight of a child
100	=	uncertainty factor, according to U.S. EPA and OOW/NAS guidelines for use with a NOAEL from an animal study
1 ℓ/day	=	assumed water consumption by a child

Assessment of Longer-term HA -- Long-term exposure to BBP causes adverse effects to the testes of male rats. The only study available for the derivation of longer-term HAs is the 26-week feeding study conducted by NTP (1985). Male F344 rats consuming a dietary level of 2.5% BBP exhibited testicular lesions characterized by atrophy of seminiferous tubules and aspermia. The corresponding dose from data given, assuming 5% food consumption/day and 200 g body weight, is 1417 mg/kg/day. At this level rats also experienced significantly depressed body weight gains and significant increases in the organ-to-body weight ratios in the brain, right kidney, right testes and liver. Rats given dietary levels of 0, 0.03, 0.09, 0.28 and 0.83% BBP for 26 weeks exhibited no grossly observable effects on male reproductive organs. Corresponding doses assuming ~300 g bw and ~17 g of food consumption/day from data presented in the report are 0, 17.0, 51.0, 159 and 470 mg/kg/day, respectively. At 0.83%, the effects noted were significantly ($p<0.05$) increased absolute liver weight, increased liver-to-body weight and liver-to-brain weight ratios and increases in mean corpuscular hemoglobin. Liver-to-body weight ratios significantly ($p<0.05$) increased for the brain, right kidney and liver at the 2.5% level; however, liver-to-brain weight ratios did not significantly ($p<0.05$) increase. The

differences may have been due to the reduced weight gain and testicular effects at 2.5% BBP. The liver may be a more sensitive endpoint than the testes since liver effects were observed at a lower level (0.83%) than testicular effects (2.5%). Therefore, 0.28% or 159 mg/kg/day will be used as a NOAEL to derive the longer-term HAs as follows:

$$\underset{\text{(child)}}{\text{Longer-term HA}} = \frac{159\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell\text{/day}} = 15.9\ \text{mg/}\ell \quad \text{(rounded to 20 mg/}\ell\text{)}$$

where:

159 mg/kg/day	=	NOAEL based on the absence of increased liver weights in rats (NTP, 1985)
10 kg	=	assumed weight of a child
100	=	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study
1 ℓ/day	=	assumed water consumption by a child

$$\underset{\text{(adult)}}{\text{Longer-term HA}} = \frac{159\ \text{mg/kg/day} \times 70\ \text{kg}}{100 \times 2\ \ell\text{/day}} = 55.65\ \text{mg/}\ell \quad \text{(rounded to 60 mg/}\ell\text{)}$$

where:

159 mg/kg/day	=	NOAEL based on the absence of increased liver weight in rats (NTP, 1985)
70 kg	=	assumed weight of an adult
100	=	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study
2 ℓ/day	=	assumed water consumption by an adult

Assessment of Long-Term Exposure Data and Derivation of a DWEL -- NTP (1985) is also the only available study for the derivation of the DWEL (U.S. EPA, 1991). The DWEL is derived as follows:

Step 1 - RfD Derivation

$$RfD = \frac{159\ mg/kg/day}{1000} = 0.159\ mg/kg/day$$
(rounded 0.2 mg/kg/day)

where:

159 mg/kg/day = NOAEL derived from orally exposed rats (NTP, 1985)

1000 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from animal data, for less than lifetime exposure and to protect sensitive members of the human population

Step 2 - DWEL Derivation

$$DWEL = \frac{0.2\ mg/kg/day \times 70\ kg}{2\ \ell/day} = 7\ mg/\ell$$

where:

0.2 mg/kg/day = RfD

70 kg = assumed weight of an adult

2 ℓ/day = assumed water consumption by an adult

The 1-day HA, 10-day HA and DWEL values calculated for BBP and the effects levels used in calculation are summarized in Table VIII-1.

Studies Considered for Noncarcinogenic Quantification -- DBP. No information was found in the available literature on the effects of DBP in humans and information on effects in animals is limited. The teratogenic effects of PAEs following oral administration were studied by Nikonorow et

al. (1973). In this study female Wistar rats were administered 120 and 600 mg/kg/day DBP in olive oil for ~3 months and during mating. Upon confirmation of conception the administration of DBP was discontinued. On day 21 the uteri and fetuses were removed. Results of this study indicated that fetal weight was significantly ($p<0.05$) reduced at 600 mg/kg/day DBP. No detectable differences were observed in the number of sternum ossification foci, the development of the bones at the base of the skull, paws on the front and hind legs, or rib fusion in fetuses from treated rats at either dose level when compared with the control animals.

Cater et al. (1977) found that DBP induced testicular atrophy in young (3-4 weeks old) male Sprague-Dawley rats. DBP was dissolved in corn oil and administered by gavage in doses of 500, 1000 and 2000 mg/kg/day for 14 days, while control animals received corn oil in a volume of 5 mℓ/kg. Testes weights were measured on days 4 and 6 for 500, 1000 and 2000 mg/kg/day doses of DBP. In addition body weight and relative liver, kidney and testes weights were measured on days 3, 7, 10 and 14 at 2000 mg/kg/day. The initial effect was a progressive reduction in weight of the testes. At 4 days, however, 500 mg/kg/day DBP did not have an effect on testes weight. A significant ($p<0.05$) reduction in the relative testes weight occurred within 6 days at 500 mg/kg/day and within 4 days at 1000 (significance $p<0.01$) and 2000 (significance $p<0.001$) mg/kg/day. By 14 days, the reduction at 2000 mg/kg/day amounted to 60-70% of the original weight. Since there was also a decrease in body weight, the authors used "relative testes weight" and found that on this basis there was still a significant loss of testes weight. There was a nonstatistically significant increase in liver weights. Histopathologic examination of testes tissue after 4 days of 2000 mg/kg DBP exposure revealed a diminution of both spermatocytes and spermatogonia.

In a dietary study DBP was fed to male and female Fischer 344 rats at 0, 0.6, 1.2 and 2.5% for 21 days (CMA, 1986). Corresponding dose levels were 0, 624, 1234 and 2156 mg/kg/day for males and 0, 632, 1261, and 2107 mg/kg/day for females. Absolute and relative liver weights were significantly increased in both male and female rats at all treatment levels. Male rats fed 2.5% DBP had severe testicular atrophy and significantly lower testes weight. Samples of liver from rats administered the 2.5% level showed "moderate" peroxisomal proliferation. In addition lauric acid 11- and 12-hydroxylase increased in males given 0.6, 1.2 and 2.5% and in females given 2.5%. Cyanide-insensitive palmitoyl CoA oxidation increased at 1.2 and 2.5% in males and 2.5% in females.

Smith (1953) studied the effects of feeding DBP to groups of 10 male 5-week-old Sprague-Dawley rats, weighing 55-65 g. Rats were fed dietary levels of 0, 0.01, 0.05, 0.25 and 1.25% DBP for 1 year. The dietary intakes for DBP were 0, 5, 25, 125 and 600 mg/kg/day, respectively, estimated from a figure depicting daily intake in mg/kg in Smith (1953). Survival rates were not reported for the three lowest dose groups. In the group fed 1.25% DBP, half (presumably 5/10) of the animals died during the first week of the experiment while the remaining animals gained weight comparable with controls. It was not indicated whether the deaths were thought to be treatment-related. Necropsies were performed when rats showed marked weight loss or signs of severe infection. Animals alive at the end of 1 year were sacrificed and examined for gross pathologic changes. While it was stated that several organs were sectioned and stained, the results of histologic evaluation were not reported. Of the animals surviving, no adverse effects on growth, survival, gross pathology or hematology were observed among those

fed diets containing 0.01, 0.05 or 0.25% DBP. The daily intake of food and plasticizer (mg/kg bw/day) decreased as the rats increased in size. No changes in hematologic parameters or gross pathology were observed at any dose level.

Shiota and Nishimura (1982) found retarded ossification in mice fed diets of 80, 180, 370, 660 and 2100 mg/kg/day DBP on days 0-18 of gestation. At the 660 mg/kg/day level, reduced fetal weight and retarded ossification were observed. Among rats fed diets of 2100 mg/kg/day, decreased maternal weight was observed along with reduced weight in the fetuses, retarded ossification and neural tube defects in the fetuses. The authors concluded that delayed ossification was related to the general underdevelopment of the fetuses. The maximum nonembryotoxic dose as stated by the authors would be 370 mg/kg/day DBP.

Quantification of Noncarcinogenic Effects -- DBP.

Assessment of Acute Exposure Data and Derivation of the 1-Day HA -- No information was found in the available literature on the acute toxicity of DBP to humans. Cater et al. (1977) found that DBP induced testicular atrophy in young (3-4 weeks old) male Sprague-Dawley rats. DBP was administered by gavage in doses of 500, 1000 and 2000 mg/kg/day for 14 days. Effects of treatment on body weight and relative liver, kidney and testes weights were measured on days 3, 7, 10 and 14 at 2000 mg/kg/day. In addition, testes weights were measured on days 4 and 6 for 500, 1000 and again for 2000 mg/kg/day. At 4 days of 500 mg/kg/day treatment testes weights were not affected. Liver weights increased but were not statistically significant. Treatment at 4 days of 1000 and 2000 mg/kg/day

significantly reduced testes weight and at 2000 mg/kg/day diminished both spermatocytes and spermatogonia. By 14 days 2000 mg/kg/day reduced the testes to 60-70% of the original weight.

In another study, CMA (1986) reported significant increases in absolute and relative liver weights after 21 days of exposure to doses of 624 and 632 mg/kg/day in male and female Fischer 344 rats, respectively.

Cater et al. (1977) identified a NOAEL of 500 mg/kg/day for testicular effects in male rats. Liver weights increased but were not statistically significant. After 21 days of exposure, CMA (1986) reported significantly increased absolute and relative liver weights. It appears that for a 1-day exposure testes are the most sensitive organ and, therefore, the NOAEL of 500 mg/kg/day will be used to derive the 1-day HA as follows:

$$\text{1-Day HA} = \frac{500\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell/\text{day}} = 50\ \text{mg}/\ell$$

where:

500 mg/kg/day	= NOAEL based on the absence of decreased testes weight from animal data (Cater et al., 1977)
10 kg	= assumed weight of a child
100	= uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study
1 ℓ/day	= assumed water consumption by a child

<u>Assessment of Acute Exposure Data and Derivation of the 10-Day HA</u> -- Cater et al. (1977) observed decreased spermatocytes and spermatogonia as well as significantly reduced testes weight after 4 days exposure to 2000 mg/kg/day DBP. In addition, testes weights were significantly reduced at

500 mg/kg/day at 6 days and 1000 mg/kg/day at 4 days of exposure (LOAEL = 500 mg/kg/day). CMA (1986) reported significant increases in absolute and relative liver weights after 21 days of exposure to ~600 mg/kg/day in rats. However, Smith (1953) reported a NOAEL of 125 mg/kg/day for growth, survival, gross pathology or hematology after 1 year exposure of DBP to male rats. In light of these data, 125 mg/kg/day appears to be a reasonable estimate of a NOAEL after 10 days of exposure and will be used to derive the 10-day HA in addition to the longer-term HAs and DWEL.

