Toxicity and Health Threats of Phthalate Esters: Review of the Literature

by John Autian*†

Introduction

In 1970 Jaeger and Rubin (*1*) reported detecting the presence of a plasticizer, di-2-ethylhexyl phthalate (DEHP), in tissues and organs of two deceased patients who previously had received blood transfusions. The blood had been stored in bags made of poly(vinyl chloride) (PVC) plasticized with DEHP. Even though other reports had implied that certain formulations of PVC medical tubings (*2,3*) and containers would, in fact, release plasticizers as well as additives to solutions, little concern had been expressed as to the possible health consequences to humans from leaching of these agents. Since Jaeger and Rubin's publication, however, considerable interest has developed concerning the possible toxicogenic effects not only of DEHP, but of all phthalate esters. More recently, interest has also centered on the potential hazards from these plasticizers in the environment. It is estimated that the yearly production of phthalate esters in this country is in the neighborhood of 900 million pounds (*4*). Since these esters have been produced commercially in greater quantities each year since the Second World War, it should not be too surprising to find that they are entering the environment. Currently no restrictions have been placed on phthalates in regard to industrial wastes, and thus large quantities are being dumped into waterways adjacent to industrial regions producing or using the esters for various applications. Phthalate esters are poorly water-soluble but are readily sequestered or absorbed by organic residues and solid surfaces in environmental water systems. Although there is no quantitative information on the extent of such phthalate ester deposition, either naturally or from industrial or municipal wastes, accumulation and slow, long-term release may significantly affect the ecology of water systems.

Since there is a growing concern that phthalate esters may be a menace to health and to our ecological system, there is a need to review the evidence on the toxicity and possible health threats that these esters present directly or indirectly to man. This paper reviews the story of the phthalate esters in a manner which hopefully separates science from fiction. Certain value judgments which are included reflect the background and experiences of the author. To those who have become interested in the phthalate esters, this

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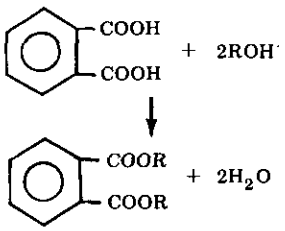
review should be a good starting point from which to pursue the subject in more detail. Unfortunately, as will be evident, there are still a large number of questions in regard to potential health threats which cannot be answered rationally on the basis of present knowledge. It is hoped that this review will stimulate research to fill the gaps of knowledge. It is hoped that this review will stimulate research to fill the gaps of knowledge needed to make sound scientific judgments in the near future as to benefits versus risks of these very important chemical agents.

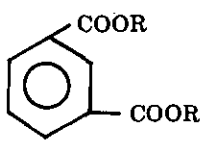
Chemistry and Properties

The term "phthalate esters" is restricted to the *ortho* form of benzenedicarboxylic acid prepared by reaction of phthalic acid with a specific alcohol to form the desired ester.

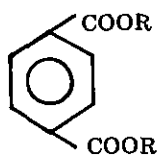


Industrially, these esters are prepared from phthalic anhydride rather than from the acid. Commercial phthalate esters generally are not pure compounds, but reasonably pure compounds can be prepared for specific applications. Most of the esters are colorless liquids, have low volatility, and are poorly soluble in water but soluble in organic solvents and oils (Table 1).

Two other isomeric forms of benzenedicarboxylic acid esters are also available having important industrial applications–the *meta* form (or isophthalate esters) and the *para* form (or terephthalate esters).



Isophthalates



Terephthalates

Terephthalic acid and esters of this acid are becoming increasingly important as starting chemicals for certain types of polymers (i.e., Dacron) and are used in animal feed to enhance the physical properties of the feed and the bioavailability of added components such as antibiotics. In 1970, some 420×10^6 lb of terephthalic acid was produced in this country with a prediction (*6*) that up to 2.9×10^9 lb will be manufactured by 1979. Isophthalates are also used industrially in the production of certain types of polymers and resins but not to the same extent as the phthalates and terephthalates. This review for the most part will deal with the phthalate esters, but this should not be taken as an indication that the isophthalates or terephthalates may not also pose health and ecological threats, but rather that sufficient information is not available on these other isomers to present a detailed review.

General Applications of Phthalate Esters

A very wide spectrum of uses has been found for the various phthalate esters. During the Second World War, dimethyl and dibutyl phthalates were used in insect repellent formulations. By far, however, the largest market for these esters is as plasticizing agents for poly(vinyl chloride), for example, the cellulosics, and certain types of elastomers. These plasticizers give the polymer the desired flexibility and softness and may account for up to 40% of the final weight of the material. They are not chemically bound to the polymer, but dispersed in the matrix of the polymer chains to decrease the interaction forces of adjacent chains, lower the glass transition temperature of the polymer, and causes chain mobility and material flexibility. Dioctyl phthalate (DOP) and its isomer, di-2-ethylhexyl phthalate (DEHP), are probably the most widely used plasticizers today, although other phthalates are used, sometimes in combinations, to give certain additional properties desired in the final material.

A large number of industrial and consumer products of the poly(vinyl chloride) type will generally contain one of the phthalate esters as a plasticizer. These products include floor tiles, various types of furnishings for house-

Table 1. Physical and chemical properties of phthalate esters.[a]

Compound	Molecular weight	Specific gravity	Bp, °C	Solubility in H_2O, g/100 ml
Dimethyl phthalate	194.18	1.189 (25/25)	282	0.5
Diethyl phthalate	222.23	1.123 (25/4)	296.1	Insoluble
Diallyl phthalate	246.27	1.120 (20/20)	290	0.01
Diisobutyl phthalate	278.3	1.040	327	Insoluble
Dibutyl phthalate	278.34	1.0465 (21)	340	0.45 (25°C)
Dimethoxyethyl phthalate	282.0	1.171 (20)	190–210	0.85
Dicyclohexyl phthalate	330.0	1.20 (25/25)	220–228	Insoluble
Butyl octyl phthalate	334.0	–	340	–
Dihexyl phthalate	334.0	0.990	–	Insoluble
Butylphthalyl butyl glycolate	336.37	1.097 (25/25)	219 /5 mm/	0.012%
Dibutoxyethyl ethyl phthalate	366.0	1.063	210	0.03
Di-2-ethylhexyl phthalate	391.0	0.985 (20/20)	386.9/5 mm	Insoluble
Diisooctyl phthalate	391.0	0.981	239/5 mm	Insoluble
Di-*n*-octyl phthalate	391.0	0.978	220 /5 mm/	Insoluble
Dinonyl phthalate	419.0	0.965	413	Insoluble

[a]Data of Patty (*5a*).

holds and transportation vehicles, food packaging systems, industrial tubings and conduits, medical tubings, catheters and blood containers, certain types of dental materials, coatings for drugs, and numerous other products.

The phthalate esters also have many other uses. For example, they are used as defoaming agents in the manufacture of paper, in cosmetic products as a vehicle (primarily diethyl phthalate) for perfumes, in lubricating oils, and in other industrial and consumer applications in which the esters add specific desired properties. There is also some evidence suggesting that the esters are present in certain plants and organisms as normal metabolites.

Several of the phthalate esters have been approved by the FDA for food-packaging applications, and thus they have been accepted as generally safe under the conditions of use.

Toxicology

Industrial Health Problems

Industrial production of very large quantities of phthalate esters has not generally caused health problems where good industrial hygiene has been practiced. As in the manu-

facture of any chemical agent, many of the starting agents in themselves may present toxic and health liabilities to workers if care and proper engineering methods are not provided.

Since one of the starting materials for the manufacture of phthalate esters is phthalic anhydride, it is important to recognize the toxic nature of this agent. Phthalic anhydride is a solid (generally crystalline) and is slightly soluble in water. It has a characteristic choking odor and can be a moderate fire and explosion hazard when exposed to heat and flame.

The chief acute toxic effects result from the very marked irritating properties of the chemical on skin, eyes, and mucous membranes of the nasal passage and upper respiratory tract. On dry skin the irritant properties are less, but in the presence of moisture or water a highly inflamed tissue will result. In susceptible workers, repeated contact with the agent may cause allergic dermatitis and perhaps pulmonary sensitization, leading to asthmatic attacks.

The oral LD_{50} in rats ranges from 800 to 1600 mg/kg; in the guinea pig the intraperitoneal LD_{50} dose is 100 mg/kg (*5b*).

If proper precautions are not taken in a working environment, the worker may inhale phthalic anhydride as a fine dust, resulting in irritation of the mucous membranes of the nose and copious nasal discharge with, at times, bleeding. The TLV (Threshold Limit Value, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio) for phthalic anhydride is 25 mg/m^3. A number of reports have appeared, primarily in European publications, on the toxic and health hazards of the anhydride, but few recent reports are available in the American literature. Further detailed information on this material may be obtained from reports by Baader (*7*), Menschick (*8*), Friebel (*9*), and Jacobs (*10*), and several reference texts (*11,12*).

