Toxicology of Phthalic Acid Esters Used in Food-Packaging Material

by S. I. Shibko* and H. Blumenthal*

Phthalic acid esters may be used as plasticizers in food-packaging materials that have direct contact with food. Under normal conditions of use small amounts of the plasticizers would be expected to migrate into the food. The safe use of the phthalic acid esters under these conditions is based on available toxicity data, as well as regulations which, in general, specify the maximum extractable fraction of plasticizer that may enter food.

Before the enactment of the Food Additives Amendment to the Federal Food, Drug and Cosmetic Act in 1958, sanctions were granted for the use of five phthalates in food-packaging material (diethyl phthalate, butylphthalyl butyl glycolate, ethylphthalyl ethyl glycolate, diisooctyl phthalate, and di-2-ethylhexyl phthalate), with the limitations that they be used in accordance with good manufacturing practice for food packaging materials, and that di-2-ethylhexyl phthalate and diisooctyl phthalate be used with foods of high water content. Foods of high water content were defined as those from which no fat would be extracted when the food was pressed against filter paper; under the conditions of this test, if a water spot was formed the food was considered aqueous and if a fat spot was formed the food was considered fatty. The restriction was applied to di-2-ethylhexyl phthalate and to diisooctyl phthalate because the available toxicological data would not support unlimited migration into fatty foods. An additional 18 phthalate esters are now listed in the Code of Federal Regulations, 21. Food and Drugs.

The regulated uses of phthalates may be classified into three categories that reflect the possible levels of direct migration to foods; namely, significant, slight, and essentially zero. Table 1 lists regulated uses that could result in migration of phthalates to foods. The uses listed in Table 1 include (a) those that will be major contributors of phthalate migration to foods (includes prior sanction), and (b) those that will result in slight migration to foods. Regulated uses that, under normal conditions, would not be expected to result in migration to foods are listed in Table 2.

Phthalate esters that are regulated for uses that would be expected to result in migration to foods are listed in Table 3. Each ester may have several uses, some of which would result in migration to foods and others which would not. Some phthalate esters are regulated only for uses that would not be expected to result in migration to foods under normal conditions of use (Table 4), e.g., adhesives. The use of adhesives is based on the requirement of a functional barrier between the adhesives and the food to prevent any migration of the adhesive.

The extent of toxicological studies required to establish the safety of authorized use of phthalate esters will depend on the level of migration to food. In the case of

*Division of Toxicology, Food and Drug Administration, Washington, D. C. 20204.

Table 1. Regulated uses of phthalate esters that could result in migration into foods.[a,b]

121.2001	Substances employed in manufacture of food packaging material (prior sanction)
121.2511	Plasticizers in polymeric substances
121.2514	Resinous and polymeric coatings
121.2526	Components of paper and paperboard in contact with aqueous and fatty foods
121.2550	Closures with sealing gaskets for food containers
121.2531	Surface lubricants used in the manufacture of metallic articles
121.2569	Resinous and polymeric coatings for polyolefin films
121.2507	Cellophane

[a]Code of Federal Regulations 21. Food and Drugs. Jan. 1, 1972.

[b]Order of listing reflects possible level of migration into foods (i.e., 121.2001, 2511, 2514, 2526 and 2550 will result in greatest migration; 121.2531, 2569, 2507, only slight migration).

phthalate esters approved by prior sanction, theoretically all the phthalate esters (except those intended for use with foods of a high water content) used in food-packaging material could migrate into fatty foods held for a period of time in the package. The amount available for migration will be limited by good manufacturing practice for food-packaging materials, which includes a restriction that the quantity of the substance used shall be reduced to the least amount reasonably possible. In addition, if all the phthalate esters were leached out of the plastic packaging material it would no longer be functional. Since levels of migration of these phthalate esters to food might be high,

Table 2. Regulated uses of phthalate esters that under normal conditions of use would not reasonably be expected to result in migration to foods, based on available scientific information and data.[a]

121.2577	Pressure-sensitive adhesives
121.2562	Rubber articles intended for repeated use
121.2571	Components of paper and paperboard in contact with dry food
121.2519	Defoaming agents used in the manufacture of paper and paperboard
121.2520	Adhesives

[a]Code of Federal Regulations 21. Food and Drugs. Jan. 1, 1972.

Table 3. Phthalate esters regulated for uses that could result in migration to foods.[a]

Diisooctyl phthalate[b]	Di-*n*-hexyl phthalate
Ethylphthalyl butyl glycolate[b]	Diphenyl phthalate
Di-2-ethylhexyl phthalate[c]	Dibutyl phthalate
Diethyl phthalate[c]	Diisobutyl phthalate
Butylphthalyl butyl glycolate[c]	Diisodecyl phthalate
Butyl benzyl phthalate	Dimethylcyclohexyl phthalate
Dicyclohexyl phthalate	Dihydroxyabietyl phthalate
Castor oil phthalate, hydrogenated	

[a]See Table 1 for regulated uses. Note: esters usually have one or more regulated uses. Most of these esters are also regulated for uses which would not be expected to result in migration to foods.

[b]Prior sanction for foods of high water content only.

[c]Prior sanction.