The 10-day HA is calculated as follows:

$$\text{10-day} = \frac{125\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell/\text{day}} = 12.5\ \text{mg}/\ell \quad (\text{rounded to } 10\ \text{mg}/\ell)$$

where:

125 mg/kg/day	=	NOAEL based on the absence of increased mortality and hematologic effects (Smith, 1953)
10 kg	=	assumed weight of a child
100	=	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study
1 ℓ/day	=	assumed water consumption by a child

<u>Derivation of Longer-Term HA</u> -- The only study available for the derivation of longer-term HAs is by Smith (1953). Male rats fed diets containing 5.0, 25 and 125 mg/kg/day for 1 year experienced no adverse effects on growth, survival, gross pathology or hematology. At a level of 600 mg/kg/day DBP, half of the animals died. The remaining animals gained weight as did the controls. The limitations of this study, such as few animals of one sex, the lack of animal survival data, animal infections and

the 50% survival rate among the high-dose group, combined with the lack of micropathologic examination, must be noted in interpreting these results.

$$\text{Longer-term HA (child)} = \frac{125\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell/\text{day}} = 12.5\ \text{mg}/\ell$$

(rounded to 10 mg/ℓ)

where:

125 mg/kg/day = NOAEL in male rats based on the absence of increased mortality and hematologic effects (Smith, 1953)

10 kg = assumed weight of a child

100 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study

1 ℓ/day = assumed water consumption by a child

$$\text{Longer-term HA (adult)} = \frac{125\ \text{mg/kg/day} \times 70\ \text{kg}}{100 \times 2\ \ell/\text{day}} = 43.75\ \text{mg}/\ell$$

(rounded to 40 mg/ℓ)

where:

125 mg/kg/day = NOAEL in male rats based on the absence of increased mortality and hematological effects (Smith, 1953)

70 kg = assumed weight of an adult

100 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study

2 ℓ/day = assumed water consumption by an adult

<u>Assessment of Long-Term Exposure Data and Derivation of a DWEL</u> -- Smith (1953) is also the only available study for the derivation of the DWEL (U.S. EPA, 1991). The DWEL is derived as follows:

$$RfD = \frac{125\ mg/kg/day}{1000} = 0.125\ mg/kg/day$$
(rounded to 0.1 mg/kg/day)

where:

125 mg/kg/day = NOAEL in male rats based on the absence of increased mortality and hematologic effects (Smith, 1953)

1000 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from a subchronic animal study

$$DWEL = \frac{0.1\ mg/kg/day \times 70\ kg}{2\ \ell/day} = 3.5\ mg/\ell$$
(rounded to 4 mg/ℓ)

where:

0.1 mg/kg/day = RfD

70 kg = assumed weight of an adult

2 ℓ/day = assumed water consumption by an adult

Studies Considered for Noncarcinogenic Quantification -- DEP. No information was available on the effects of DEP in humans. Information on DEP toxicity in animals is limited. In a 2-year study (Food Research Laboratories, Inc., 1955), groups of 30 rats (15/sex) were fed 0.5, 2.5 or 5.0% levels of DEP (250, 1250 or 2500 mg/kg bw/day, respectively) in the diet. No effects were observed at levels of 0.5 or 2.5%. DEP at the 5.0% dose level resulted in a small, but significant decrease in the growth rate of the rats without any effect on food consumption. Thus, 5.0% DEP appeared to affect the efficiency of food conversion to body mass. No information was available on the numbers of rats surviving (42% or more of each sex survived) the 2-year study period and histopathologic examination was performed only on the 5.0% dose group. Statistical analysis was only

conducted on organ weights and excluded statistically higher rats from the respective group averages. Also, as part of this study, 13 young mongrel dogs were fed DEP in the diet at levels of 0, 0.5, 1.5, 2.0 and 2.5% for 1 year. Problems were encountered with palatability of DEP in the diet. As a result, the dogs received varying exposures to DEP before each dog attained stabilization at the highest tolerated dietary level. Accordingly, three dogs were maintained at 0.5%, one each at 1.5 and 2.0%, and three at the 2.5% level. The average weekly intakes of DEP were computed and found to be 0.8, 2.4, 3.5 and 4.4 g/kg/week in order corresponding to increasing dietary level. No effects were noted in dogs as a result of DEP exposures.

Brown et al. (1978) also studied the long-term oral toxicity of DEP in rats. Groups of 15 CD strain rats of each sex were given diets containing 0, 0.2, 1.0 or 5.0% DEP for 16 weeks. The authors estimated the mean intakes to be 0, 150, 770 and 3160 mg/kg/day in males and 0, 150, 750 and 3710 mg/kg/day in females, respectively. Autopsies and histologic examinations were conducted at the end of 16 weeks. No changes in behavioral patterns or clinical signs of toxicity were observed. Female rats fed diets containing 1% DEP and both sexes fed diets of 5% DEP gained significantly less weight than the controls. Mean food consumption of rats of both sexes given 5% DEP and females given 1% DEP was significantly lower than that of control rats. In order to judge whether palatability was the possible cause in decreased weight gain, a paired-feeding study was conducted. Test rats fed 5% DEP consumed more food (total) and gained less weight than controls. Weights of the brain, heart, spleen and kidney were significantly lower in male and female rats fed 5% DEP. Female rats given 5% DEP showed a statistically significant increase in full caecum weight. There were no

significant changes in the absolute weights of any organs below the 5% DEP dietary level. Relative weights of the brain, liver, kidney, stomach, small intestine and full caecum were significantly higher in both sexes at the 5% dietary level when compared with the controls. These changes were attributed to the compound-related effect on growth rate since dose-related changes in gross or microscopic pathology were not observed. No other effects were observed.

Quantification of Noncarcinogenic Effects -- DEP.

Assessment of Acute Exposure Data and Derivation of the 1-Day HA -- There were no satisfactory studies for the derivation of a 1-day HA.

Assessment of Acute Exposure Data and Derivation of the 10-Day HA -- There were no satisfactory studies for the derivation of a 10-day HA.

Assessment of Longer-Term HA -- There is only one subchronic study appropriate for the derivation of longer-term HAs. Brown et al. (1978) fed CD rats diets containing 0.2, 1.0 and 5.0% DEP (150, 770 and 3160 mg/kg/day, male; 150, 750 and 3710 mg/kg/day, females) for 16 weeks. Female rats fed 1% DEP and both sexes fed 5% DEP gained significantly less weight than the controls. A paired-feeding study showed this weight difference was not from palatability. Both the liver and kidneys were histologically normal at all DEP dietary levels. Relative kidney weights at the 5% dose level were 0.67 and 0.69 g/100 g bw in males and females, respectively, compared with control values of 0.57 and 0.62 g/100 g bw in males and females, respectively. Although slight but significant ($p<0.05$) changes were seen in females at the 1% level, the use of multiple T tests for the comparisons

(without correction) and the small magnitude of the changes indicates that the 1% feeding level (750 mg/kg/day) represents a NOAEL in this study. Therefore, the NOAEL (750 mg/kg/day, females) determined from the Brown et al. (1978) study will be used to derive the longer-term HAs as follows:

$$\underset{\text{(child)}}{\text{Longer-term HA}} = \frac{750\ \text{mg/kg/day} \times 10\ \text{kg}}{100 \times 1\ \ell\text{/day}} = 75\ \text{mg/}\ell \quad \text{(rounded to 80 mg/}\ell\text{)}$$

where:

750 mg/kg/day	=	NOAEL, based on the lack of kidney and body weight effects. The dose level of 750 mg/kg/day was chosen since the females were considered more sensitive than the males (Brown et al., 1978).
10 kg	=	assumed weight of a child
1 ℓ/day	=	assumed water consumption by a child
100	=	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study

for a 70 kg adult:

$$\underset{\text{(adult)}}{\text{Longer-term HA}} = \frac{750\ \text{mg/kg/day} \times 70\ \text{kg}}{100 \times 2\ \ell\text{/day}} = 262.5\ \text{mg/}\ell \quad \text{(rounded to 300 mg/}\ell\text{)}$$

where:

750 mg/kg/day	=	NOAEL, based on the lack of kidney and body weight effects. The dose level of 750 mg/kg/day was chosen since the females were considered more sensitive than the males (Brown et al., 1978).
2 ℓ/day	=	assumed water consumption by an adult
100	=	uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from an animal study

The longer-term HA values for a 10 kg child and a 70 kg adult are 80 mg/ℓ and 300 mg/ℓ, respectively.

Assessment of Long-term Exposure Data and Derivation of a DWEL -- There are two possible long-term studies for derivation of a lifetime DWEL. The Brown et al. (1978) 16-week study as described for the longer-term HA is also considered in deriving the DWEL. In a 2-year dietary study, Food Research Laboratories, Inc. (1955) observed similar results at 5.0% DEP as in the Brown et al. (1978) study. They reported a NOEL at 2.5% or 1250 mg/kg/day. Deficiencies in the reporting of the study reduce confidence in the use of this data, since complete histopathologies were not conducted and no information was available on the number of rats surviving the 2-year study by Food Research Laboratories, Inc. (1955). Therefore, the NOAEL (750 mg/kg/day, females) determined from the Brown et al. (1978) study will be used to derive the lifetime DWEL (U.S. EPA, 1991).

Step 1 - RfD Derivation

$$RfD = \frac{750\ mg/kg/day}{1000} = 0.75\ mg/kg/day$$
(rounded to 0.8 mg/kg/day)

where:

750 mg/kg/day = NOAEL in orally exposed rats based on lack of kidney and weight gain effects (Brown et al., 1978)

1000 = uncertainty factor, according to U.S. EPA and ODW/NAS guidelines for use with a NOAEL from a subchronic animal study

Step 2 - DWEL Derivation

$$DWEL = \frac{0.8\ mg/kg/day \times 70\ kg}{2\ \ell/day} = 28\ mg/\ell$$ (rounded to 30 mg/ℓ)

where:

0.8 mg/kg/day = RfD

70 kg = assumed weight of an adult

2 ℓ/day = assumed water consumption by an adult

Studies Considered for Noncarcinogenic Quantification -- DMP. No information was available on the effects of DMP in humans. The only studies available on acute oral toxicity in animals used lethality as the toxic end-point. The only long-term oral data was from an unpublished review article (Lehman, 1955).

Quantification of Noncarcinogenic Effects -- DMP. The 1-day, 10-day or longer-term HAs and a lifetime DWEL for DMP cannot be derived due to insufficient information.

Carcinogenic Effects

There are very few animal carcinogenic studies on PAEs considering the number of esters. The available human studies are inadequate due to the small numbers of subjects studied and the lack of quantitative information on levels and duration of exposure. The human studies were designed to assess toxic effects caused by PAEs. However, there is adequate data to consider DEHP to be a Group B2 compound (i.e., probable human carcinogen) based on significant increases in liver tumor responses in rats and mice of both sexes. BBP has been classified as a Group C compound (i.e., possible human carcinogen) based on mononuclear cell leukemia in female rats. DBP, DEP and DMP are classified as Group D (i.e., not classifiable) since pertinent data regarding carcinogenicity was not located in the available literature (U.S. EPA, 1986; These classifications have all been verified by the CRAVE Work Group.

Studies Considered for Carcinogenic Quantification -- DEHP. In an NTP study (1982a), 50 male and 50 female Fisher 344 rats per group were fed 6000

or 12,000 ppm DEHP in the diet for 103 weeks. Similarly, groups of 50 male and 50 female B6C3F1 mice were given 3000 or 6000 ppm DEHP in the diet for 103 weeks. In this study rodent meal was provided in such a way that measured food consumption actually represented significant spillage and waste rather than true food intake. For this reason a standard food consumption rate of 13% of mouse and 5% of rat body weight was used in the dose conversion. Corresponding dose levels are 300 and 600 mg/kg/day for rats and 390 and 780 mg/kg/day for mice (low and high dose, respectively). Doses were those estimated to be maximally and one-half maximally tolerated in preliminary 90-day subchronic feeding studies. The animals were ~6 weeks old when the study began, and survivors were sacrificed at 105 weeks. All animals were necropsied, and a histologic examination of tissues was made. Treated animals were compared with 50 matched controls of each sex.

Median survival times were >104 weeks for all groups. Body weight loss was evident in low- or high-dose animals in each treatment group.

A statistically significant ($p<0.05$ or better) increase in the incidence of hepatic neoplasms in both rats and mice treated with DEHP was found as described in Table VIII-2. Hepatic tumors are described as hepatocellular carcinomas and neoplastic nodules in rats and hepatocellular carcinomas and hepatocellular adenomas in mice. Metastasis of liver carcinoma to the lung in mice was found in 5/50 high-dose males, 7/49 low-dose males, 7/50 high-dose females and 2/50 low-dose females (NTP, 1982a).

Carpenter et al. (1953) evaluated the chronic toxicity of DEHP in Sherman rats. The untreated control group and each treatment group con-

TABLE VIII-2

Results of a 2-Year Carcinogenicity Bioassay of DEHP in Rats and Mice[a]

Species	Sex	Types of Liver Tumor	Lifetime Dose (mg/kg/day)		Incidence[b]		
			Low-Dose Group	High-Dose Group	Control Group	Low-Dose Group	High-Dose Group
Rat	M	hepatocellular carcinomas	300	600	1/50	1/49 NS	5/49 NS
		hepatocellular carcinomas and neoplastic nodules			3/50	6/49 NS	12/49 p=0.01
	F	hepatocellular carcinomas	300	600	0/50	2/49 NS	8/50 p=0.003
		hepatocellular carcinomas and neoplastic nodules			0/50	6/49 p=0.012	13/50 p=0.001
Mouse	M	hepatocellular carcinomas	390	780	9/50	14/48 NS	19/50 p=0.022
		hepatocellular carcinomas and hepatocelluar adenomas			14/50	25/48 p=0.013	29/50 p=0.002
	F	hepatocellular carcinomas	390	780	0/50	7/50 p=0.006	17/50 p<0.001
		hepatocellular carcinomas and hepatocellular adenomas			1/50	12/50 p=0.001	18/50 p<0.001

[a]Source: NTP, 1982a

[b]One-tailed Fisher Exact Probability Test was used to compare the significance of tumors in treated groups with those in control groups.