Depending on the specific phthalate ester manufactured, various alcohols are used, each of which presents specific toxic properties to workers if general safety precautions are not taken. The acute systemic toxicity of the aliphatic alcohols increases with chain length, reaching a maximum and then declining parabolically due to decreased solubility and changing intrinsic toxicity. Inhalation of vapors of the alcohols will lead to narcosis, which can cause accidents in the work environment. Unsaturated alcohols are generally more toxic than saturated, and branched-chain alcohols are more volatile and generally produce a greater central nervous system depression than their straight-chain isomers. Irritant effects on eyes and membranes depend on the specific esterified alcohol; diallyl phthalate, for example, is a lacrimating agent. For the most part, the alcohols have not presented serious industrial hazards.

Acute Toxicity of Phthalate Esters

Up to the present, published literature on the subject of phthalate esters has indicated that these chemical agents are relatively nontoxic and should not present any health hazards to man. Animal experiments dating back to the Second World War have shown that the most frequently used phthalate esters have an extremely low order of acute toxicity (see Table 2). Esters of higher molecular weight, such as dioctyl and di-2-ethylhexyl phthalate, show a very low order of acute toxicity. Lethal effects from absorption through the skin of guinea pigs and rabbits are also quite low, with reports of LD_{50} values greater than 5, 10, or even 20 ml/kg.

Most of the phthalate esters have a low volatility, and thus inhalation of these agents generally does not present problems of an acute nature. Experiments in which groups of animals were exposed to various concentrations of the esters produced very few effects except at relatively high chamber concentrations. In many instances it was necessary to heat the ester to 100 or 200°C to reach sufficiently high concentrations in the chamber to note toxic effects.

As a group, the phthalate esters produce little irritant response (perhaps with the exception of diallyl phthalate) when placed in contact with animal and human skin. Few reports have noted the irritant properties of

Table 2. Acute toxicity of phthalate esters: LD_{50} in animals.

Compound	Animal	Route	LD_{50}, g/kg	Reference
Dimethyl phthalate	Mouse	Oral	7.2	(*5c*)
	Mouse	IP	3.6	(*5c*)
	Mouse	IP	1.58	(*13*)
	Rat	Oral	2.4	(*5c*)
	Rat	IP	3.38[a]	(*14*)
	Guinea pig	Oral	2.4	(*5c*)
	Rabbit	Dermal	10.0[a]	(*5c*)
Diethyl phthalate	Mouse	IP	2.8	(*5c*)
	Mouse	IP	2.8	(*13*)
	Rat	IP	5.06[a]	(*14*)
	Rabbit	Oral	1.0	(*5c*)
Dimethoxyethyl phthalate	Mouse	Oral	3.2–6.4	(*5c*)
	Mouse	IP	2.51	(*13*)
	Rat	Oral	4.4	(*5c*)
	Rat	IP	3.7	(*13*)
	Guinea pig	Oral	1.6–3.2	(*5c*)
	Guinea pig	Dermal	10.0[a]	(*5c*)
Diallyl phthalate	Mouse	IP	0.7	(*5c*)
	Rat	Oral	1.7	(*5c*)
	Rabbit	Oral	1.7	(*5c*)
	Rabbit	Dermal	3.4[a]	(*5c*)
Dibutyl phthalate	Mouse	IP	4.0	(*13*)
	Rat	IP	3.05[a]	(*14*)
	Rat	IM	8.0	(*5c*)
	Rabbit	Dermal	20.0[a]	(*5c*)
Diisobutyl phthalate	Mouse	Oral	12.8	(*5c*)
	Mouse	IP	4.50	(*13*)
	Rat	IP	3.75[a]	(*14*)
	Guinea pig	Dermal	10.0[a]	(*5c*)
Butyl carbobutoxy-methyl phthalate	Rat	Oral	14.6[a]	(*5c*)
	Rat	IP	6.89	(*14*)
Dihexyl phthalate	Rat	Oral	30.0	(*5c*)
	Rabbit	Dermal	20.0[a]	(*5c*)
Dioctyl phthalate	Mouse	Oral	13.0	(*5c*)
	Rat	IP	50.0[a]	(*14*)
	Guinea pig	Dermal	5.0[a]	(*5c*)
Di-2-ethylhexyl phthalate	Mouse	IP	14.2	(*13*)
	Rat	Oral	26.0	(*5c*)
	Rat	IP	50.0[a]	(*14*)
	Rabbit	Oral	34.0	(*5c*)
	Guinea pig	Dermal	10.0	(*5c*)
Butylbenzyl phthalate	Mouse	IP	3.16	(*13*)
Dicapryl phthalate	Mouse	IP	14.2	(*13*)
Dinonyl phthalate	Rat	Oral	2.00	(*5c*)
Dibutyl (diethylene glycol bisphthalate)	Mouse	Oral	11.2	(*15*)
	Mouse	IP	~11.2	(*15*)
	Rat	Oral	11.2	(*15*)
	Rat	IP	~11.2	(*15*)
Dialkyl 79 phthalate	Mouse	Oral	>20.00	(*16*)
	Rat	IP	>20.00	(*16*)

[a] LD_{50} in ml/kg.

these compounds to the eye (again with the exception of diallyl phthalate). However, intradermal injections of some phthalate esters in rabbits can lead to irritant responses (Table 3) (*13*).

Table 3. Irritative response in rabbits to intradermal injections of phthalate esters.[a]

Material injected	Degree of extravasation[b] 10 min	15 min	26 min
Dimethyl phthalate	+ +	+ + +	+ + +
Diethyl phthalate	+ + +	+ + +	+ + +
Dibutyl phthalate	+	+	+ +
Diisobutyl phthalate	–	–	+ +
Dimethoxyethyl phthalate	+ +	+ +	+ +
Butylbenzyl phthalate	–	+	+ +
Di-2-ethylhexyl phthalate	+ + +	+ + +	+ + +
Dicapryl phthalate	–	+	+
0.85% NaCl (negative control)	–	–	–
20% Ethyl alcohol (positive control)	+ + +	+ + +	+ + +

[a]Data of Calley, Autian, and Guess (*13*).

[b]Inflammatory response indicated by degree of dye extravasation: – no color (negative response); + mild; + + moderate; + + + marked.

When poly(vinyl chloride) containing di-2-ethylhexyl phthalate is implanted subdermally or intramuscularly in rats or rabbits, no inflammatory response is seen (Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, Tennessee 38103, unpublished data). As yet, no reports have stated that phthalate esters act as sensitizing agents in animals or man. If there is an inflammatory or sensitizing response to a material, it most likely results from other additives or contaminants.

Many of the commercially available phthalate esters contain impurities, including homologs and isomers. Nematollahi, Guess, and Autian (*17*) synthesized and purified a number of dialkyl and dicycloalkyl phthalates, isophthalates, and terephthalates and evaluated them for acute toxicity by a tissue culture test and by intraperitoneal (IP) injection into mice.

The mice, three in each group, received a dose of 5 mole/kg in either cottonseed oil or castor oil, depending on the solubility of the specific compound, and were observed for 4 days or until death. The results (Table 4) showed cytotoxic and toxic effects from the lower molecular weight compounds but not from esters with C_6 to C_9 alkyl groups. The cyclohexyl phthalate, isophthalate, and terephthalate esters were toxic, as was cyclohexyl as the phthalate or isophthalate ester. However, the terephthalate ester was not. The n-$C_{10}H_{21}$ phthalate and terephthalate esters were toxic, while the isophthalate isomer was not. Some toxicity was manifested by the n-$C_{12}H_{25}$ phthalate esters, a slight toxic effect by the terephthalate ester, but none by the isophthalate.

Subacute and Chronic Toxicity of Phthalate Esters

Repeated exposure to phthalate esters—orally, IP, dermally, and by inhalation—appears to produce few if any toxic effects in animals except at very high doses or high inhalation concentrations. Since the Second World War the number of reports published on groups of phthalate esters or on specific esters have been relatively few, most being published from 1945 to 1955. Since the total quantity of the di-2-ethylhexyl phthalate manufactured is much greater than that of the other phthalate esters, more interest has been shown in this plasticizer than in the others. In the section to follow, several of the phthalate esters are reviewed, but comments are brief because of the paucity of information available. More attention is, however, directed to DEHP, not only because of its great commercial importance but also because of the availability of toxicity data dealing with subacute and chronic studies.