NS = Not significant at the 0.05 level

sisted of 32 males and 32 female animals, which were 60 days old when treatment began. Dosage groups were given 400, 1300 or 4000 ppm DEHP in the diet for 1 year. After 1 year of treatment, each group was reduced to a maximum of eight males and eight females, and treatment was continued for another year until sacrifice of the survivors.

A filial (F_1) generation of animals was produced from animals in the control and 4000 ppm groups, which had been given the appropriate diet for 120 days. Each litter was reduced to two males and two females when the pups had reached 15 days of age, and 32 males and 32 females in the F_1 generation were assigned to each of the control and 4000 ppm groups. All surviving F_1 animals were sacrificed after being maintained on control or 4000 ppm diets for 1 year.

All animals were subjected to necropsy and histopathologic examination. Only 40-47% of the animals in each group, including F_1 animals, survived 1 year. Of the animals allowed to be on study for 2 years, 61-71% died before termination of the study at 2 years. Lung infection was diagnosed as the primary cause of death.

No malignant tumors were observed in this study. One to five rats in each group (males and females combined) had tumors with no treatment-related trend in evidence; however, the tumor types were not identified.

A carcinogenic effect of DEHP was not evident in this study. However, this study is weakened by the fact that of the 32 animals of each sex in each group of the study (excluding the F_1 animals, all of which were allowed to survive 1 year), only eight were allowed to survive beyond 1 year

of treatment. Furthermore, mortality was high with respect to all groups. Hence, an insufficient number of animals were available for a lifetime feeding study of DEHP carcinogenicity in rats.

Carpenter et al. (1953) also investigated the toxicity of DEHP in long-term studies in guinea pigs and dogs. Groups of 23 or 24 guinea pigs of each sex were fed 1300 or 4000 ppm DEHP in the diet for 1 year until termination of the study. Four dogs were dosed 5 days/week by oral administration of DEHP in capsules at a dosage of 0.03 mℓ/kg for the initial 19 doses followed by 240 doses at 0.06 mℓ/kg. The dogs were sacrificed at the end of the 1-year dosing period. Pathologic evaluation of the guinea pigs and dogs did not reveal a carcinogenic effect of DEHP. However, the treatment and survival periods for these animals were considerably below their lifetimes.

Since the animal evidence is considered by U.S. EPA to be sufficient and there is no human data, according to the U.S. EPA Guidelines for Carcinogen Risk Assessment, DEHP is classified as a B2 carcinogen (U.S. EPA, 1986). This classification was verified (10/07/87) by the CRAVE Work Group (U.S. EPA, 1991).

<u>Quantification of Carcinogenic Effects -- DEHP</u>. The risk calculation is based on the liver tumor data from the NTP study (1982a) on DEHP. Hepatocellular carcinoma and hepatocellular adenoma incidence were reported in both male and female rats and male and female mice. However, male mice were the most sensitive group. The combined incidence of hepatocellular carcinomas and adenomas in male mice (see Table VIII-2) was 14/50 for con-

trol animals, 25/48 for 390 mg/kg/day animals and 29/50 for 780 mg/kg/day animals.

NTP (1982a), Kluwe et al. (1982a), U.S. EPA (1987b) and IARC (1982) concluded that these results provide sufficient evidence of di(2-ethylhexyl) phthalate-induced carcinogenicity in rats and mice. This conclusion, however, is disputed. Northrup et al. (1982) claim that the NTP (1982a) results are equivocal since the MTD was exceeded in some treatment groups, incidences of liver tumors varied within different control groups of the same species and sex, and treated animals may have been malnourished. Northrup et al. (1982) also claimed that the rodent data cannot be used to predict carcinogenic risk in humans because DEHP is metabolized differently in rats than in humans. In response, Kluwe et al. (1983) noted that the MTD was not exceeded since there were no compound-related effects on survival, the incidence of liver tumors was increased in DEHP-treated animals regardless of the control data used and the differences in metabolism between rodents and humans would not affect the carcinogenic response in rodents. More recently, Turnbull and Rodricks (1985) concluded that using NTP (1982a) data to estimate DEHP-induced carcinogenic risk to humans will probably overestimate actual risk. This conclusion was based on the differences between rodents and primates in the metabolism of DEHP, a nonlinear relationship between the administered dose of DEHP to the dose of the "proximate carcinogenic species" in rodents, the fact that the "proximate carcinogenic species," which is hypothesized to induce cancer, is produced to a greater extent in rodents than in primates and that there are differences in target-site sensitivity between humans and rodents for liver tumors in general.

The dose-response data used in the potency calculations included rats with either hepatocellular carcinomas or neoplastic nodules and mice with either hepatocellular carcinomas or adenomas in the NTP (1982a) bioassay. Male and female response data from the rat and mouse were used to calculate a q_1^* value (Table VIII-3). The oral slope factors were 3.18×10^{-3} and 4.52×10^{-3} $(mg/kg/day)^{-1}$ for male and female rats, and 1.41×10^{-2} and 1.03×10^{-2} $(mg/kg/day)^{-1}$ for male and female mice. The value of 1.41×10^{-2} represents the most sensitive response and hence is selected as the potency value for DEHP. This value and the following risk/concentration calculations should be viewed as interim since it would appear that metabolism and pharmacokinetic considerations should be accounted for in the dose response analysis. The examination of these factors has been done by Turnbull and Rodricks (1985), but has not been further evaluated in this document. These refinements will be further evaluated before the risk values are put to final use. The upper-bound estimate of the cancer risk due to the ingestion of 2 ℓ of water for a 70-year lifetime with a concentration of contaminant is 4.0×10^{-7} $(\mu g/\ell)^{-1}$. Since risk is assumed to be linear with dose in this range, risk factors of 10^{-4}, 10^{-5} and 10^{-6} correspond to 300, 30 and 3 $\mu g/\ell$, respectively.

<u>Studies Considered for Carcinogenic Quantification -- BBP</u>. A bioassay was performed to evaluate the carcinogenic potential of BBP in rats and mice (Kluwe et al., 1982b; NTP, 1982b). Dietary levels of 6000 and 12,000 ppm (780 and 1560 mg/kg/day) BBP were each fed to groups of 50 male and 50 female F344 rats and 50 male and 50 female B6C3F1 mice. Untreated groups of 50 males and 50 females of each species were used as controls. The female

TABLE VIII-3

Cancer Risk Calculations

Animal/Sex	Animal Dose (mg/kg/day)		Human Equivalent (Dose mg/kg/day)		Human Potency Oral Slope Factor $(mg/kg/day)^{-1}$
	Low	High	Low	High	
Rat/male	300	600	52	100	3.18×10^{-3}
Rat/female	300	600	46	89	4.52×10^{-3}
Mouse/male	390	780	32	65	1.41×10^{-2}
Mouse/female	390	780	31	59	1.03×10^{-2}

rats and both sexes of mice were maintained on these diets for 103 weeks; however, ingestion rates and average weights were not available from the study. The male rats at both dose levels experienced high mortality within the first 30 weeks of the study, at which time the male rat study was terminated. No chronic or carcinogenic effects were observed in male or female mice. Among female rats, however, an increase in mononuclear cell leukemia was observed at the higher dose level.

Quantification of Carcinogenic Effects -- BBP. The available data meets the criteria for limited animal evidence based on mononuclear cell leukemia in female rats. Hence BBP is considered to be a Group C, possible human carcinogen according to U.S. EPA Guidelines for Carcinogen Risk Assessment. This classification has been verified (08/26/87) by the CRAVE Work Group (U.S. EPA, 1991). A bioassay was performed by the NTP (1982b) to evaluate the carcinogenicity of BBP in both rats and mice. The male rats at both dose levels experienced high mortality within the first 30 weeks of the study due to apparent internal hemorrhaging; all male rats were terminated at 30 weeks. Among female rats a statistically significant increase in mononuclear cell leukemia was observed at the high-dose level by comparison with both concurrent controls and historical controls. The conclusions reached by the peer review group of this study indicate that BBP was "probably" carcinogenic in female rats. Although the increase in leukemia was statistically significant, the biological relevance of this finding was questioned due to the background incidence of mononuclear cell leukemia in Fischer 344 rats. The NTP is currently repeating the rat portion of the cancer bioassay for BBP. Testing began in June, 1989 (NTP, 1991).

Studies Considered for Carcinogenic Quantification -- DBP. Pertinent data regarding the carcinogenicity of DBP could not be located in the available literature. According to U.S. EPA guidelines DBP is classified as Group D, not classifiable. This classification was verified (08/26/87) by the CRAVE Work Group (U.S. EPA, 1991).

Studies Considered for Carcinogenic Quantification -- DEP. Pertinent data regarding the carcinogenicity of DEP could not be located in the available literature. According to U.S. EPA guidelines DEP is classified as Group D, not classifiable. This classification was verified (08/26/87) by the CRAVE Work Group (U.S. EPA, 1991).

Studies Considered for Carcinogenic Quantification -- DMP. Pertinent data regarding the carcinogenicity of DMP could not be located in the available literature. According to U.S. EPA guidelines DMP is classified as Group D, not classifiable. This classification was verified (08/26/87) by the CRAVE Work Group (U.S. EPA, 1991).

Existing Criteria and Standards

The American Conference of Industrial Hygienists has set a TLV of 5 mg/m^3, as an 8-hour TWA, for DEHP, DBP, DEP and DMP (ACGIH, 1985).

The RfD Work Group verified the following RfDs: 0.02 mg/kg/day for DEHP (01/22/86); 0.2 mg/kg/day for BBP (06/15/89); 0.1 mg/kg/day for DBP (01/22/86); and 0.8 mg/kg/day for DEP (07/16/87). These assessments are all available on IRIS (U.S. EPA, 1991). Quantitative data are not available for DMP. The CRAVE Work Group has verified the following cancer classifica-

tions: Group B2 for DEHP (10/07/87); Group C for BBP (08/26/87); and Group D for DBP, DEP and DMP (08/26/87). The oral slope factor for DEHP, the only one of these five phthalates to have a quantitative cancer risk assessment, is 4×10^{-7} $(\mu g/\ell)^{-1}$. These assessments are also available on IRIS (U.S. EPA, 1991).

Interactions with Other Chemicals

PAEs have been shown to interact with other compounds in a synergistic or antagonistic manner. Carbon tetrachloride, barbiturates and organophosphate insecticides (applied following PAE exposure) were shown to act synergistically with PAEs (Seth et al., 1979; Rubin and Jaeger, 1973; Al-Badry and Knowles, 1980). Antagonistic effects were noted between PAEs (and testicular zinc levels), methylenedioxyphenyl compounds, paraoxon and simultaneously applied organophosphate insecticides (Cater et al., 1977; Foster et al., 1980; Melancon and Lech, 1979; Al-Badry and Knowles, 1980). DEHP has been shown to increase antipyrine metabolism in rats, possibly by inducing hepatic microsomal enzymes (Pollack and Shen, 1984). Interaction between DEHP and ethanol in rats has been studied by Agarwal et al. (1982a). DEHP produces changes in the pharmacologic response to ethanol by altering the activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Agarwal et al. (1982b) examined the effects of DEHP administration on phenobarbital-induced sleeping time in rats. The authors concluded that PAEs interfere with biotransformation mechanisms of hepatic microsomal drug-metabolizing enzymes. The effects of DEHP on the activity of various enzymes differed between oral and intraperitoneal exposure routes.

Special Groups at Risk

Patients receiving blood transfusions or hemodialysis constitute a high-risk subpopulation for PAE exposure. This group may receive excessive quantities of PAEs during transfusion or hemodialysis due to leaching of PAE plasticizers from plastic blood bags or plastic tubing.

Hillman et al. (1975) studied the occurrence of necrotizing enterocolitis and DEHP tissue concentrations in infants who had received treatment using arterial catheters containing DEHP. Higher DEHP content was found in catheterized infants with necrotizing enterocolitis than in infants that had been catheterized but did not develop this disease. While the study did not show a causal relationship, it did demonstrate that DEHP accumulated in the tissues of critically ill infants.

Gibson et al. (1976) estimated that the amount of DEHP delivered to a patient during hemodialysis ranged from 1.5-150 mg for dialysis lasting 15 minutes to 5 hours. Another study suggested that exposure to diethyl phthalate during hemodialysis may be linked to development of hepatitis (Neergaard et al., 1971). However, evidence of the causal relationship was not conclusive.

It is also possible that workers in the manufacture of PAEs or in the plastics industry constitute a high-risk population. However, little information is available for these groups. The only prospective cohort study looked at workers exposed to DEHP for periods of 3 months to 24 years. This study did not demonstrate any compound-related injury or disease. Therefore, the degree of risk to workers cannot be quantified (Thiess et al., 1978b).