Dimethyl Phthalate: Repeated inhalation of the vapor of dimethyl phthalate will cause irritation of the nasal mucous membrane and the upper respiratory tract. Prolonged inhalation may lead to central nervous system depression and eventual paralysis. Dose levels of 4 to 8% in the diet of female rats over a

Table 4. Toxicity evaluation of the dialkylbenzene dicarboxylate on the mammalian cell cultures and mice.[a]

R	Phthalates			Isophthalate			Terephthalates		
	Chick embryo cells	L-cells	Mice	Chick embryo cells	L-cells	Mice	Chick embryo cells	L-cells	Mice
CH_3	+	+	+	+	+	+	+	+	+
C_2H_5	+	+	+	+	+	+	+	+	+
n-C_3H_7	+	+	+	+	+	+	+	+	+
i-C_3H_7	+	+	+	+	+	-	+	+	+
n-C_4H_9	±	+	+	+	+	+	+	+	+
i-C_4H_9	+	+	±	+	+	+	-	-	±
n-C_5H_{11}	-	±	-	+	+	-	+	+	+
i-C_5H_{11}	+	+	+	+	+	-	±	+	-
Cyclo-C_5H_9	+	+	±	+	+	+	±	±	±
n-C_6H_{13}	-	-	-	-	-	-	-	-	-
Cyclo-C_6H_{11}	-	-	-	-	-	-	-	-	-
n-C_7H_{15}	-	-	-	-	-	-	+	+	-
Cyclo-C_7H_{13}	+	+	-	+	+	-	-	-	-
n-C_8H_{17}	-	-	-	-	-	-	-	-	-
Cyclo-C_8H_{15}	-	-		-			±	±	
n-C_9H_{19}	-	-	-	±	+	-	-	-	-
n-$C_{10}H_{21}$	±	+	±	-	-	-	+	+	-
n-$C_{11}H_{23}$	-	-	-	±	±	-	-	-	-
n-$C_{12}H_{25}$	±	+	-	-	-	-	-	-	±

[a]For cell culture (chick embryo and L-cells) + indicates a cytoxic response, - indicates noncytotoxic, and ± a questionable response. For mice + indicates 2 or 3 deaths, ± indicates 1 death; and - indicates no deaths. Data of Nematollahi, Guess, and Autian (*17*).

2-yr period produced a slight reduction in growth, but 2% did not (*5d*). At the 8% dose level there was noticeable kidney damage. Single applications of dimethyl phthalate to the skin of rabbits indicated a dermal LD_{50} value of greater than 10 ml/kg, which, however, was only somewhat greater than 4 ml/kg when repeated applications were made over a 90-day period.

Diethyl Phthalate: The small amount of information available from chronic toxicity studies and human experience of a quarter of a century of use suggest an extremely low toxic potential of diethyl phthalate. Like dimethyl phthalate, prolonged inhalation of vapors of this ester may produce irritant responses in the nasal passage and on the mucous membrane of the upper respiratory tract. Generally, however, only if the ester is heated will sufficient concentration of the vapor become available to produce an irritant response (*5e*).

Dibutyl Phthalate: In chronic toxicity studies, Smith (*18*) found the lethal oral dose of dibutyl phthalate to be approximately 8.0 g/kg in rats. Rats fed diets containing 0.01, 0.05, and 0.25% dibutyl phthalate showed no toxic reactions after 1 yr. Of those fed 1.25%, half died during the first week of the experiment, but those which survived grew normally, as compared to untreated controls.

Dibutyl(Diethylene Glycol Bisphthalate): Dibutyl(diethylene glycol bisphthalate) (DDGB) was synthesized as a promising new plasticizer for moisture-proof coatings on cellulose film. Hall, Austin, and Fairweather (*15*) found the oral LD_{50} of a commercial sample of DDGB that contained approximately 80% DDGB, 15% dibutyl phthalate, and 5% di(diethylene glycol) phthalate to be greater than 11.2 g/kg and the IP LD_{50} to be approximately 11.2 g/kg. The acute toxicity of the product was greater when it was administered as a 50% solution in arachis oil.

In a 12-week subacute toxicity study, rats fed a diet containing 0, 0.25, and 2.5% of the product showed a marked retardation of growth as compared to control animals (*15*). Enlargement of liver and heart was noted at the 1.0 and 2.5% level in male rats and enlarged brain in both male and female animals. Oxaluria and hematuria were noted in both male and female rats at the 2.5% level, the oxaluria being assumed to be a direct result of the *in vivo* liberation of diethylene glycol (a known producer of oxalate stones in the bladder). Since dose levels below 0.25% were not tested, no information is available as to the "no adverse effect" level.

Dialkyl 79 Phthalate: Dialkyl 79 phthalate is a plasticizer which contains a mixture of phthalate esters of alcohols having chain lengths of 7 to 9 carbons. Elizarova (*19*) reported that the oral LD_{50} for mice is greater than 44 ml/kg and for rats is in excess of 70-80 ml/kg. In other acute toxicity studies with doses of 20.0 g/kg dialkyl 79 phthalate, Gaunt et al. (*16*) found no deaths when mice or rats were given doses of 20.0 g/kg orally or by intraperitoneal injection. A 90-day feeding study in rats demonstrated no adverse effects at the 0.125% level, but 0.5 and 1.0% resulted in increased liver weights even though histopathologic changes were not evident. The authors concluded after imposing a 100-fold safety factor, that a 60-kg adult could ingest 36 mg/day without any apparent harm.

Di-2-ethylhexyl Phthalate: Di-2-ethylhexyl phthalate (DEHP) has an extremely low order of acute toxicity. The LD_{50} values in several species of animals (Table 2) indicate that extremely large doses, 14.2 to greater than 50 g/kg, of the compound are necessary to produce lethal effects. In fact, this agent would be classified as "practically nontoxic" by the toxicity rating system of Gleason et al. (*20*). No irritant response from dermal application or sensitizing potential has been noted in animals or humans. The ester is very poorly absorbed through skin, very large concentrations (approximately 25 ml/kg) being necessary to bring about death in rabbits. In early experiments by Shaffer and associates (*21*), in which groups of rats were exposed to DEHP mists generated by bubbling air through DEHP heated to 170°C in a chamber maintained at room temperature, there were no fatalities in 2 hr. but all the animals died in 4 hr.

Much of the information on the subacute and chronic toxicity of DEHP has come from reports by Shaffer et al. (*21*), Carpenter et al. (*22*), and Harris et al. (*23*), spanning a period of approximately 12 years (1945 to 1956). Since 1956 no published data on chronic toxicity have appeared in the American literature. Because of the recent resurrection of interest in DEHP, newer subacute and chronic studies have been initiated, but these data are not available as publications yet although a few may be available as preliminary reports.

Shaffer and associates, as early as 1945 (*21*), reported a subacute study in rats on the toxicity of DEHP, which was administered in the diet at concentrations of 3.0, 1.5, 0.75, and 0.375% for 90 days. At the three highest levels there was a slight decrease in growth as compared to control rats, and at the two highest doses tubular atrophy and degeneration in the testes were observed. However, no abnormal blood picture was noted at any of the doses. The authors concluded that no injury resulted from oral administration of 0.2 g/kg per day or less, while a slight retardation in growth occurred at 0.4 g/kg per day. Metabolism experiments conducted by Shaffer are discussed below.

In 1953, Carpenter, Weil, and Smyth (22) published a chronic oral toxicity study on DEHP, using rats, guinea pigs, and dogs. The rat experiment was designed as follows. Groups composed of 32 male and 32 female Sherman rats constituted the parental (P_1) generation rats, which were maintained for a maximum of 2 yr on diets containing 0.4, 0.13, and 0.04% DEHP. In addition, approximately 80 first filial generation (F_1) rats were maintained for 1 yr on a diet containing 0.4% DEHP. Appropriate controls for each group received the basal diet without the chemical additive. The criteria of effect chosen for statistical evaluation by direct

comparison with the untreated controls were mortality, life expectancy, body weight, food consumption, liver and kidney weights, micropathological changes, neoplasm incidence, hematology, and fertility.

The plasticizer was added to the diet when animals attained an age of 60 days. Male and female rats on the 0.4% DEHP were mated, and pregnant females were isolated until they gave birth and the pups were weaned or died. The pups were removed from the mother after they had reached an age of 15 days, at which time they received a diet similar to the mother's. A portion of the animals in P_1 were sacrificed at the end of 1 yr of feeding of the plasticizer, and the rest at the end of the second yr. All animals in F_1 were sacrificed at the age of 1 year.

Over the 2-yr period there were a number of deaths in both the P_1 experimental animals and P_1 controls, but there was no indication that the treated animals had higher mortalities than the control animals. The majority of deaths were ascribed to lung infections. In the F_1 groups, the treated (at 0.4% DEHP) and nontreated animals had a similar mortality rate during the 1-yr study. It was interesting that the life expectancy of the P_1 groups receiving diets containing 0.4 and 0.13% DEHP exceeded that of the control group even though these were found not to differ significantly from the control animals.

At the 0.4% level both the P_1 and F_1 male animals had significantly lower body weights than the control animals, but the lower levels of DEHP appeared not to affect the weight gain. Food consumptions for the P_1 and F_1 animals were not significantly different from those of the controls at the end of the first year. Also at the 0.4% DEHP level, the mean liver and kidney weights were significantly greater for male P_1 rats after 1 yr, but controls did not differ significantly at any of the DEHP levels.

No tissue or organ pathology was evident which could be attributed to DEHP at any of the levels tested in the P_1 and F_2 groups. No hematologic changes were observed in the treated animals which were significantly different from controls. Fertility did not appear to be altered in the treated animals except in the case of F_1 rats at the 0.4% DEHP level and this effect was not considered significant by the authors.

In a separate study, guinea pigs were administered 0.13% and 0.04% DEHP in the diet for 1 yr (22). No real differences in deaths, growth, life expectancy, food consumption, liver and kidney weights, and pathology were observed which could be ascribed to the esters. The only unusual effect noted was that the kidneys of females on the 0.13 and 0.04% dose levels were larger than those of the controls, but the authors did not judge this as being deleterious.