9. REFERENCES

Abe, S. and M. Sasaki. 1977. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst. 58: 1635-1641.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. Documentation of the Threshold Limit Values and Biological Exposure Indices, 2nd ed. Cincinnati, OH.

Agarwal, D.K. 1986. Letters to the Editor. Toxicol. Appl. Pharmacol. 82: 383-385.

Agarwal, D.K., S. Agarwal and P.K. Seth. 1982a. Effect of di-(2-ethylhexyl) phthalate on drug metabolism, lipid peroxidation, and sulfhydryl content of rat liver. Drug Metab. Dispos. 10(1): 77-80.

Agarwal, D.K., S. Agarwal and P.K. Seth. 1982b. Interaction of di-(2-ethylhexyl) phthalate with the pharmacological response and metabolic aspects of ethanol in mice. Biochem. Pharmacol. 311(21): 3419-3423.

Agarwal, D.K., R.R. Maronpot, J.C. Lamb, IV and W.M. Kluwe. 1985a. Adverse effects of butylbenzyl phthalate on the reproductive and hematopoietic systems of male rats. Toxicology. 35: 189-206.

Agarwal, D.K., W.H. Lawrence and J. Autian. 1985b. Antifertility and mutagenic effects in mice from parenteral administration of di-2-ethylhexyl phthalate (DEHP). J. Toxicol. Environ. Health. 16: 71-84.

Agarwal, D.K., W.H. Lawrence, L. J. Nunez and J. Autian. 1985c. Mutagenicity evaluation of phthalic acid esters and metabolites in Salmonella typhimurium cultures. J. Environ. Health. 16: 61-69.

Agarwal, D.K., S. Eustis, J.C. Lamb IV, J.R. Reel and W.M. Kluwe. 1986. Effects of di(2-ethylhexyl)phthalate on the gonadal pathophysiology, sperm morphology and reproductive performance of male rats. Environ. Health Perspect. 65: 343-350.

Al-Badry, M.S. and C.D. Knowles. 1980. Phthalate-organophosphate interactions: Toxicity, penetration and metabolism studies with houseflies. Arch. Environ. Contam. Toxicol. 9: 147-161.

Albro, P.W. 1986. Absorption, metabolism and excretion of di(2-ethylhexyl) phthalate by rats and mice. Environ. Health Perspect. 65: 293-298.

Albro, P.W. and B. Moore. 1974. Identification of the metabolites of simple phthalate diesters in rat urine. J. Chromatogr. 94: 209-218.

Albro, P.W., R. Thomas and L. Fishbein. 1973. Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr. 76: 321-330.

Albro, P.W., J.T. Corbett, J.L. Schroeder, S. Jordan and H.B. Matthews. 1982. Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. Environ. Health Perspect. 45: 19-25.

Albro, P.W., J.T. Corbett, J.L. Schroeder and S.T. Jordan. 1983a. Incorporation of radioactivity from labeled di-(2-ethylhexyl) phthalate into DNA of rat liver in vivo. Chem.-Biol. Interact. 44: 1-16.

Albro, P.W., I. Tondeur, D. Marbury, S. Jordan, J. Schroeder and J.T. Corbett. 1983b. Polar metabolites of di-(2-ethylhexyl) phthalate in the rat. Biochem. Biophys. Acta. 760: 283-292

Aronson, C.E., E.R. Serlick and G. Preti. 1978. Effects of di-(2-ethylhexyl) phthalate on the isolated perfused rat heart. Toxicol. Appl. Pharmacol. 44: 155-169. (Cited in Bell, 1982).

Ashby, J., F.J. deSerres, M. Draper, et al. 1985. Overview and conclusions of the IPCS collaborative study on in vitro assay systems. In: Evaluation of Short-Term Tests for Carcinogens, J. Ashby, F.J. deSerres, M. Draper, et al., Ed. Elsevier Science Publishers, Amsterdam. p. 117-174.

Atlas, E., R. Foster and C.S. Giam. 1982. Air-sea exchange of high molecular weight organic pollutants: Laboratory studies. Environ. Sci. Technol. 16: 283-286.

Autian, J. 1973. Toxicity and health threats of phthalate esters: Review of the literature. Environ. Health Perspect. 3: 3-6.

Autian, J. 1982. Antifertility effects and dominant lethal assays for mutagenic effects of DEHP. Environ. Health Perspect. 45: 115-118.

Bell, F.P. 1982. Effects of phthalate esters on lipid metabolism in various tissues, cells and organelles in mammals. Environ. Health Perspect. 45: 41-50.

Bell, F.P. and D.A. Buthala. 1983. Biochemical changes in liver of rats fed the plasticizer di-(2-ethylhexyl) phthalate. Bull. Environm. Contam. Toxicol. 31: 177-182.

Bell, F.P. and D.J. Nazir. 1976. Effect of di-(2-ethylhexyl) phthalate on lipid biosynthesis in selected tissues from rat in vitro. Lipids. 11: 216-221. (Cited in Seth, 1982).

Bell, F.P., C.S. Patt, B. Brundage, P.J. Gillies and W.G. Phillips. 1977. Studies on lipid biosynthesis and cholesterol content of liver and serum lipoproteins in rats fed various phthalate esters. Lipids. 13: 66-74. (Cited in Seth, 1982).

Blickensdorfer, P. and L. Templeton. 1930. A study of the toxic properties of diethylphthalate. J. Am. Pharm. Assoc. 19: 1179-1181.

Bratt, H. and P. Batten. 1982. Imperial Chemical Industries PLC. (Cited in CPSC, 1985)

Brown, D., K.R. Butterworth, I.F. Gaunt, P. Grasso and S.D. Gangolli. 1978. Short-term oral toxicity study of diethylphthalate in the rat. Food Cosmet. Toxicol. 16: 415-422.

Callahan, M.A., M.W. Slimak, N.W. Gabel, et al. 1979. Fate of 129 Priority Pollutants Vol. II. Office of Water Planning and Standards, U.S. EPA, Washington, DC. (December). p. (94)1-28.

Calley, D., J. Autian and W. L. Guess. 1966. Toxicology of a series of phthalate esters. J. Pharmacol. Sci. 55: 158-162. (Cited in Gangolli, 1982; Seth, 1982).

Carpenter, C.P., C.S. Weil and H.F. Smith, Jr. 1953. Chronic oral toxicity of di-(2-ethylhexyl) phthalate for rats, guinea pigs and dogs. AMA Arch. Ind. Hyg. Occup. Med. 8: 219-226.

Cater, B.R., M.W. Cook, S.D. Gangolli and P. Grasso. 1977. Studies on dibutyl phthalate-induced testicular atrophy in the rat: Effect on zinc metabolism. Toxicol. Appl. Pharmacol. 41: 609-618.

Christian, M.S., Ed. 1985. Final report on the safety assessment of dibutyl phthalate, dimethyl phthalate and diethyl phthalate. J. Am. College Toxicol. 4(3): 267-303.

CMA (Chemical Manufacturing Association). 1985. A 21-day feeding study of butyl benzyl phthalate to rats: Effects on the liver and liver lipids. Report No. 0495/1/84.

CMA (Chemical Manufacturing Asssociation. 1986. A 21-day feeding study of di-N-butyl phthalate to rats: Effects on the liver and liver lipids. Report No. 0495/3/85.

Cohen, A.J. and P. Grasso. 1981. Review of the hepatic response to hydrolipidaemic drugs in rodents and assessment of its toxicological significance to man. Food Cosmet. Toxicol. 19: 585-605.

Corcoran, E.F. 1973. Gas-chromatographic detection of phthalic acid esters. Environ. Health Perspect. 3: 13-15.

Coward, W.A., J.Mc.C. Howell, G.A.J. Pitt and J.N. Thompson. 1966. Effect of hormones on reproduction in rats fed a diet deficient in retinol (vitamin A alcohol) but containing methyl retinoate (vitamin A acid methyl ester). J. Reprod. Fertil. 12: 309-317.

CPSC (Consumer Products Safety Commission). 1983. Children's Chemical Hazards Risk Assessment on Di(2-Ethylhexyl) Phthalate in Children's Products. Chemical Hazards Program Directorate for Health Sciences. August.

CPSC (Consumer Products Safety Commission). 1985. U.S. Consumer Product Safety Commission. Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on di(2-ethylhexyl)phthalate (DEHP).

Curto, K.A. 1984. Effects of di-(2-ethylhexyl) phthalate and monoethylhexyl phthalate on male rodent gonad. Dissert. Abst. Int. B. 45(1): 146.

Daniel, J.W. and H. Bratt. 1974. The absorption, metabolism and tissue distribution of di-(2-ethylhexyl) phthalate in rats. Toxicol. 2: 51-65. (Cited in Seth, 1982).

Darby, J. and J. Sears. 1969. Plasticizers. In: Encyclopedia of Polymer Science Technology, Vol. 10, John Wiley and Sons, New York. p. 228-306.

Dean, Ed. 1979. Lange's Handbook of Chemistry, 12th ed. McGraw-Hill Book Co., New York.

Deangelo, A.B. and C.T. Garrett. 1983. Inhibition of development of preneoplastic lesions in the livers of rats fed a weakly carcinogenic environmental contaminant. Cancer Lett. 20: 199-205.

Deangelo, A.B., C.T. Garrett, L.A. Manolukas and T. Yario. 1986. Di-n-octyl phthalate (DOP), a relatively ineffective peroxisome inducing straight chain isomer of the environmental contaminant di(2-ethylhexyl) phthalate (DEHP), enhances the development of putative preneoplastic lesions in rat liver. Toxicology. 41: 279-288.

DeHaan, R.L. 1971. Toxicity of tissue culture media exposed to polyvinylchloride plastic. Nature. 231: 85-86. (Cited in Bell, 1982)

Dilling, W.L. 1977. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11(4): 405-410.

Dillingham, E.O. and J. Autian. 1973. Teratogenicity, mutagenicity and cellular toxicity of phthalate esters. Environ. Health Perspect. January. p. 81-89.

Dostal, L.A., W.L. Jenkins and B.A. Schwetz. 1987a. Hepatic peroxisome proliferation and hypolipidemic effects of di(2-ethylhexyl) phthalate in neonatal and adult rats. Toxicol. Appl. Pharmacol. 87:81-90.

Dostal, L.A., R.P. Weaver and B.A. Schwetz. 1987b. Transfer of di(2-ethylhexyl)phthalate through rat milk and effects on milk composition and mammary gland. Toxicol. Appl. Pharmacol. 91: 315-325.

Douglas, G.R., A.P. Hugenholtz and D.H. Blakey. 1986. Genetic toxicology of phthalate esters: Mutagenic and other genotoxic effects. Environ. Health Perspect. 65: 255-262.

Dvoskin, I.A.G., N.A. Rokhmanina, F.V. Demiyauko, T.A. Menshikova, L.F. Ervreva and A.V. Lashkina. 1969. Hygienic assessment of certain polymers (provinols). Gig. Sanit. Jan: 34(1): 7-11. (Translated title) (Ger.)

Eigenberg, D.A., H.P. Bozigian, D.E. Carter and I.G. Sipes. 1986. Distribution, excretion and metabolism of butylbenzl phthalate in the rat. J. Toxicol. Environ. Health. 17: 445-456.

Ekwall, B., C. Nordensten and L. Albanus. 1982. Toxicity of 29 plasticizers to HeLa cells in the MIT-24 system. Toxicology. 24: 199-210.

Engelhardt, G. and P.R. Wallnofer. 1978. Metabolism of di- and mono-n-butyl phthalate by soil bacteria. Appl. Environ. Microbial. 35: 243-246.

Engelhardt, G., P.R. Wallnoeter and O. Hutzinger. 1975. Microbial metabolism of dibutyl phthalate and related dialkyl phthalates. Bull. Environ. Contam. Toxicol. 13: 342-347.

Engelhardt, G., G. Tillmanns, P.R. Wallnöfer and O. Hutzinger. 1977. Biodegradation of di-isobutyl phthalate and related dialkyl phthalates by Penicillium lilacinum. Chemosphere. 6: 347-354.

Ewing, B. and E. Chian. 1977. Monitoring to detect previously unrecognized pollutants in surface waters. Office of Toxic Substances, U.S. EPA, Washington, DC. EPA 560/7-77/15a.

Food Research Laboratories, Inc. 1955. Data submitted to U.S. FDA by Celanese Corporation of America. Report No. 67567.

Foster, P.M., L.V. Thomas, M.W. Cook and S.D. Gangolli. 1980. Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rats. Toxicol. Appl. Pharmacol. 54: 392-398.

Foster, P.M.D., M.W. Cook, L.V. Thomas, et al. 1982. Differences in urinary metabolic profile from di-n-butyl phthalate-treated rats and hamsters. Drug Metab. Dispos. 11(1): 59-61.

Gangolli, S.D. 1982. Testicular effects of phthalate esters. Environ. Health Perspect. 45: 77-84.

Ganning, A.E., U. Brunk and R. Nilsson. 1981. Induction of mitochondria and peroxisomes with phthalate esters. Biochem. Soc. Trans. 9: 251P.

Ganning, A.E., U. Brunk and G. Dallner. 1983. Effects of dietary di-(2-ethylhexyl) phthalate on the structure and function of rat hepatocytes. Biochem. Biophys. Acta. 763: 72-82.

Ganning, A.E., U. Brunk and G. Dallner. 1984. Phthalate esters and their effect on the liver. Hepatology. 4(3): 541-547.

Garvey, L.K., J.A. Swenberg, T.E. Hamm, Jr. and J.A. Popp. 1987. Di(2-ethylhexyl)phthalate: Lack of initiating activity in the liver of female F-344 rats. Carcinogenesis. 8(2): 285-290.

Giam, C.S., H.S. Chan, G.S. Neff and E.L. Atlas. 1978. Phthalate ester plasticizers: A new class of marine pollutants. Science. 199: 419-421.

Gibson, T.P., W.A. Briggs and B.J. Boone. 1976. Delivery of di-2-ethylhexyl phthalate to patients during hemodialysis. J. Lab. Clin. Med. 87(3): 519-524.

Gledhill, W.E., R.G. Kaley, W.J. Adams, et al. 1980. An environmental safety assessment of butyl benzyl phthalate. Environ. Sci. Technol. 14: 301-305.

Gleiberman, S.E., I.A. Kotova, G.M. Nikolaev and V.V. Yurchenko. 1978. Pharmacokinetics of dimethyl phthalate. Med. Parazitol. Parazit. Bolezni. 47(3): 58-63. (CA90: 80639m)

Gollamudi, R., H.R. Prasanna, R.H. Rao, et al. 1983. Impaired metabolism of di-(2-ethylhexyl) phthalate (DEHP) in old rats--an in vitro study. J. Toxicol. Environ. Health. 12: 623-632.

Gollamudi, R., R.H. Rao, W.H. Lawrence and J. Autian. 1985. Developmental changes in the conversion rates of di(2-ethylhexyl) phthalate to monoethylhexyl phthalate in rats. J. Toxicol. Environ. Health. 15: 459-465.

Graham, P.R. 1973. Phthalate ester plasticizers - why and how they are used. Environ. Health Perspect. 3: 3-12.

Gray, T.J.B. n.d. No title provided. Unpublished work. (Cited in Foster et al., 1982)

Gray, T.J.B. and J.A. Beamand. 1984. Effect of some phthalate esters and other testicular toxins on primary cultures of testicular cells. Food Chem. Toxic. 22(2): 123-131.

Gray, T.J.B. and S.D. Gangolli. 1986. Aspects of the testicular toxicity of phthalate esters. Environ. Health Perspect. 65: 229-235.

Gray, T.J.B., K.R. Butterworth, I.F. Gaunt, P. Grasso and S.D. Gangolli. 1977. Short-term toxicity study of di-(2-ethylhexyl) phthalate in rats. Food Cosmet. Toxicol. 15: 389-399. (Cited in Gangolli, 1982; Seth, 1982).

Gray, T.J.B., I.R. Rowland, P.M.D. Foster and S.D. Gangolli. 1982. Species differences in the testicular toxicity of phthalate esters. Toxicol. Lett. 11: 141-147.

Gray, T.J.B., B.G. Lake, J.A. Beamand, et al. 1983. Peroxisomal effects of phthalate esters in primary cultures of rat hepatocytes. Toxicology. 28: 167-179.

Gunn, S.A. and T.C. Gould. 1970. Cadmium and other mineral elements. In: The Testis, R. D. Johnson, W. R. Gomes and N. L. Vandenmark, Eds., Vol. 3, Academic Press, New York. p. 377-481. (Cited in Gangolli, 1982).

Hamano, Y., A. Kuwano, K. Inove et al. 1977. Studies on toxicity of phthalic acid esters. Part I. Teratogenic effects in mice when administered orally. Osaka Furitsu Koshu Eisei Kenkyusho Kenkyu hokoku, Shokuhin Eisei Hen. 8: 123-124. (Abstract)

Harris, R.S., H.C. Hodge, E.A. Maynard and H.J. Blanchet, Jr. 1956. Chronic toxicity of 2-ethylhexyl phthalate in rats and dogs. Am. Med. Assoc. Arch. Ind. Health. 13: 259-264.

Hattori, Y., Y. Kuge and S. Nakagawa. 1975. Microbial decomposition of phthalate esters in environmental water. Pollut. Control Cent. Osaka Prefect. Mizu Shori Gijutsu. 16: 951-954.

Hawley, G.G., Ed. 1981. The Condensed Chemical Dictionary, 10th ed. Van Nostrand Reinhold Co., New York.

Hillman, L.S., S.L. Goodwin and W.R. Sherman. 1975. Identification and measurement of plasticizer in neonatal tissue after umbilical catheters and blood products. New Engl. J. Med. 292(8): 381-386.

Hites, R. 1973. Phthalates in the Charles and Merrimack Rivers. Environ. Health Perspect. 3: 17-21.

Hites, R. and K. Bieman. 1972. Water pollution: Organic compounds in the Charles River, Boston. Science. 178: 158.

Hodgson, J.R., B.C. Myhr, M. McKeon and D.J. Brusick. 1982. Evaluation of di-(2-ethylexyl) phthalate and its major metabolites in the primary rat hepatocyte unscheduled DNA synthesis assay. Environ. Mutagen. 4: 388.

Hopkins, J. 1983. Is diethylhexyl phthalate genotoxic? Food Chem. Toxicol. 21(5): 684-687.

Horowitz, A., D.R. Shelton, C.P. Cornell and J.M. Tiedje. 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Ind. Microbiol. 23: 435-444.

Howard, P.H., S. Banerjee and K.H. Robillard. 1985. Measurement of water solubilities, octanol/water partition coefficients and vapor pressures of commercial phthalate esters. Environ. Toxicol. Chem. 4: 653-661.

Huff, J.E. and W.M. Kluwe. 1984. Phthalate esters carcinogenicity in F344/N rats and $B6C3F_1$ mice. Prog. Clin. Biol. Res. 141: 137-154.

IARC (International Agency for Research on Cancer). 1982. IARC Monographs on the evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs. Volume 29. p. 193-201, 269-275.

Ishidate, M., Jr. and S. Odashima. 1977. Chromosome tests with 134 compounds on Chinese hamster cells in vitro -- A screening test for chemical carcinogens. Mutat. Res. 48: 337-354.

Ishikawa, Y., K. Honda, S. Sasakawa, K. Hatada and H. Kobayashi. 1983. Prevention of leakage of di-(2-ethylhexyl) phthalate from blood bags by glow discharge treatment and its effect on aggregability of stored platelets. Vox Sang. 45: 68-76.

Jacobson, M.S., R. Parkman, L.N. Button, R.J. Yaeger and S.V. Kevy. 1974. The toxicity of human serum stored in flexible polyvinylchloride containers on human fibroblast cell cultures: An effect of di-(2-ethylhexyl) phthalate. Res. Comm. Chem. Pathol. Pharmacol. 9(2): 315-323.

Jacobson, M.S., S.V. Kevy and R.J. Grand. 1977. Effects of a plasticizer leached from polyvinyl chloride on the subhuman primate: A consequence of chronic transfusion therapy. J. Lab. Clin. Med. 89: 1066-1079. (Cited in Seth, 1982).

Jaeger, R.J. and R.J. Rubin. 1970. Plasticizers from plastic devices: Extraction, metabolism and accumulation by biological systems. Science. 170: 460-462.

Jaeger, R.J. and R.J. Rubin. 1972. Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its location in human tissues. New Engl. J. Med. 287: 1114-1118.

Jaeger, R.J. and R.J. Rubin. 1973. Di-(2-ethylhexyl) phthalate, a plasticizer contaminant of platelet concentrates. Transfusion. 13(2): 107-108.

Johnson, E.M. and B.E.G. Gabel. 1983. An artificial "embryo" for detection of abnormal developmental biology. Fundam. Appl. Toxicol. 3: 243-249.

Johnson, B.T. and W. Lulves. 1975. Biodegradation of di-n-butyl phthalate and di-2-ethylhexyl phthalate in freshwater hydrosoil. J. Fish Res. Board Can. 32(3): 333-340.

Johnson, B.T., M.A. Heitkamp and J.R. Jones. 1984. Environmental and chemical factors influencing the biodegradation of phthalic-acid esters in freshwater sediments. Environ. Pollut. Ser. B. Chem. Phys. 8(2): 101-118.

Jones, A.E., R.H. Kahn, J.T. Groves and E.A. Napier, Jr. 1975. Phthalate ester toxicity in human cell cultures. Toxicol. Appl. Pharmacol. 31: 283-289.

Kaneshima, H., T. Yamaguchi, T. Okui and M. Naitoh. 1978. Studies on the effects of phthalate esters on the biological system (Part 2) - in vitro metabolism and biliary excretion of phthalate esters in rats. Bull. Environ. Contam. Toxicol. 19: 502-509.

Katoh, H., S. Nakajima, Y. Kawashima, et al. 1984. Induction of rat hepatic long-chain acyl-CoA hydrolases by various peroxisome proliferators. Biochem. Pharmacol. 33(7): 1081-1085.

Kevy, S.V., L.N. Button and M.S. Jacobson. 1978. Toxicology of plastic devices having contact with blood. Rep. No. 1 HB 5-2906, National Heart, Lung and Blood Institute, Bethesda, Maryland.

Khan, S.U. 1980. Determining the role of humic substances in the fate of pesticides in the environment. International Symposium on Hazards of Pesticides to the Environment and Human Health, Alexandria, Egypt, Nov. 1-3, 1978. J. Environ. Sci. Health Pestic. Food Contam. Agric. Wastes. 15(6): 1071-1090.

Khawaja, J. and G. Dallner. 1982. Oral administration of di-(2-ethylhexyl) phthalate reduces liver protein synthesis in vivo. IRCS Med. Sci. 10(8): 639.

Kihlström, I. 1983. Placental transfer of diethylhexyl phthalate in the guinea pig placenta perfused in situ. Acta Pharmacol. Toxicol. 53: 23-27.

Kirby, P.E., R.F. Pizzarello, T.E. Lawlor, S.R. Haworth and J.R. Hodgson. 1983. Evaluation of di-(2-ethylhexyl) phthalate and its major metabolites in the Ames test and L51784 mouse lymphoma mutagenicity assay. Environ. Mutagen. 5(5): 657-664.

Klausmeier, R.E. and W.A. Jones. 1960. Microbial degradation of plasticizers. Develop. Ind. Microbial. 2: 47-53.

Kluwe, W.M. 1982a. Overview of phthalate ester pharmacokinetics in mammalian species. Environ. Health Perspect. 45: 3-10.

Kluwe, W.M. 1982b. Introduction. Environ. Health Perspect. 45: 1.

Kluwe, W.M. 1986. Carcinogenic potential of phthalic acid esters and related compounds: Structure-activity relationships. Environ. Health Perspect. 65: 271-278.

Kluwe, W.M., J.K. Haseman, J.F. Douglas and J.E. Huff. 1982a. The carcinogenicity of dietary di-(2-ethylhexyl) phthalate (DEHP) in Fischer 344 rats and $B6C3F_1$ mice. J. Toxicol. Environ. Health. 10(4-5): 797-815.

Kluwe, W.M., E.E. McConnell, J.E. Huff, et al. 1982b. Carcinogenicity testing of phthalate esters and related compounds by the National Toxicology Program and the National Cancer Institute. Environ. Health Perspect. 45: 129-133.

Kluwe, W.M., J.K. Haseman and J.E. Huff. 1983. The carcinogenicity of di-(2-ethylhexyl) phthalate (DEHP) in perspective. J. Toxicol. Environ. Health. 12: 159-169.

Komarova, E.N. 1979. Materials on the toxicology of dibutyl phthalate, dioctyl phthalate, dibutyl sebacate and butyl stearate. Tokasikol. Sanit. Khim. Plastmass. 3: 12-15 (translated).

Komitowski, D., P. Schmezer, B. Schmitt, V. Ehemann and S. Muto. 1986. Quantitative analysis of the early changes of hepatocyte nuclei after treating Syrian golden hamsters with di(2-ethylhexyl) phthalate. Cancer Res. Clin. Oncol. 111: 103-107.