The same investigators also included a 1-yr dog study in which the animals were administered, in capsules, 0.03 ml/kg per day, 5 days a week, for the first 19 doses and then 0.06 ml/kg per day until 240 doses were given (22). The results of this study demonstrated that DEHP had no significant effect on body weight, nor was there any significant difference between liver and kidney weights of treated animals and those of controls. One dog in the experiment initially received 0.06 ml/kg for a total of 77 doses with no ill effects and then received 0.09 ml/kg until an additional 169 doses had been administered. In this dog fatty vacuolation and limited congestion were observed in the liver as well as moderate congestion of the kidney. No apparent gross or microscopic pathology of tissues or organs of dogs was noted with the 0.06 ml/kg dose as compared with controls. The authors ascertained that a no-effect dose over a 2-yr period fell between 0.06 and 0.2 g/kg per day for rats and was approximately 0.06 g/kg per day for the guinea pig and dog. No evidence developed implicating DEHP as a carcinogenic agent.

In 1956, Harris, Hodge, Maynard, and Blanchet (23) published results of a chronic toxicity study on DEHP in rats and dogs undertaken in order to verify the results of previous studies. The ester was administered to rats, both male and female, in the feed at 0.5%, 0.1%, and 0.0% levels. Groups of animals were sacrificed at 3, 6, 12, and 24 months, and body weights, food consumptions, and organ weights (liver, testes, kidneys, lungs, brains, stomach, heart, and

spleen) were noted. Gross and histopathological studies were carried out on selected tissues and organs. Even though mortality of the treated animals was high over the 2-yr period, the number of deaths did not differ significantly from those in the control group. After 1 yr at the highest dose level (0.5%), the average weights of the animals were approximately 50 g less than those of the controls, and in the second year the average weights of the animals in the 0.5%, 0.1%, and 0.0% (control) groups were approximately the same. However, the total number of animals in all of the groups were quite small due to deaths and sacrifices. No significant differences were noted in food consumption up to 6 months for the treated animals as compared to the controls, but at the end of the first year the food consumption decreased drastically in the 0.5% group with little difference in the 0.1% group as compared to controls. At 6 months, enlarged livers and kidneys were observed in the animals on the 0.5% DEHP diet.

Two dogs were also used in this study; one was administered 5 g/kg per day for 14 weeks. A no-effect dose of 0.1 g/kg per day was ascertained. Some adverse effects were noted in the dog receiving 5.0 g/kg. Results of the studies in rats and dogs were in general similar to those reported by Carpenter et al. (*22*) in 1953.

Brown, Muir, and Thorpe (*24*) administered daily oral doses of 5 mg/kg of phthalates, having alcohol chain lengths varying from 7 to 11, to groups of rats for 7 consecutive days. There were no overt signs of toxicity other than general depression and persistent wetness of the hair. Histopathological studies of the organs showed periportal cytoplasmic vacuolation due to fat deposition in the liver.

In an unpublished report, Lawrence conducted a subacute toxicity study on DEHP by administering three dose levels of the ester subcutaneously to groups of rats, once a week for 12 weeks i.e., 5.0 g/kg, 100 mg/kg, and 25 mg/kg (W. H. Lawrence, Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, Tennessee, unpublished data). Weight gain, food consumption, and general health of the animals were observed, and at the end of the study, organ-to-body weight ratios were determined and hematological studies, BSP liver tests, and gross and histopathologic examination of tissues and organs were performed. No significant differences were noted for any of the criteria measured, at any of the doses, compared to the control animals. Selected organs were extracted, and thin-layer chromatography was used to detect the presence of DEHP. At the 5.0 g/kg and 100 mg/kg levels, DEHP was detected in the liver and kidney. The ester was also detected in the serum of the rats receiving the highest dose level. Since a total of 12 doses was administered at weekly intervals, the results cannot be compared to oral feeding studies lasting a year in which daily doses were given.

As already stated, the acute toxicity of DEHP is quite low as reflected by the LD_{50} (Table 2). In order to determine whether repeated administration of DEHP and DOP (dioctyl phthalate) would produce a cumulative toxic effect in mice, Lawrence administered several dose levels of these compounds IP to groups of mice 5 days per week for 11 weeks. The LD_{50} values, calculated on a weekly basis, are shown in Table 5.

At the end of the first week the LD_{50} of DOP was 6.40 ml/kg, which gradually decreased and plateaued at 10 weeks at 1.37 ml/kg. These results indicate that in some manner DOP has a cumulative toxic effect in these animals, since the compound was 4.67 times as toxic at 10 weeks as at the end of the first week. The LD_{50} of DEHP for the first week was found to be 25.41 ml/kg, which declined to 3.06 ml/kg by the end of 11 weeks; i.e., the material was 8.31 times as toxic at the end of 11 weeks as at the end of 1 week.

Since it is possible that animals and workers may be repeatedly exposed to DEHP by inhalation, Lawrence conducted an experiment in which air was bubbled through DEHP at a temperature of 50°C; the vapors were then cooled and passed into an inhalation chamber containing mice. The mice were exposed to these vapors for 1 hr three times a week for 12 weeks and observed for deaths

Table 5. Effect of repeated administrations of DOP and DEHP on LD_{50} in mice.[a]

Week	Apparent LD_{50}, ml/kg (95% confidence level)	
	Dioctyl phthalate (DOP)	Di-2-ethylhexyl phthalate (DEHP)
1	6.4017 (5.0120-8.1772)	25.4111 (20.3338-31.7557)
2	3.6288 (2.8945-4.5497)	12.7544 (10.8275-15.0334)
3	2.8453 (2.2843-3.5440)	9.2212 (7.2300-11.7681)
4	2.0579 (1.7125-2.4710)	7.8408 (6.3055- 9.7500)
5	1.8968 (1.5669-2.2963)	7.5292 (6.0053- 9.4400)
6	1.6796 (1.4127-1.9960)	6.4019 (3.4953- 5.6516)
7	1.6128 (1.3777-1.8881)	4.4446 (3.4953- 5.6516)
8	1.4872 (1.2826-1.7246)	3.3548 (2.6356- 4.2486)
9	1.4271 (1.2227-1.6680)	3.2134 (2.5507- 4.0482)
10	1.3714 (1.1714-1.6055)	3.0856 (2.4304- 3.9177)
11	1.3714 (1.1714-1.6055)	3.0586 (2.4304- 3.9177)

[a]Data of H. L. Lawrence, Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, unpublished data.

and other untoward responses. During this time, no deaths or unusual behavior were observed. At the end of the study the animals were sacrificed. The lungs, removed for gross and histopathologic examination, showed signs of diffuse chronic inflammation similar to a burn reaction. Since the chamber temperature did not exceed 27–28°C, it may be concluded that the pathology of the lungs was due directly to DEHP and not to a thermal effect.

Absorption, Distribution, Excretion, and Metabolism

Absorption: Phthalate esters can be absorbed from the intestinal tract, the intraperitoneal cavity, and the lungs. Even though dermal absorption is low with the higher molecular weight phthalate esters, recent evidence indicates that those of lower molecular weight, such as diethyl phthalate, may pass through the skin more rapidly than originally suspected. In particular, this was observed in rabbit experiments in which ^{14}C-diethyl phthalate was placed on the skin, and approximately 9% of the activity was recovered in the urine after 24 hr (E. O. Dillingham and M. Pesh-Imam, Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, Tennessee, unpublished data).

Distribution: Jaeger and Rubin (*1*) found that organs of two patients who had received large volumes of blood IV from DEHP-plasticized PVC blood bags contained significant quantities of DEHP. Presumably this substance was released to the blood during storage in PVC blood bags or by blood passing through PVC tubings. DEHP was found in spleen, liver, lung, and abdominal fat, with concentrations ranging from 0.025 mg/g in spleen to 0.270 mg/g in abdominal fat. In another study by these same investigators, 27% of an IV-administered dose of DEHP was recovered in the lungs of rats after 24 hr (R. J. Rubin, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, personal communication).

Dillingham and Pesh-Imam, in studies with ^{14}C-DEHP in which mice were injected with a single IV dose (unpublished data), found that after 7 days the highest specific activity was in lungs, with lesser amounts in the brain, fat, heart, and blood and no apparent preferential deposition in fatty tissue. In a similar experiment, except that ^{14}C-DEHP was administered IP, after 14 days the lungs still retained the highest specific activity as compared to the other organs and tissue. This information thus parallels the results of Jaeger and Rubin. In a third experiment, a large dose of unlabeled DEHP was administered IP,

followed 3 days later by an IV dose of ^{14}C-DEHP. Seven days later the mice were sacrificed and the same organs and tissues examined for the presence of labeled DEHP. The lungs again showed the highest concentration (activity) of DEHP. A similar 14-day experiment involving IP administration only and a 7-day experiment involving IV administration only showed essentially the same results, although the absolute amounts of phthalate ester (radioactivity) remaining in the organs at 14 days was less.