Kozumbo, W.J., R. Kroll and R.J. Rubin. 1982. Assessment of the mutagenicity of phthalate esters. Environ. Health Perspect. 45: 103-109.

Krauskopf, L.G. 1973. Studies on the toxicity of phthalates via ingestion. Environ. Health Perspect. 3: 61-72.

Kurane, R., T. Suzuki and Y. Takahara. 1979a. Microbial degradation of phthalate esters. IV. Removal of phthalate esters by activated sludge inoculated with a strain of Nocardia erythropolis. Agric. Biol. Chem. 43: 421-427.

Kurane, R., T. Suzuki and Y. Takahara. 1979b. Microbial population and identification of phthalate ester-utilizing microorganisms in activated sludge inoculated with microorganisms. Agri. Biol. Chem. 43: 907-917.

Lake, B.G., S.D. Gangolli, P. Grasso and S. Lloyd. 1975. Studies on the hepatic effects of orally administered di-(2-ethylhexyl) phthalate in the rat. Toxicol. Appl. Pharmacol. 32: 355-367. (Cited in Seth, 1982).

Lake, B.G., P.G. Brantum, S.D. Gangolli, K.R. Butterworth and P. Grasso. 1976. Studies on the effects of orally admistered di-(2-ethylhexyl) phthalate in the ferret. Toxicol. 6: 341-356. (Cited in Seth, 1982).

Lake, B.G., J.C. Philips, J.C. Linnell and S.D. Gangolli. 1977. The in vitro hydrolysis of some phthalate esters by hepatic and intestinal preparations from various species. Toxicol. Appl. Pharmacol. 39: 239-248.

Lake, B.G., R.A. Harris, P. Grasso and S.D. Gangollia. 1978. Studies on the metabolism and biological effects of n-butyl benzyl phthalate in the rat. Prepared by British Industrial Biological Research Association for Monsanto, Report No. 232/78, June 1978.

Lake, B.G., T.J.B. Gray, J.R. Foster, et al. 1984a. Comparative studies on di-(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation in the rat and hamster. Toxicol. Appl. Pharmacol. 72: 46-60.

Lake, B.G., W.R. Pels Rijcken, T.J.B. Gray, et al. 1984b. Comparative studies of the hepatic effects of di- and mono-n-octyl phthalates, di-(2-ethylhexyl) phthalate and chlofibrate in the rat. Acta Pharmacol. Toxicol. 54: 167-176.

Lake, B.G., T. Gray and S.D. Gangolli. 1986. Hepatic effects of phthalate esters and related compounds - in vivo and in vitro correlations. Environ. Health Perspect. 67: 283-290.

Lamb, J.C., IV, R.E. Chapin, J. Teague, A.D. Lawton and J.R. Reel. 1987. Reproductive effects of four phthalic acid esters in the mouse. Toxicol. Appl. Pharmacol. 88(2): 255-269.

Lawrence, W.H. and S.F. Tuell. 1979. Phthalate esters: The question of safety - an update. Clin. Toxicol. 15(4): 447-466.

Lawrence, W.H., M. Malik, L.C. Turner, A.R. Singh and J. Autian. 1975. A toxicological investigation of some acute, short-term and chronic effects of administering di-(2-ethylhexyl) phthalate (DEHP) and other phthalate esters. Environ. Res. 9: 1-11.

Leah, T.D. 1977. Environmental Contaminants Inventory Study No. 4: The Production, Use and Distribution of Phthalic Acid Esters in Canada. Report Ser. No. 47. Inland Waters Directorate, Ontario Region, Water Planning and Management Branch, Burlington, Ontario.

Lefaux, R. 1968. Practical Toxicology of Plastics, CRC Press, Chemical Rubber Co., Cleveland, Ohio. (Cited in Krauskopf, 1973).

Lehman, A.J. 1955. Insect repellents. Food Drug Office Q. Bull. 19: 87-99.

Lehman, A.J. 1959. Appraisal of the safety of chemicals in foods, drugs and cosmetics. Assoc. Food Drug Officials United States

Lewandowski, M., J. Fernandes and T.S. Chen. 1980. Assessment of the teratogenic potential of plasma-soluble extracts of diethylhexyl phthalate plasticized polyvinyl chloride plastics in rats. Toxicol. Appl. Pharmacol. 54: 141-147.

Lhuguenot, J.C., A.M. Mitchell, G. Milner, E.A. Lock and C.R. Elcombe. 1985. The metabolism of di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate (MEHP) in rats: In vivo and in vitro dose and time dependency of metabolism. Toxicol. Appl. Pharmacol. 80: 11-22.

Lindgren, A., N.G. Lindquist, A. Lyden, et al. 1982. A whole body autoradiographic study on the distribution of ^{14}C-labelled di-(2-ethylhexyl) phthalate in mice. Toxicology. 23: 149-158.

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co., New York. p. 4-9, 15-16.

Mabey, W.R., J.H. Smith, R.T. Podoll, et al. 1982. Aquatic fate process data for organic priority pollutants. Prepared by SRI International for U.S. EPA, Office of Water Regulations and Standards, Washington, DC. EPA 440/4-81-014.

Mallette, F.S. and E. Von Haam. 1952. Studies on the toxicity and skin effects of compounds used in the rubber and plastics industries. I. Plasticizers. Arch. Ind. Hyg. Occup. Med. 6: 231-236.

Mangham, B.A., J.R. Foster and B.G. Lake. 1981. Comparison of the hepatic and testicular effects of orally administered di-(2-ethylhexyl) phthalate and dialkyl 79 phthalate in the rat. Toxicol. Appl. Pharmacol. 61: 205-214.

Marcel, Y.L. 1973. Determination of di-(2-ethylhexyl) phthalate in human blood plasma and cryoprecipitates. Environ. Health Perspect. 3: 119-121. (Cited in Pollack et al., 1985b)

Marcel, Y.L. and S.P. Noel. 1970. A plasticizer in lipid extracts of human blood. Chem. Phys. Lipids. 4: 418-419.

Mark, H.F., D.F. Othmer, C.G. Overberger, G.T. Seaborg, Ed. 1982. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 17. Wiley Interscience Publication, John Wiley and Sons. p. 745.

Mason, K.E. 1933. Differences in testes injury and repair after vitamin A deficiency, vitamin E deficiency and inanition. Am. J. Anat. 52: 153-239.

Mathur, S.P. 1974. Phthalate esters in the environment: Pollutants or natural products? J. Environ. Qual. 3: 189-197.

Matsuda, K. and M. Schnitzer. 1971. Reactions between fulvic acid, a soil humic material and dialkyl phthalates. Bull. Environ. Contam. Toxicol. 6: 200-204.

Mayer, F.L. 1976. Residue dynamics of di-(2-ethylhexyl) phthalate in fathead minnows (Pimephales promelas). J. Fish. Res. Board Can. 33: 2610-2613.

Melancon, M.J., Jr. and J.J. Lech. 1979. Structural requirements for the inhibition of phthalate ester hydrolysis in rainbow trout by methylenedioxyphenyl compounds. Xenobiotica. 9: 317-322.

Melnick, R.L. and C.M. Schiller. 1982. Mitochondrial toxicity of phthalate esters. Environ. Health Perspect. 45: 51-56.

Men'shikova, T.A. 1971. Hygienic evaluation of dibutyl phthalate in relation to the use of polymeric materials for finishing living quarters on ships. Gig. Sanit. 36: 23-27 (Translation)

Milkov, L.E., M.V. Aldyreva, T.B. Popova, et al. 1973. Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins. Environ. Health Perspect. 3: 175-178.

Mitchell, F.E., S.C. Price, R.H. Hinton, P. Grasso and J.W. Bridges. 1985. Time and dose-response study of the effects on rats of the plasticizer di(2-ethylhexyl) phthalate. Toxicol. Appl. Pharmacol. 81: 371-392.

Monsanto. 1972. Unpublished work. (Cited in Krauskopf, 1973)

Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips and D.A. Hungerford. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20(3): 613-616. (Cited in Thiess and Fleig, 1979)

Murakami, K. and K. Nishiyama. 1986. Toxicity of dibutyl phthalate and its metabolites in rats. Jap. J. Hyg. 41(4): 775-780.

Mushtaq, M. and K.K. Datta. 1981. Effect of di-2-ethylhexyl phthalate on rat testis. Indian J. Biochem. Biophys. 18(4,Suppl.): 159. (Abstract)

Nair, N. and C.K.R. Kurup. 1986. A comparative study of the effects of administration of diethylhexyl phthalate on hepatic mitochondria of the rat and the mouse. Indian J. Biochem. Biophys. 23: 270-273.

NAS (National Academy of Science). 1977. Drinking Water and Health. National Academy Press, Washington, DC. Vol. I. p. 19-63.

NAS (National Academy of Science). 1980. Drinking Water and Health. National Academy Press, Washington, DC. Vol. 3. p. 25-67.

Nazir, D., A.P. Alcaraz, B.A. Bieri, M. Beroza and P.P. Nair. 1971. Isolation, identification and specific localization of di-(2-ethylhexyl) phthalate in bovine heart muscle mitochondria. Biochemistry. 10: 4425-4429.

Neergaard, J., B. Nielsen, V. Faurby, D.H. Christensen and O.F. Nielsen. 1971. Plasticizers in PVC and the occurence of hepatitis in a hemodialysis unit. Scand. J. Urol. Nephrol. 5: 141-145.

Nikonorow, M., H. Mazur and H. Piekacz. 1973. Effect of orally administered plasticizers and polyvinyl chloride stabilizers in the rat. Toxicol. Appl. Pharmacol. 26: 253-259. (Cited in Seth, 1982)

NIOSH (National Institute for Occupational Safety and Health). 1985. RTECS (Registry of Toxic Effects of Chemical Substances) master file listing as of March, 1984. U.S. Department of Health and Human Services, Public Health Services, Center of Disease Control, NIOSH, Cincinnati, OH.

Northup, S., L. Martis, R. Ulbricht, et al. 1982. Comment on the carcinogenic potential of di-(2-ethylhexyl) phthalate. J. Toxicol. Environ. Health. 10: 493-518.

NTP (National Toxicology Program). 1982a. Carcinoginesis Bioassay of di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study). NTP Tech. Rep. NIN/PUB-82-1773, NTP-80-37. NTIS PB82-184011.

NTP (National Toxicology Program). 1982b. Carcinogenesis Bioassay of Butylbenzylphthalate (CAS No. 85-68-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study). NTP Tech. Rep. NTIS PB83-118398, Research Triangle Park, NC. 98 p.

NTP (National Toxicology Program). 1984a. Di-(2-ethylhexyl) phthalate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. Final Report. NTP, Research Triangle Park, NC.

NTP (National Toxicology Program). 1984b. Di(n-Butyl)Phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed. Final Report. NTP, Research Triangle Park, NC.

NTP (National Toxicology Program). 1984c. Diethylphthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed. Final Report. NTP, Research Triangle Park, NC.

NTP (National Toxicology Program). 1985. Twenty-six week subchronic study and modified mating trial in F344 rats. Butylbenzylphthalate. Final report. Project No. 12307-02, -03. Hazelton Laboratories America, Inc. Unpublished report.

NTP (National Toxicology Program). 1986. Addendum to final report. Bone marrow differential results -- 26-week study. LBI/HLA Project No. 12307-02. Hazelton Laboratories America, Inc. Unpublished report.

NTP (National Toxicology Program). 1991. Chemical Status Report. 07/09/91.

Ogner, G. and M. Schnitzer. 1970. Humic substances: Fulvic acid-dialkyl phthalate complexes and their role in pollution. Science. 170: 317-318.

O'Grady, D.P., P.H. Howard and A.F. Werner. 1985. Activated sludge biodegradation of 12 commercial phthalate esters. Appl. Environ. Microbiol. 49(2): 443-445.

Ohta, Y. and M. Nakamoto. 1979. Metabolism of di-n-butyl phthalate by Aeromonas sp. Hakkokogaku. 57: 50-53.

Oishi, S. 1984a. Effects of di-2-ethylhexyl phthalate on lipid composition of serum and testis in rats. Toxicol. Lett. 23: 67-72.

Oishi, S. 1984b. Testicular atrophy of rats induced by di-(2-ethylhexyl) phthalate: Effects of vitamin A and zinc concentrations in the testis, liver and serum. Toxicol. Lett. 20: 75-78.

Oishi, S. 1986. Testicular atrophy induced by di(2-ethylhexyl)phthalate: Changes in histology, cell specific enzyme activities and zinc concentrations in rat testes. Arch. Toxicol. 59:290-295.