In a further set of experiments by Dillingham and Pesh-Imam using ^{14}C-DEP (diethyl phthalate), the ester was applied to the skin of rabbits and tissue distribution was determined qualitatively by autoradiography of tissue sections. After 3 days of dermal exposure, radioactivity was detected in lung, heart, liver, kidney, gonads, and spleen. The radioactivity was not random but showed distinct intracellular localization. Surprisingly enough, skin and subdermal fatty tissue at the site of application showed no radioactivity. The presence of the compound (or its metabolite) in the brain indicated the blood-brain barrier had been penetrated.

Excretion: Shaffer et al.(*21*), in one of the few studies on the excretion of phthalate esters, as early as 1945 reported the presence of phthalates (as phthalic acid) in the urine of man, dogs, and rabbits after single oral doses of DEHP, indicating that the ester is absorbed from the intestine. The human experiment was performed on two males: one receiving 10 g of DEHP and the second 5 g. Excretion of phthalate equivalent in the urine after 24 hr came to 4.5% of the total dose administered for the subject having received the highest dose and 2.0% for the subject with the lower dose. Results of the dog experiments followed the same general trend of excretion as noted for the human subjects. These investigators noted that rabbits excrete high levels of DEHP, equivalent to 26 to 65.4% of the original dose, in 3 days. Although quantitative determinations were not made, considerable amounts of DEHP equivalent were apparently excreted in the urine by the rats.

Urinary excretion of ^{14}C-DEHP by mice receiving pure phthalate ester IP and saturated saline solution of the ester IV was studied by Dillingham and Pesh-Imam (unpublished data). Excretion following IV administration was only slightly higher and closely paralleled that observed following IP administration, 68% and 63% respectively of the total dose having been excreted by 7 days (Fig. 1). The IV dose was lower than the IP by a factor of 10^4. A first-order decline in the daily excretion rate was observed following IV administration (Fig. 2). After reaching a peak, excretion followed a first-order decline with IP administration. The combined IP-IV experiment used the same dose levels as the independent IP and IV experiments and was carried out in the same manner except that the IP-administered DEHP was unlabeled and the IV administration of ^{14}C-DEHP was on the third day following the IP administration. The daily excretion rate (Fig. 2) and the cumulative (Fig. 3) following IV administration of the labeled compound were calculated on the assumption that the IV-administered ester reached rapid and com-

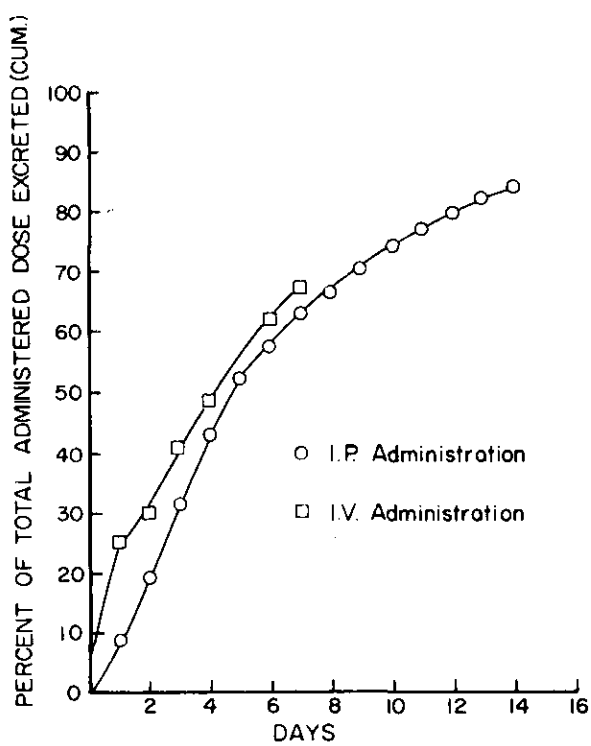


Figure 1. Cumulative urinary excretion of ^{14}C-DEHP by mice: (○) IP administration; (□) IV administration.

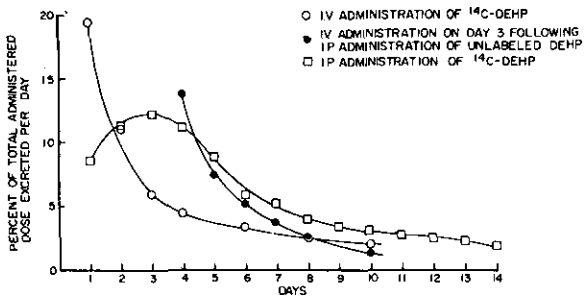


Figure 2. Rate of urinary excretion of ^{14}C-DEHP by mice: (○) IV administration of ^{14}C-DEHP; (●) IV administration on day 3 following IP administration of unlabeled DEHP; (□) IP administration of ^{14}C-DEHP.

plete equilibrium with the residual unlabeled DEHP pool (approximately 77% of the IV-administered dose) at 3 days. The close agreement of the cumulative IV and IP curves is consistent with the assumption of rapid equilibrium and with the conclusion that the DEHP pool at 3 days had not been metobolized to any large degree.

Approximately 83% of the total IP-administered dose was excreted by 14 days, and no significant radioactivity was found in the urine of mice after 35 days. A very interesting aspect of this study is that despite rapid excretion at 1400 days, there remained in the mice an amount of DEHP beyond that necessary to saturate the aqueous compartment of the body. An unresolved question is why the cumulative excretion rate (Fig. 3) shifts from zero order to first order at approximately 4 days at a time when there is approximately 20 times the amount of DEHP present required to saturate the aqueous compartment. A small amount of the ester (radioactivity) was detected in the feces but was not enough to account for any significant depletion of the pool. Also, the vapor pressure of the ester is too low to account for any significant losses by expiration, although this was not investigated.

A significant diuretic effect was observed following IV administration of the DEHP-saturated saline solution and a distinct suppression of urinary excretion following the IP injection of the pure ester. It was noted, however, that IV administration of the DEHP-saturated solution in the combined IP-IV experiment did not lead to diuresis, the urinary excretion volumes remaining approximately the same as for the IP experiment.

Rabbits with dermally applied labeled diethyl phthalate were found to excrete (in the urine) approximately 9% of the labeled activity after 24 hr, 14% after 48 hr, and approximately 16–20% within 72 hr. (Dillingham and Pesh-Imam, unpublished data). Excretion by other routes, such as in the feces or through exhaled air, was not followed. Some of the loss of the compound may have occurred at the site of application due to sorption into the cotton patches used to cover the application sites.

A very important question which still needs to be answered is the possible accumulation of phthalate esters in the body. If these esters are sequestered in some manner in specific organs and tissues of the body, will the body burden be exceeded, bringing about subtle toxic effects which may not be apparent until many months or years later? As of now, no information is available to indicate that body burdens are being exceeded or even what may be considered as a tolerable body burden.

Metabolism: It would be expected that esters of phthalic acid, under appropriate conditions, would hydrolyze, releasing phthalic acid and an alcohol. As with other biological aspects of phthalic esters, information on metabolism is minimal and at times

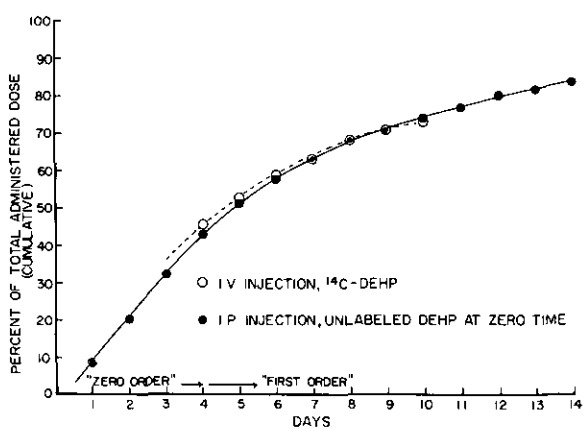


Figure 3. Urinary excretion of ^{14}C-DEHP administered IV to mice on day 3 after IP injection of nonlabeled DEHP: (○) IV injection, ^{14}C-DEHP: (●) IP injection, unlabeled DEHP at zero time.

quite vague. One of the few published studies on metabolism, in this case on DEHP, appeared in the experiments by Shaffer et al. (*21*). These investigators noted that urine of man, dogs, rabbits, and rats contained phthalic acid after administration of DEHP orally. By their methods they were not able to identify the ester as such in the urine. Thus, the question may be raised whether, if DEHP is metabolized in the body, the acute toxicity of the administered ester would be due to (1) the alcohol, (2) phthalic acid, or (3) perhaps a combination of alcohol, phthalic acid, and ester. Shaffer et al. (*21*) in further experiments on the acute toxicity of phthalic acid, 2-ethylhexanol, and DEHP, found LD_{50} values in rats of 7.9 g/kg (slope 16.11 ± 7.80), 7.1 g/kg (slope 4.09 ± 2.64), and 30.6 g/kg (slope 1.92 ± 0.98), respectively. The slopes of the LD_{50} curves of the alcohol and ester were similar, but the slope of the acid curve differed markedly, indicating that the alcohol and ester may have similar modes of action. It appears that, at least in rats, phthalic acid is not metabolized further, but this may not be the case for other species. Shaffer and associates concluded that the toxicity of the original ester is most likely due to the alkyl portion of the molecule rather than to the ester or acid.

Even though Hall and associates (*15*) did not specifically study the metabolism of dibutyl(diethylene glycol bisphthalate), they made a tentative conclusion that the oxaluria produced by high doses of DDGB was a result of the release of diethylene glycol, indicating that the plasticizer was biotransformed in the body.

In the more recent paper by Jaeger and Rubin(*1*), the conclusion was reached that butyl glycolylbutyl phthalate (BGBP) can be metabolized by the liver, whereas DEHP cannot. However, these experiments were run in isolated rat liver preparations. If deesterification takes place in the kidney in the process of excretion, the results are not incompatible. As more metabolic studies are performed in animals and humans with the various esters, more light may be shed on what actually does take place in the body.

Teratogenic, Mutagenic, and Carcinogenic Effects

Teratogenic Effects: Verrett et al. (*25*) demonstrated that a number of compounds, including phthalic acid, phthalimide, and phthalamide, related to thalidomide had teratogenic potential in the developing chick embryo. In another study by Guess et al. (*26*) on the subtle toxicity of a number of agents and ingredients used in the preparation of polymeric materials, chicks whose embryos had been exposed to phthalate esters showed deformities. These results suggested to Bower, Haberman, and Minton (*27*) that a more definitive study should be undertaken on the teratogenic and embryotoxic effects of the more useful phthalate esters, using chick embryos as the test organism. In such a study conducted on eight esters, dibutoxyethyl phthalate produced congenital malformations described as crania bifida and anophthalmia. Marked exophthalmia was also observed in some of the chicks. Central nervous system damage was noted in newborn chicks receiving dibutoxyethyl phthalate, di-2-methoxyethyl phthalate, and octyl isodecyl phthalate.

Singh, Lawrence, and Autian (*14*) studied the embryotoxicity and teratogenic effects of several phthalate esters in rats, which were injected IP at three different dose levels (1/10, 1/5, and 1/3 of the acute LD_{50}). Two of the compounds, i.e., dioctyl phthalate and di-2-ethylhexyl phthalate, because of their very low acute toxicity, were administered in doses of 5 and 10 ml/kg. All treatments took place on the 5th, 10th, and 15th day of gestation. After 20 days, the rats were sacrificed with an overdose of ether, and the uterine horns and ovaries were surgically exposed to permit counting and recording of the number of corpora lutea, resorption sites, and viable and dead fetuses. Additionally, the fetuses, both viable and nonviable, were excised, weighed, and examined for gross malformations. One third to one half of the fetuses, using those which showed no gross malformation whenever possible, were prepared as transparent specimens to permit visualizations of skeletal deformities. All the

esters, to some degree, produced gross or skeletal abnormalities which were dose-related (Table 6). Dead fetuses were found in groups treated with dimethyl, dimethoxyethyl, and diisobutyl phthalates. The most embryotoxic agent was dimethoxyethyl phthalate. All the esters decreased the size of the fetuses, as evidenced by lower weights than controls. Dioctyl and di-2-ethylhexyl phthalate seemed to have the least adverse effect on embryo-fetus development. Since lower doses were not used, a no-adverse-effect dose could not be ascertained.

Mutagenic Effects: No published information is available on the mutagenic effects of phthalate esters. In preliminary unpublished studies, Singh and associates of these laboratories, using mice and dominant-lethal assay, noted that both DEHP and DMEP appeared to act as mutagenic agents at the higher dose levels. Further confirming information, however, is still needed to establish that these esters are indeed mutagenic or may present this hazard to man.

Carcinogenic Effects: No animal data have yet demonstrated that any of the phthalate

Table 6. Gross and sketetal abnormalities produced by phthalate esters in rats.[a]

Treatment	Injected, ml/kg	Resorptions		Gross abnormalities[b]		Skeletal abnormalities[b]	
		No.	%	No.	%	No.	%
None	None	0		0		0	
Distilled water	10.00	4	6.8	0		0	
Normal saline	10.00	7	11.5	1	1.9	4	14.3
Cottonseed oil	10.00	4	6.8	1	1.8	3	10.7
	5.00	3	6.4	0		0	
Dimethyl	1.125	17	32.1	4	11.1	9	75.0
phthalate	0.675	0		4	7.5	6	35.3
	0.338	21	33.3	4	9.5	4	25.0
Dimethoxyethyl	1.245	55	96.5	2	100.0	2	100.0
phthalate	0.747	52	89.7	5	83.3	4	100.0
	0.374	16	27.6	1	2.4	13	92.9
Diethyl	1.686	2	3.6	0		13	81.3
phthalate	1.012	0		0		8	47.1
	0.506	28	44.4	0		5	26.3
Dibutyl	1.017	23	36.5	0		8	33.3
phthalate	0.610	2	3.6	0		7	24.1
	0.305	4	7.3	0		6	20.7
Diisobutyl	1.250	16	25.8	0		8	33.3
phthalate	0.750	3	5.5	2	3.9	5	17.2
	0.375	5	9.6	0		4	14.8
Butyl carbo-	2.296	13	24.1	1	2.4	5	21.7
butoxymethyl	1.378	8	14.5	1	2.1	4	16.0
phthalate	0.689	4	7.8	0		4	13.8
Dioctyl	10.00	5	8.3	15	27.3	0	
phthalate	5.00	2	3.8	8	15.7	0	
Di-2-ethylhexyl	10.00	15	26.8	9	22.0	0	
phthalate	5.00	5	8.2	0		0	

[a]Data of Singh, Lawrence, and Autian (*14*).

[b]Percentage resorption based on total number of implantations, gross abnormalities based on total number of fetuses, and skeletal abnormalities based on total number of stained fetuses.

esters act as carcinogenic agents. Likewise, their role as possible co-carcinogens has not been established.

Cellular Toxicity

As is apparent from this review, the acute toxicity of the phthalate esters in animals and humans is quite low under the experimental conditions used in evaluating the biological effects. There may, however, be more subtle toxic effects which up to now have not been revealed. For this reason, information at a cellular level may be helpful to those interested in the toxicity of phthalate esters in the broadest sense. Information in this specific area, as with many of the other areas of phthalate ester toxicity, is still quite limited.

Effect on Mouse Fibroblasts: Dillingham, Chambers, and Autian determined the effects of several phthalates on growth inhibition of mouse fibroblasts (L-cells) in tissue culture (unpublished data). By plotting the percentage growth vs. concentration, they obtained dose-response curves from which the ID_{50} values (or dose required to inhibit growth by 50%) could be estimated. From the slopes of these curves, the "intrinsic toxicities" T_i of the esters in cell culture were evaluated (shown in Table 7 with LD_{50} values for comparison). The *in vivo* toxicity, as evident from LD_{50} values, increased in general with molecular weight except for dibutyl phthalate, which was the most toxic. Tissue culture toxicity, as evident from ID_{50} values, increased with increasing molecular weight with the exception of dimethoxyethyl phthalate, which was the least toxic. The intrinsic toxicity in tissue culture followed the same trend but had a much wider range of variation, approximately 7×10^5 fold.

On the assumption that the *in vivo* toxicity should be proportional to the product of the intrinsic cellular toxicity and the water solubility (or maximum effective water concentration *in vivo*), the authors found the relation for the phthalate esters investigated to be as follows.

$$T_i M_s / LD_{50} = \text{Constant}$$

The relative constancy of the ratios (Table 8) suggests that for these compounds the intrinsic toxicity *in vitro* (in cell culture) and *in vivo* (in the rat) are basically the same. It also suggests that the water solubility of the compounds is directly related to the effective concentrations attained *in vivo* under the conditions used in the LD_{50} assay. In other words, the toxicity potential of the ester at a cellular level, whether in cell culture or in the intact animal, is related to the concentration of the agent in the aqueous environment in contact with the cells and the intrinsic toxicity of the compound. Although this simple empirical relation between *in vivo* and *in vitro* toxicity raises a number of basic questions relative to the nature and mechanism of expression of toxicity in the two systems, the authors have demonstrated a similar relation between *in vivo* and *in vitro* toxicity for a series of halogen- and methyl-substituted alcohols (*28*).

Warren and Glick (*29*) reported that the specific activity of the membrane and particulate fractions of mouse fibroblasts in labeled medium increased at a rate independent of cell division. Further, they showed a rapid

Table 7. LD_{50}, ID_{50}, and intrinsic toxicity of a group of phthalates.