Oishi, S. and K. Hiraga. 1982. Distribution and elimination of di-(2-ethylhexyl) phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) after a single oral administration of DEHP in rats. Arch. Toxicol. 51: 149-155.

Oishi, S. and K. Hiraga. 1983. Effects of di-(2-ethylhexyl) phthalate on lipid composition of liver, testis and serum of male rats. Jpn J. Pharm. 33(Suppl.): 153. (Abstract)

Okita, R. and C. Chance. 1984. Induction of laurate ω-hydroxylase by di-(2-ethylhexyl) phthalate in rat liver microsomes. Biochem. Biophys. Res. Comm. 121(1): 304-309.

Osumi, T. and T. Hashimoto. 1978. Enhancement of fatty acyl-CoA oxidizing activity in rat peroxisomes by di-(2-ethylhexyl) phthalate. J. Biochem. 83: 1361-1365. (Cited in Seth, 1982).

Parmar, D., S.P. Srivastava, S.P. Srivastava and P.K. Seth. 1985. Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to di-(2-ethylhexyl)phthalate through mother's milk. Drug. Metab. Dispos. 13(3): 368-370.

Parmar, D., S.P. Srivastowa and P.K. Seth. 1986. Effect of di(2-ethylhexyl)phthalate (DEHP) on spermatogenesis in adult rats. Toxicology. 42: 47-55.

Peakall, D.B. 1975. Phthalate esters: Occurrence and biological effects. Residue Rev. 54: 1-41.

Peck, C.C. and P.W. Albro. 1982. Toxic potential of the plasticizer di-(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. Environ. Health Perspect. 45: 11-17.

Peck, C.C., D.G. Odom, H.I. Friedman. 1979. Di-(2-ethylhexyl) phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion. 19: 137-146.

Perez, J.A., M.A. Hernandex, R.A. Ruiz and P.J. Brown. 1977. The utilization of the plasticizer dimethyl phthalate by an isolated strain of Enterobacter aerogenes. Bull. Environ. Contam. Toxicol. 18: 104-107.

Peters, J.W. and R.M. Cook. 1973. Effects of phthalate esters on reproduction in rats. Environ. Health Perspect. 3: 91-94.

Petersen, R.V., D. Lyman, D.B. Roll and E. Swinyard. 1972-1975. Toxicology of plastic devices having contact with blood. Final report. NIH, Bethesda, MD. NIH-NHLI73-2908-B. (Cited in Bell, 1982)

Phillips, B.J., T.E.B. James and S.D. Gangolli. 1982. Genotoxicity studies of di-(2-ethylhexyl) phthalate and its metabolities in CHO cells. Mutat. Res. 102: 297-304.

Pillai, K.S.R. and P.K. Seth. 1978. Influence of low protein diet on the toxicity of di-(2-ethylhexyl) phthalate. Ind. J. Biochem. Biophys. 16(Suppl.) Abstr. No. 243. (Cited in Seth, 1982)

Plasterer, M.R., W.S. Bradshaw, G.M. Booth, M.W. Carter, R.L. Schuler and B.D. Hardin. 1985. Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, O-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea and four glycol ether derivatives. J. Toxicol. Environ. Health. 15: 25-38.

Pollack, G.M. and D.D. Shen. 1984. Effect of renal failure and bis(2-ethylhexyl) phthalate pretreatment on the dispostion and metabolism of antipyrine in the rat. J. Pharm. Sci. 73(1): 29-33.

Pollack, G.M., R.C. Li, J.C. Ermer and D.D. Shen. 1985a. Effects of route of administration and repetitive dosing on the disposition kinetics of di(2-ethylhexyl) phthalate and its mono-de-esterified metabolite in rats. Toxicol. Appl. Pharmacol. 79: 246-256.

Pollack, G.M., J.F. Buchanan, R.L. Slaughter, R.K. Kohlli, D.D. Shen. 1985b. Circulating concentrations of di-(2-ethylhexyl) phthalate and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. Toxicol. Appl. Pharmacol. 79: 257-267.

Putman, D.L., W.A. Moore, L.M. Schechtman and J.R. Hodgson. 1983. Cytogenetic evaluation of di-2-ethyl phthalate and its major metabolites in Fischer 344 rats. Environ. Mutagen. 5(2): 227-232.

Reddy, J.K., D.E. Mody, D.L. Azarnoff and M.S. Rao. 1976. Di(2-ethylhexyl) phthalate: An industrial plasticizer induces hypolipidemia and enhances hepatic catalase and carnitine acetyltransferase activities in rats and mice. Life Sci. 18: 941-945. (Cited in Seth, 1982).

Reddy, J.K., M.K. Reddy, M.I. Usman, N.D. Lalwani, and S. Ras. 1986. Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl)phthalate and di(2-ethylhexyl) adipate with a hypolipidemic drug. Environ. Health Perspect. 65: 317-327.

Rhodes, C., T.C. Orton, I.S. Pratt, et al. 1986. Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: Extrapolation of effects in rodents to man. Environ. Health Perspect. 65: 299-308.

Rodricks, J.V. and D. Turnbull. 1987. Interspecies differences in peroxisomes and peroxisome proliferation. Toxicol. and Ind. Health. 3(1): 197-212.

Rowland, I.R. 1974. Metabolism of di-(2-ethylhexyl) phthalate by the contents of the alimentary tract of the rat. Food Cosmet. Toxicol. 12: 293-302.

Rowland, I.R., R.C. Cottrell and J.C. Phillips. 1977. Hydrolysis of phthalate esters by the gastrointestinal contents of the rat. Food Cosmet. Toxicol. 15: 17-21.

Rubin, R.J. 1975. Metabolism and acute lung toxicity of solubilized di-(2-ethylhexyl) phthalate (DEHP) in rats. In: Mechanisms of Toxicity and Metabolism. Proc. Sixth Int. Congr. Pharmacol. Helsinki, Finland. 6: 205-213.

Rubin, R.J. 1976. Transcript of proceedings. Workshop on adenine and red cell preservation. Food Drug Admin. Bureau Biol., DHEW.

Rubin, R.J. and Chang. 1978. Effect of intravenous administration of the solubizilized plasticizer, di(2-ethylhexyl)phthalate, on the lung and survival of transfused rats. Presented at 17th Ann. Meet. Society of Toxicology. (Abstract)

Rubin, R.J. and R.J. Jaeger. 1973. Some pharmacologic and toxicologic effects of di-(2-ethylhexyl) phthalate (DEHP) and other plasticizers. Environ. Health Perspect. 3: 53-59. (Cited in Bell, 1982).

Rubin, R.J. and P.P. Nair. 1973. Plasticizers in human tissues. New Engl. J. Med. 288: 915-916.

Rubin, R. and C. Schiffer. 1976. Fate in humans of the plasticizer, di-(2-ethylhexyl) phthalate, arising from transfusion of platelets stored in vinyl plastic bags. Transfusion. 16: 330-335.

Rubin, R.J., W. Kozumbo and R. Kroll. 1979. Ames mutagenic assay or a series of phthalic acid esters: Positive response of the dimethyl and diethyl esters in TA 100. Soc. Toxicol. Ann. Meet., New Orleans, March 11-15. p. 11. (Abstract)

Ruddick, J.A., D.C. Villeneure, I. Chu, E. Nestman and D. Miles. 1981. An assessment of the teratogenicity in the rat and mutagenicity in Salmonella of mono-2-ethylhexyl phthalate. Bull. Environ. Contam. Toxicol. 27: 181. (Cited in Thomas and Thomas, 1984; Hopkins, 1983)

Saeger, V.W. and E.S. Tucker. 1973a. Phthalate esters undergo ready biodegradation. Plast. Eng. (August) p. 46-49.

Saeger, V.W. and E.S. Tucker. 1973b. Biodegradation of phthalate esters. Technical Paper for Regulatory Technology Conference of the Society of Plasticizers. England Palisades Section. March 20-22. p. 105-113.

Saeger, V. and E. Tucker. 1976. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microbiol. 31: 29-34.

Sakurai, T., S. Miyazawa and T. Hashimoto. 1978. Effects of di-(2-ethylhexyl) phthalate administration on carbohydrate and fatty acid metabolism in rat liver. J. Biochem. (Tokyo). 83: 313-320.

Sasaki, S. 1978. The scientific aspects of the chemical substance control law in Japan. In: Aquatic Pollutants: Transformation and Biological Effects, O. Hutzinger, L.H. Von Letyoeld and B.C.J. Zoeteman, Ed. Pergamon Press, Oxford. p. 283-298.

Schmid, P. and C. Schlatter. 1985. Excretion and metabolism of di(2-ethylhexyl)phthalate in man. Xenobiotica. 15: 251-256.

Schultz, C.O. and R.J. Rubin. 1973. Distribution, metabolism and excretion of di-(2-ethylhexyl) phthalate in the rat. Environ. Health Perspect. 3: 123-129.

Schultz, C.O., R.J. Rubin and G.M. Hutchins. 1975. Acute lung toxicity and sudden death in rats following intravenous administration of the plasticizer, di-(2-ethylhexyl) phthalate, solubilized with Tween surfactants. Toxicol. Appl. Pharmacol. 33: 514-525.

Seed, J.L. 1982. Mutagenic activity of phthalate esters in bacterial liquid suspension assays. Environ. Health Perspect. 45: 111-114.

Seth, P.K. 1982. Hepatic effects of phthalate esters. Environ. Health Perspect. 45: 27-34.

Seth, P.K., S.P. Srivastava, M. Mushtag, D.K. Agarwal and S.V. Chandra. 1979. Effect of di-(2-ethylhexyl) phthalate on rat liver injured by chronic carbon tetrachloride treatment. Acta Pharmacol. Toxicol. 44: 161-167.

Seth, P.K., D.K. Agarwal and S. Agarwal. 1981. Effect of phthalic acid esters on drug metabolizing enzymes. Bull. Environ. Contam. Toxicol. 26: 764-768.

Shaffer, C.B., C.P. Carpenter and H.F. Smyth, Jr. 1945. Acute and subacute toxicity of di-(2-ethylhexyl) phthalate with note upon its metabolism. J. Ind. Hyg. Toxicol. 27(5): 130-135.

Shahin, M.M. and R.C. von Borstel. 1977. Mutagenic and lethal effects of benzene hexachloride, dibutyl phthalate and trichloroethylene in Saccharomyces cerevisiae. Mut. Res. 48: 173-180.

Shelton, D.R., S.A. Boyd and J.M. Tiedje. 1984. Anaerobic biodegradation of phthalic acid esters in sludge. Environ. Sci. Technol. 18(2): 93-97.

Shiota, K. and S. Mima. 1985. Assessment of the teratogenicity of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in mice. Arch. Toxicol. 56: 263-266.

Shiota, K. and H. Nishimura. 1982. Teratogenicity of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) in mice. Environ. Health Perspect. 45: 65-70.

Short, R.D., E.C. Robinson, A.W. Lington and A.E. Chin. 1987. Metabolic and peroxisome proliferation studies with di(2-ethylhexyl)phthalate in rats and monkeys. Toxicol. Ind. Health. 3(1): 185-195.

Simmon, V., K. Kauhanen and R. Tardiff. 1977. Mutagenic activities of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2: 249-258.

Singh, A.R., W.H. Lawrence and J. Autian. 1972. Teratogenicity of phthalate esters in rats. J. Pharmacol. Sci. 61: 51-55.

Singh, A.R., W.H. Lawrence and J. Autian. 1974. Mutagenic and antifertility sensitivities of mice to di-(2-ethylhexyl) phthalate (DEHP) and dimethoxyethyl phthalate (DEMP). Toxicol. Appl. Pharmacol. 29: 35-46.

Singh, A.R., W.H. Lawrence and J. Autian. 1975. Maternal-fetal transfer of ^{14}C-di-(2-ethylhexyl) phthalate and ^{14}C-diethyl phthalate in rats. J. Pharmacol. Sci. 64(8): 1347-1350. (Cited in Kluwe, 1982a).

Sjöberg, P., U. Bondesson and M. Hammorlund. 1985a. Non-linearities in the pharmacokinetics of di-(2-ethylhexyl) phthalate and metabolites in male rats. Arch. Toxicol. 58: 72-77.

Sjöberg, P., N.G. Linquist, G. Montin and L. Plöen. 1985b. Effects of repeated intravenous infusions of the plasticizer di-(2-ethylhexyl) phthalate in young male rats. Arch. Toxicol. 58: 78-83.

Sjöberg, P., U. Bondesson, L. Kjellen, N.-G. Linquist, G. Montin and L. Plöen. 1985c. Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. Acta Pharmacol. Toxicol. 56: 30-37.