Phthalate	Mol. wt.	Water solubility (M_s),mole/l	LD_{50}, mole/kg	ID_{50}, mole/l.	$T_i \times 10^3$, %/M
Dimethyl	194	0.0263	0.0235	0.007	14.3
Diethyl	222	0.0048	0.0202	0.003	47.0
Dibutyl	278	0.008	0.0138	0.0001	184,000
Dimethoxyethyl	282	0.0400	0.0159	0.0084	5.6
Di-2-ethylhexyl	390	0.0004	0.0151	0.00005	3,750,000

Table 8. T_iM_s/LD_{50} Values of a group of phthalate esters.[a]

Phthalate	T_iM_s/LD_{50}
Dimethyl	16,004
Diethyl	11,169
Dibutyl	12,000
Dimethoxyethyl	14,088
Di-2-ethylhexyl	11,680

[a] T_i is the intrinsic toxicity in cell culture and M_s is the molar solubility in water; LD_{50} is taken from rat experiments. The units are included in Table 7.

turnover of protein in nondividing cells. This led them to surmise that a protein synthesis rate in excess of that required by cell division may account for increased instability of the cell and increased susceptibility to toxicants. Dillingham, Wu, and Autian (published data) noted, in studies on dimethyl and dimethoxyethyl phthalate in cell culture, that these agents were highly toxic during the time that the cells were undergoing significant protein turnover. Even though these results cannot now be directly extrapolated to toxic events in animals, they suggest that any tissue which exhibits periodic increases in protein turnover related to changes in cell division rate and metabolic activity (protein synthesis) has an increased susceptibility to the toxic effects of phthalate esters. This may account for the teratogenic and embryotoxic effects of several of the phthalate esters reported in rats, since differentiating embryonic tissues have periodic major changes in cell division rates and metabolic activity.

Effect on Formed Elements of Blood: Some concern has been raised that perhaps phthalate esters which can enter patients through the use of poly(vinyl chloride) tubings and blood bags may be acting on formed elements of blood such as platelets, leukocytes, and erythrocytes. Circumstantial evidence suggests that at least some of the esters may change membrane properties, leading to adhesions of platelets and leukocytes (*30*). If these events can occur, microemboli may form and become lodged in the capillary tissues of the lungs. Occurrence of microemboli in patients who are highly debilitated may account for the syndrome of "shocked lungs," leading to death of the patient. Much more research is needed, however, in this specific area before factual information is available to substantiate what has been suggested.

Phthalates and Medical Devices

A large number of tubings, blood bags, infusion containers, surgical gloves, catheters, surgical drapes, and other medical items are of poly(vinyl chloride), containing rather large concentrations of phthalate esters, primarily DEHP. Cellulosic membranes used in dialysis units may also contain a phthalate ester. When these items are in contact with blood or infusion solutions or directly with tissue, there is a possibility that the esters as well as other additives may migrate from the material to the solution or tissue. As early as 1960, Meyler, Willebrands, and Durrer (*31*), in isolated rat heart experiments, noted that certain types of PVC tubings used in the perfusion apparatus would release additives from the plastic to the solution, which would affect the heart. Since in addition to the phthalate plasticizer the tubings contained stabilizers such as organotin compounds, the cardiotoxic effect may have been due to the plasticizer, to the organotin compound, or to both. The authors tentatively concluded that the organotin was most likely responsible for the cardiotoxic effects.

Cruickshank et al.(*32*) investigated the toxicity of rubbers and plastics used in transfusion sets. They noted that specific rubbers and certain PVC tubings were highly cytotoxic to cell cultures. Again, the toxic effects from the PVC may have been derived from the additives in the plastic rather than from the phthalate ester. In these experiments there was no attempt to isolate and identify the offending agents.

Braun and Kummel (*33*) in 1963 reported their results on a group of plastic containers, including PVC, used for storing blood and transfusion solutions. Most of their tests, both physical and chemical, were done on aqueous extracts of the plastic. They noted that the PVC material released phthalate ester as well as other additives to the extracting water.

This extract also produced hemolysis of red blood cells in *in vitro* tests. Since the phthalate esters, of themselves, are not considered hemolytic agents, the hemolysis noted by Braun and Kummell most likely resulted from the presence of one of the additives.

In the mid-sixties, Lawrence et al. (*34*), testing a large number of PVC tubings which were parts of commercially available administration devices, noted that a large number of these tubings produced various degrees of irritation when samples were implanted in the muscle of rabbits for short times. It was assumed that an organotin stabilizer was the offending agent and that the plasticizer, assumed to be DEHP, acted as a vehicle carrying the organotin into the immediate tissue.

Trimble et al. (*35*) reported that butyl phthalylbutyl glycolate (BPBG) was being extracted from PVC tubings, which were being used for extraction of blood and brown fat of groundhogs. The extracts, in turn, showed biological activity which was attributed originally to substances from the animal but later to the contamination of the extract with BPBG.

Guess, Jacob, and Autian (*2*), in 1967, published results on chemical contamination of a number of American PVC blood bags containing anticoagulant solution (ACD). All the anticoagulant solutions stored in these bags for up to 1 year were found to contain small concentrations of DEHP, 2-ethylhexanol, phthalic anhydride, phthalic acid, and some unidentified chemicals. The authors suggested that steps should be taken to prevent the contamination. Or, if the contamination was limited, a complete toxicological profile (gross and subtle) should be developed for these substances to demonstrate their safety.

Other investigators have now noted that PVC tubings and devices can release phthalate plasticizers to solutions, with the quantity of plasticizer extracted increasing as the solution becomes more lipoidal (blood, serum, etc.). For example, Jaeger and Rubin (*36*), in 1970, reported that blood and its anticoagulant solution (ACD) may contain 6 mg of DEHP/100 ml after being stored in PVC blood bags at 4°C for 21 days. In a more recent study, Jaeger and Rubin (*1*) noted that several phthalate esters could be extracted by blood from PVC tubings and blood bags. This report also showed for the first time that phthalate esters were present in tissues and organs of two deceased patients previously having received large volumes of blood, presumably stored in PVC blood bags.

During the past year in this country, PVC infusion containers have been introduced into medical practice. These containers may contain 250, 500, or 1000 ml of different types of aqueous solutions which will be administered to patients requiring large volumes of these specific fluids. The solutions are aqueous, and questions have been raised as to the possibility of plasticizers being released to these solutions. One specific manufacturer (Viaflex Plastic Container, Travenol Laboratories, Inc., Morton Grove, Ill.) includes on a label insert the following. "Solutions in contact with the plastic container can leach out certain of its chemical components in very small amounts within the expiration period, e.g., dioctylphthalate (DOP), up to 5 parts per million; however, the safety of the plastic has been confirmed in tests in animals according to U.S.P. biological standards for plastic containers as well as by tissue culture toxicity studies." Even though the plasticizer above has been identified as DOP, it is most likely DEHP.

Rubin has studied a number of these newer PVC infusion containers containing such solutions as: 0.9% saline, 5% dextrose, and 5% protein hydrolyzates (*37*). The solutions were stored in the containers for longer than 1 year under different ambient temperatures. The author was not able to detect the presence of DEHP in any of the three solutions greater than the blank value of the assay system used. These results thus differ from those of Guess et al. (2), who found DEHP in ACD solutions stored in blood bags. This suggests that the newer PVC containers are an improved version of those used by Guess and associates. It should, however, still be kept in mind that blood and solutions that are lipoidal in nature will extract microquantities of phthalate esters (as well as perhaps other additives) and

that these agents will then enter the patients. Marcel and Noel (*38*), for example, found high concentrations of DEHP in lipid extracts of human blood that had been stored in a PVC transfusion pack.

Even though the implication is given that phthalate esters may play a role in causing adhesiveness of platelets and leukocytes leading to microemboli, it should be noted that other types of containers may also cause the formation of microemboli. Swank (*39*) and Connell and Swank (*40*) have studied this problem and have suggested that "aged" bank blood be filtered in a specific manner prior to transfusion.

In 1971 a report by Neegaard et al. (*41*) implied that diethyl phthalate was the cause of several cases of hepatitis in a hemodialysis unit in a hospital. The plasticizer was present in a PVC tubing used in the dialysis apparatus. Removal of these specific PVC tubings for other PVC tubings (not containing diethyl phthalate) eliminated the health hazard. Since organotin compounds may have been present in the diethyl phthalate-PVC tubing, it is possible that these agents may have played a role in causing hepatitis (*42*). Present experimental evidence does not indicate that diethyl phthalate alone will cause hepatic damage in animals.

With the advent of the plastic blood bag (principally PVC with plasticizers and additives) in the 1950's, a number of studies were conducted to determine the effect these new containers had on whole blood or blood components as compared to these products stored in glass. Since the exact formula of these PVC containers was generally never revealed, it is now nearly impossible to attempt to compare results from one study to another. Wall et al. (*43*) observed that erythrocytes stored as whole blood in glass containers survive better than when stored in two types of plastic containers (from two different manufacturers of blood containers and assumed to be PVC). Strumia et al. (*44*) reported in 1955 that certain plastic containers appeared to damage red cells, reducing the post-transfusion survival rate, while other plastic containers produced a definite beneficial effect on red cells. A study published in 1964 by Ozge, Baldini, and Goldstein (*45*) indicated that platelet-rich plasma stored in plastic lost its clot-retracting and serotonin-uptake properties more rapidly than when the product was stored in silicone-coated or uncoated glass containers. In the ensuing years, improvements have been made in the PVC blood bags, and thus previous observation on the potential deleterious effects of PVC on stored blood and blood products may no longer be valid.

Environmental Problems

The approximately 900 × 10^6 lb of phthalate esters produced each year in this country dwarfs the production of both DDT and the polychlorinated biphenyls (PCP), which have now been established as threats to the environment. Since the phthalate esters are used in a large number of applications, from plasticizers to defoaming agents, the potential for their release to the environment is apparent.

Interest in the environmental aspects of phthalate esters is relatively recent. Mayer, Stalling, and Johnson (*46*) presented a report in 1971 implicating the phthalate esters as environmental pollutants. For example, in a limited survey in which they were measuring organochlorine insecticides and PCP residues, they noted that phthalate esters were also present. Fish contained dibutyl phthalate ranging from 0 to 500 μg/kg, while DEHP concentrations were as high as 3200 μg/kg. It was assumed that these fish had been exposed to industrial wastes containing the esters. These authors rationalized the source to be the diet, which probably had previously been contaminated in some way with the ester.

The same report stated that dibutyl phthalate has a relatively low level of acute toxicity to fish. But, since chronic toxicity studies had not been conducted, no information could be given as to the long-term effects of the ester on fish. The same authors did, however, report that the crustacean *Daphnia magna*, when exposed to 0.1 μg/l. of labeled dibutyl phthalate in a continuous-flow apparatus, accumulated phthalate residue amounting to 600 μg/kg within 10 days (or a

6000-fold increase). When the *Daphnia* was placed in uncontaminated water, 50% of the labeled phthalate was excreted in 3 days. Perhaps more significant was the discovery that a concentration of only 3 μg/l. of DEHP in the water was sufficient to significantly decrease growth and reproduction of the crustacean. In view of these observations and of the demonstrated teratogenic and mutagenic properties of phthalate esters in mice and rats and their toxicity in tissue culture, a question is raised as to the effect of these compounds on the reproductive processes of higher aquatic organisms.

Miyoshi and Harada (*47*) published a paper in 1970 which suggests very strongly that certain microorganisms have the ability to biosynthesize phthalate esters. Specifically, they found that a soil bacterium (bacterial strain BD_{34}) could utilize 2-butyne-1-4-diol to form two compounds, one being dibutyl phthalate. It is not apparent from the article, however, what precautions were taken to prevent contamination during the processing and analysis of the compounds.

Phthalate esters have been reported as being present in kewda (*48*), tobacco leaf (*49*) and lily of the valley (*50*), but the chemical structures of the esters have not been elucidated. Hayashi and associates (*51*) were able to identify a number of phthalate esters present in a perennial vegetable cultivated or growing wild in many regions of Japan, viz., dimethyl, diisobutyl, isobutyl *n*-butyl, dibutyl, diisoamyl, diamyl, dihexyl, and di-2-ethylhexyl. The authors did not state if they believed that the esters were biosynthesized, nor was there any indication as to the possibility that contamination had or had not occurred.

It appears plausible to assume that a variety of biological species may have the ability to biosynthesize phthalate esters even though information on this subject is quite limited. Sugiyama et al. (*52*) in 1966 reported the isolation and identification of diphenyl phthalate from a fungus (*Alternaria kikuchiana Tanaka*) which is pathogenic to pears grown in Japan. The authors concluded that the phthalate ester as well as a number of other agents found in the fungus were normal metabolites.

Since phthalate esters are being reported in different locations in the world and, in particular, in water bodies far removed from industrial centers, the question of how the phthalates reach these sites keeps arising. Information is now becoming available to indicate that trash no longer is confined to populated regions but that solid wastes are being seen thousands of miles from land. This past year there was a report that various types of plastic items have been found in ocean waters, indicating that these items are becoming man-made pollutants far removed from the source of manufacture and use (*53*). Since a number of these plastic items may contain phthalate esters as plasticizers, it is possible that these esters are being released to the ocean and, in turn, are being ingested by various types of marine organisms, including fish.

A more probable mechanism of transport of phthalate esters from sources of manufacture and use to remote sections of the country and world is through complexation with natural organic substances. For example, the esters may interact with fulvic acid which is present in humic substances in soils and waters. Since the fulvic acid-phthalate ester complex is soluble in water, the relatively insoluble esters can readily be carried off in water to other sections of streams, lakes, and oceans. Ogner and Schnitzer (*54*) have, in fact, found four phthalate esters (di-2-ethylhexyl, dibutyl, dicyclohexyl, and benzyl butyl phthalate) in humic substances as complexes with fulvic acid. These authors were extremely careful to preclude the possibility that these esters were contaminates and took very stringent steps in their processing to prevent contamination. Even though they leaned on the theory that the phthalate esters were most likely due to pollution, they did not completely rule out the fact that one or more of these same esters may have been biosynthetically produced in the humic material. It is clear, however, from their study that, if phthalate esters are discharged in one or more ways to soil and water, they can

combine with fulvic acid and thus be transported away from the original site of pollution.

The work of Jaeger and Rubin (*1*) showed that phthalate esters could indeed be entering man by exposure to large volumes of blood stored in PVC blood containers. Since this publication, however, phthalate esters have been noted in the blood and urine of persons known not to have received large-volume parenteral therapy from PVC containers (Rubin and Jaeger, personal communication). These phthalates may have entered the human body by several means, including ingestion in the diet, absorption through the skin, and inhalation. In certain other instances, the phthalate ester may have been biologically synthesized by the body. Nazir et al. (*55*), for example, isolated di-2-ethylhexyl phthalate from the mitochondrial fractions of bovine, rat, rabbit, and dog heart muscle, taking special pains to prevent contamination of their samples with objects containing phthalate esters. Other tissues which were examined were devoid of the ester. Not yet proved, however, is the possibility that the DEHP may have originally entered the animals through the diet or, perhaps, through inhalation.

Even though most of the phthalate esters have low volatility, they will volatilize from plastic materials, as, for example, in the case of automobiles containing vinyl furnishings. In hot climates the phthalate is released from the material and apparently coats microparticles, which then become distributed through the car environment, often coating the surface of the window (*56*).* This has apparently caused some problems by decreasing vision, and one major automobile company has now eliminated the use of DEHP in their interior furnishings. Implications of potential health hazards from inhaling these particles of DEHP have still not been defined, but one must certainly be concerned that long-term exposure could bring about subtle toxic effects not evident clinically at this time. Since humans may also be absorbing phthalate esters by other means such as from the diet or through the use of various types of cosmetic items in which phthalates may be used as perfume bases, the body burden may be adequate to produce a health hazard under conditions of prolonged exposure.

* The news item did not give the exact name of the plasticizer in PVC, but it may be assumed to be one of the phthalates.

Health Threats

Do the phthalate esters present a health threat to the population, either as a direct consequence of exposure or indirectly through ecological disturbance? Definitive answers to this question cannot be given at this time. From the review, however, it can be stated that in general the phthalate esters have a very low degree of acute toxicity to animals and man. Long-term feeding studies in several species of animals, which indicate that a no-effect dose may range from approximately 0.1 to 0.2 g/kg per day, have for the most part used phthlate ester (DEHP). Assuming that this range is also applicable to humans, and imposing a 100-fold safety factor, a 70-kg man in a normal state of health could ingest from 70 to 140 mg of DEHP on a daily basis for long periods of time without its presenting an appreciable health hazard. Unfortunately, what is still not known is the level of exposures man has to DEHP and, in general, to all the phthalate esters, keeping in mind that it is now known that the esters can enter the body through a number of routes including the mouth, the skin, the lungs, and even, on occasions, through parenteral administration.

Even though it is highly unlikely that pregnant females will be exposed to sufficiently high levels of phthalate esters to produce embryotoxic effects and birth defects, caution should prevail, and expectant mothers should be afforded maximum protection against these esters. Circumstantial evidence also suggests that the phthalate esters may act as mutagenic agents, but this cannot be established as fact until more research is conducted in this specific area of

toxicology. Much less is known on the possible subtle toxic effects that low doses of phthalate esters may have on man, but research at the cellular level does indicate that the higher molecular weight esters have an extremely high intrinsic toxicity.

Ill patients, in particular debilitated patients requiring large volumes of blood or other infusion solutions, should not be exposed to any of the phthalate esters if at all practical. Here "benefit to risk" must be the deciding factor, but as yet the phthalate risk is poorly defined.

A very real situation appears to be developing in regard to phthalate esters as environmental pollutants. Even though minimal evidence is at hand demonstrating environmental damage by phthalates, the information does point to the possible toxic effect of the phthalate esters on marine life. To prevent potential ecological organisms and their surroundings, steps should be taken to restrict the quantity of phthalate esters entering our streams, rivers, lakes, and oceans.

Even though at the present time phthalate esters do not pose an imminent health threat to our environment and to man directly, their continued and expanding use and disposition without some form of controls could pose long-range tragedies for mankind. The high benefit to risk ratio in the past may not prevail in the near and distant future. Only through continued and expanded toxicological research and research dealing with the relation of these esters to man's environment will a more accurate and realistic "benefit to risk" be established for these esters in the future. One step which most likely should be taken now is to start encouraging industry to restrict the dumping of phthalate esters into adjacent water bodies.

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