Sjöberg, P., U.G. Bondesson, E.G. Sedin and I.P. Gustaffson. 1985d. Exposure of newborn infants to plasticizers: plasma levels of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate during exchange transfusion. Transfusion. 25: 424-428.

Sjöberg, P., U.G. Bondesson, T.J. Gray and L. Ploen. 1986a. Effects of di(2-ethylhexyl)phthalate and five of its metabolites on rat testis in vivo and in vitro. Arch. Toxicol. 58: 72-77.

Sjöberg, P., N.G. Lindqvist and L. Plöem. 1986b. Age-dependent response of the rat testes to di(2-ethylhexyl)phthalate. Environ. Health Perspect. 65: 237-242.

Smith, C.C. 1953. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate and methoxyethyl oleate. Arch. Ind. Hyg. Occup. Med. 7: 310-318.

Smith, J.C., E.G. McDaniel, F.F. Fan and J.A. Hasted. 1973. Zinc: A trace element essential in vitamin A metabolism. Science. 181: 954-955.

Srivastava, S.P., D.K. Agarwal and P.K. Seth. 1977. Effects of di-(2-ethylhexyl) phthalate on activity of succinic dehydrogenase and adenosine triphosphatase of some vital organs of rat. Toxicology. 7: 163-168. (Cited in Seth, 1982).

Srivastava, S.P., D.K. Agarwal, M. Mushtag and P.K. Seth. 1978. Effect of di-(2-ethylhexyl) phthalate (DEHP) on chemical constituents and enzymatic activity of rat liver. Toxicology. 11: 271-275. (Cited in Seth, 1982).

Stalling, D., J.W. Hogan and J.L. Johnson. 1973. Phthalate ester residues - their metabolism and analysis in fish. Environ. Health Perspect. 3: 159-173.

Stenchever, M.A., M.A. Allen, L. Jerominski and R.V. Peterson. 1976. Effects of bis-(2-ethylhexyl) phthalate on chromosomes of human leukocytes and human fetal lung cells. J. Pharm. Sci. 65: 1648-1651.

Sugatt, R.H., D.P. O'Grady, S. Banerjee, P.H. Howard and W.E. Gledhill. 1984. Shake flask biodegradation of 14 commercial phthalate esters. Appl. Environ. Microbiol. 47: 601-606.

Sullivan, K.F., E.L. Atlas and C.S. Giam. 1982. Adsorption of phthalic acid esters from seawater. Environ. Sci. Technol. 16: 428-432.

Tabak, H.H., S.A. Quave, C.I. Mashni and E.F. Barth. 1981. Biodegradability studies for predicting the environmental fate of organic priority pollutants. In: Test protocols for Environmental Fate and Movement of Toxicants. Proc. Symp. Assoc. of Official Analytical Chemistry, 94th Ann. Meeting, Washington, DC. p. 267-328.

Tanaka, A., T. Adachi, T. Takahashi and T. Yamaha. 1975. Biochemical studies on phthalic esters. I. Elimination, distribution and metabolism of di-(2-ethylhexyl) phthalate in rats. Toxicology. 4: 253-264.

Tanaka, A., A. Matsumoto and T. Yamaha. 1978. Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. Toxicology. 9:109-123.

Taylor, B.F., R.W. Curry and E.F. Corcoran. 1981. Potential for biodegradation of phthalic acid esters in marine regions. Appl. Environ. Microbiol. 42(4): 590-595.

Teirlynck, O.A. and F. Belpaire. 1985. Disposition of orally administered di(2-ethylhexyl)phthalate and mono (2-ethylhexyl)phthalate in the rat. Arch. Toxicol. 57: 226-230.

Thiess, A.M. and I. Fleig. 1979. Chromosomen untersuchungen bei Mitarbeitern mit Exposition gegenüber Di-2-äthylhexylphthalat (DOP). Berichtigung Zbl. Arbeitsmed., Bd. 29, H. 4, April 1979, S. 120, p. 351-355. (Ger.)

Thiess, A.M., A. Korte and H. Fleig. 1978a. Untersuchengen zur morbidität bei Mitarbeitern mit Exposition gegenüber Di-2-äthylhexylphthalat (DOP). Vortr. Anl. d. Jahrestg. d. Deutschen Gesellschaft f. Arb. Med. in Frankfurt V. 25. - 27.5.78. p. 137-151. (Ger.)

Thiess, A.M., R. Frentzel-Beyme and R. Wieland. 1978b. Mortality study in workers exposed to di-(2-ethylhexyl) phthalate (DOP) (Ger.). In: Moglichkerten und Grenzen des Biological Monitoring. Arbeitsmedizinische Probleme des Dienstleistungsqewerbes. Arbeitsmedizinisches kolloquium [Possibilities and Limits of Biological Monitoring. Problems of Occupational Medicine in Small Industries. Colloquium in Occupational Medicine], Frankfurt/M., May 1978. Stuttgart, A.W. Gentner, p. 155-164.

Thomas, J.A. and M.J. Thomas. 1984. Biological effects of di-(2-ethylhexyl) phthalate and other phthalic acid esters. CRC Crit. Rev. Toxicol. 13(4): 283-317.

Thomas, J.A., T.D. Darby, R.F. Wallin, P.J. Garvin and L. Martis. 1978. A review of the biological effects of di-(2-ethylhexyl) phthalate. Toxicol. Appl. Pharmacol. 45: 1-27.

Thomas, J.A., K.A. Curto and M.J. Thomas. 1982. MEHP/DEHP: Gonadal toxicity and effects on rodent accessory sex organs. Environ. Health Perspect. 45: 85-88.

Tomita, I., Y. Nakamura, Y. Yagi and K. Tutikawa. 1982a. Teratogenicity/fetotoxicity of DEHP in mice. Environ. Health Perspect. 45: 71-75.

Tomita, I., Y. Nakamura, N. Aoki and N. Inui. 1982b. Mutagenic/carcinogenic potential of DEHP and MEHP. Environ. Health Perspect. 45: 119-125.

Tsuchiya, K. and K. Hattori. 1977. Chromosomal study on human leukocyte cultures treated with phthalic acid ester. Hokkaidoritus Eisei Kenkyusho Ho. 26: 114. (Abstract)

Turnbull, D. and J.V. Rodricks. 1985. Assessment of possible carcinogenic risk to humans resulting from exposure to di(2-ethylhexyl)phthalate (DEHP). J. Amer. Coll. Roxicol. 4: 111-145.

Turner, J.H., J.C. Petricciani, M.L. Crouch and S. Wenger. 1974. An evaluation of the effects of diethylhexyl phthalate on mitotically capable cells in blood packs. Transfusion. 14: 560-566.

Tyl, R.W., C.J. Price, M.C. Marr and C.A. Kimmel. 1988. Developmental toxicity evaluation of dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. Fund. Appl. Toxicol. 10(3): 395-412.

U.S. EPA. 1978. Chemical Hazard Information Profile Draft Report: Alkyl Phthalates. Office of Toxic Substances, Washington, DC.

U.S. EPA. 1980. Ambient Water Quality Criteria for Phthalate Esters. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA 440/5-80-067. NTIS PB81-117780.

U.S. EPA. 1982. Aquatic Fate Process Data for Organic Priority Pollutants. Office of Water Regulations and Standards, Washington, DC. EPA 440/4-81-014.

U.S. EPA. 1985. TSCAPP -- Toxic Substance Control Act Plant Production: Online.

U.S. EPA. 1986. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1987a. Health and Environmental Effects Profile for Phthalic Acid Alkyl, Aryl and Alkyl/Aryl Esters. Prepared by Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/X-87/384. NTIS PB89-120158/AS.

U.S. EPA. 1987b. Health Effects Assessment for Selected Phthalic Acid Esters. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA/600/8-88/053. NTIS PB88-178934/AS.

U.S. EPA. 1991. Integrated Risk Information System (IRIS). Online. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. April.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. United States Production and Sales, 1982. U.S. Government Printing Office, Washington, DC.

USITC (U.S. International Trade Commission). 1985. Synthetic Organic Chemicals. United States Production and Sales, 1984. U.S. Government Printing Office, Washington, DC. p. 165.

von Däniken, A., W.K. Lutz, R. Jackh and C. Schlatter. 1984. Investigation of the potential for binding of di-(2-ethylhexyl) phathalate (DEHP) and di-(2-ethyhexyl) adipate (DEHA) to liver DNA in vivo. Toxicol. Appl. Pharmacol. 73: 373-387.

Waddell, W.M., C. Marlowe, J.E. Miripol and P.J. Garvin. 1977. The distribution in mice of intravenously administered plasma solutions of ^{14}C-di-(2-ethylhexyl) phthalate determined by whole-body autoradiography. Toxicol. Appl. Pharmacol. 39: 339-353.

Wallin, R.F., B. Klamer, R.W. Nicora and C.R. Thompson. 1974. Di-(2-ethylhexyl) phthalate (DEHP) metabolism in animals and post-transfusion tissue levels in man. Bull. Parenteral Drug Assoc. 28: 278-287.

Walseth, F., R. Toftgard and O.G. Nilsen. 1982. Phthalate esters. I: Effects on cytochrome P-450 mediated metabolism in rat liver and lung, serum enzymatic activities and serum protein levels. Arch. Toxicol. 50: 1-10.

Ward, J.M., J.M. Rice, D. Creasia, et al. 1983. Dissimilar patterns of promotion by di-(2-ethylhexyl) phthalate and phenobarbital of hepatocellular neoplasia initiated by diethylnitrosamine in $B6C3F_1$ mice. Carcinogenesis. 4(8): 1021-1029.

Ward, J.M., B.A. Diwan, M. Ohshima, H. Hu, H.M. Schuller and J.M. Rice. 1986. Tumor-initiating and promoting activities of di(2-ethylhexyl) phthalate in vivo and in vitro. Environ. Health Perspect. 65: 279-291.

Warren, J.R., N.D. Lalwani and J.K. Reddy. 1982. Phthalate esters as peroxisome proliferator carcinogens. Environ. Health Perspect. 45: 35-40.

Watts, P. 1985. Di-2-ethylhexylphthalate metabolism in man. Food Chem. Toxicol. 23: 1023.

Weast, R.C., Ed. 1983. Handbook of Chemistry and Physics, 63rd ed. CRC Press, Inc., Boca Raton, FL.

Wilbourn, J. and R. Montesano. 1982. An overview of phthalate ester carcinogenicity testing results: The past. Environ. Health Perspect. 45: 127-128.

Wildbrett, G. 1973. Diffusion of phthalic acid esters from PVC milk tubing. Environ. Health Perspect. 3: 29-35.

Williams, D.T. and B.J. Blanchfield. 1975. The retention, distribution, excretion and metabolism of dibutyl phthalate-7-^{14}C in the rat. J. Agric. Food Chem. 23: 854-857. (Cited in U.S. EPA, 1980; Gangolli, 1982).

Williams, G.M., H. Maruyama and T. Tanaka. 1987. Lack of rapid initiating, promoting or sequential syncarcinogenic effects of di(2-ethylhexyl)phthalate in rat liver carcinogenesis. Carcinogenesis. 8(7): 875-880.

Wolfe, N.L., W.C. Steen and L.A. Burns. 1980. Phthalate ester hydrolysis: Linear free energy relationships. Chemosphere. 9: 403-408. EPA 600/J-80-016.

Yanagita, T., S. Kuzuhara, N. Enomoto, T. Shimada and M. Sugano. 1979. Effects of di-(2-ethylhexyl) phthalate on the content and composition of hepatic mitochondrial and microsomal phospholipids in the rat. Biochem. Pharmacol. 28: 3115-3122.

Yoshikawa, K., A. Tanaka, T. Yamaha and H. Kurata. 1983. Mutagenicity study of nine monoankyl phthalates and a dialkyl phthalate using Salmonella typhimurium and Escherichia coli. Food Chem. Toxicol. 21(2): 221-223.

Yurchenko, V.V. 1977. Cytogenetic study of mutagenic properties common to the repellants dimethyl phthalate and N,N-diethylamide of phenoxyacetic acid. Farmakol. Toksikol. (Moscow). 4: 454-457. (Rus.)

Yurchenko, V.V. and S. Gleiberman. 1980. Study of long-term effects of repellant use. Part III. Study of mutagenic properties of dimethyl phthalate and phenoxyacetic acid N,N-diethylamide by dominant lethal mutations. Med. Parazitol. Parizit. Boleani. 49: 58-61. (Rus.)

Zeiger, E., S. Haworth, W. Speck and V. Mortlemans. 1982. Phthalate ester testing in the National Toxicology Program's environmental mutagenesis test development program. Environ. Health Perspect. 45: 99-101.