Table 4. Phthalate esters regulated for uses that under normal conditions of use would not reasonably be expected to migrate into foods.[a]

Dibutoxyethyl phthalate
Di-2-ethylhexyl hydrophthalate
n-Octyl *n*-decyl phthalate
Dioctyl phthalate
Butyl octyl phthalate
Dimethyl phthalate
n-Amyl *n*-decyl phthalate
Methylphthalyl ethyl glycolate

[a]See Table 2 for list of regulated uses.

chronic toxicity data were obtained for four of the five phthalate esters whose use was authorized by prior sanction (diethyl phthalate, di-2-ethylhexyl phthalate, ethylphthalyl ethyl glycolate, butylphthalyl butyl glycolate).

The source and level of phthalate migration into foods resulting from their regulated uses is relatively well defined by food additive and supporting data submitted to the FDA. In general, the possible levels of migration have been derived by using the standard petition extractive tests outlined in the FDA 1966 guidelines *(1)*. The safety basis for phthalate esters whose regulated use would not result in migration to foods, e.g., those in adhesives, may be based primarily on lack of migration rather than detailed toxicity studies. In the case of low levels of migration, the phthalates may be considered as a class because the subacute and chronic studies of a number of compounds in this group show a low order of toxicity and because any toxicity may be related to the alcohol moiety. Further, metabolism of these phthalate esters would be expected to give rise to phthalic acid and alcohols, which have been adequately studied. The availability of these data permits a general approach to the toxicity of these compounds. Since the large number of phthalates and their regulated uses could result in many phthalate esters migrating to foods, another approach has been to base the possible hazard from these residues on that which might occur if the sole migrating phthalate was the most toxic of the group. Di-2-ethylhexyl phthalate may be used as the standard.

The major migration of phthalates from packaging material will occur with fatty foods. Fatty foods usually constitute only about one tenth of the total diet in the United States *(2)*. On this basis, the level of phthalates possibly present in the total diet will be reduced. In addition to concern for the type of food which may contain phthalate residues as a result of the migration from packaging, it is important to consider the section of the population that may be exposed to the phthalate esters. For example, CFR 121.2562(h) states that rubber articles intended for repeated use specifically exclude rubber nursing bottle nipples.

Toxicity data relating to the phthalic acid esters used in food packaging include: (a) acute oral LD_{50} (Table 5); (b) subacute toxicity studies in one or more species of laboratory animals for 13 phthalate esters; and (c) chronic toxicity studies for seven of the phthalate esters. A summary of the available data is presented in Table 6. Some special studies, including metabolism, teratogenicity, and chick embryo studies, have been reported for a number of phthalate esters. Reproduction studies have been carried out with some phthalate esters. Toxicity data from studies with dibutyl phthalate, diisodecyl phthalate, di-2-ethylhexyl phthalate, and ethylphthalyl ethyl glycolate, will be presented in detail to provide information on the most widely used plasticizers and to present a reasonable toxicity profile for the presently regulated phthalates.

Dibutyl Phthalate

The acute oral LD_{50} in the rat is 8–16 g/kg. When rats were dosed twice weekly with dibutyl phthalate (1 ml/kg of body weight of a solution in oil) for a period of 6

Table 5. Acute oral LD_{50} values for phthalate esters in the rat.

Phthalate ester	LD_{50}, g/kg body wt
Di-2-ethylhexyl phthalate	31
Diethyl phthalate	9.5 - 31
Butylphthalyl butyl glycolate	7
Butyl benzyl phthalate	18
Dicyclohexyl phthalate	>40
Di-*n*-hexyl phthalate	29.6
Diphenyl phthalate	8
Dibutyl phthalate	8 - 16
Diisobutyl phthalate	15
Diisodecyl phthalate	64
Dimethyl phthalate	6.9

Table 6. Available data on no-effect levels of phthalates for rats and dogs (oral administration)

Phthalate	No-effect level, mg/kg of body weight/day[a]				
	Subacute		Chronic		
	Rat	Dog	Rat	Dog	Reference[b]
Dimethyl phthalate	—	—	1000(104)	—	*(3)*
Diethyl phthalate	2500(6)	1250	1250(104)	625(52)	FDA
Dibutyl phthalate	50(16)	—	125(52)	18(52)	*(4)*, *(5)*, FDA[a]
Dialkyl 79 phthalate	60(13)	—	—	—	*(6)*
Di-*n*-hexyl phthalate	50(13)	125(13)	—	—	FDA
Diisobutyl phthalate	50(16)	25-500(18)	—	—	FDA
Diisooctyl phthalate	100(4)	100(14)	—	—	FDA
Diisodecyl phthalate	150(13)	75(13)	—	—	FDA
Diphenyl phthalate	1000(13)	500(13)	—	—	FDA, *(7)*
Dicyclohexyl phthalate	—	—	27(104)	14(52)	*(5)*, FDA
Methylphthalyl ethyl glycolate	240(4)	—	750(104)	—	FDA
Ethylphthalyl ethyl glycolate	500(17	—	250(104)	250(52)	*(8)*, *(8)*, *(8)*
Butylphthalyl butyl glycolate	—	—	450(104)	140(104)	FDA
Di-2-ethylhexyl phthalate	200(13)	500(13)	65(104)	60(52)	(9), *(10)*, *(11)*, *(11)*
Dibutoxyethyl phthalate	500(4)	—	—	—	*(12)*
Butyl benzyl phthalate	500(13)	250(13)	—	—	*(7)*

[a] Figures in parentheses represent duration of study in weeks.

[b] Reference numbers are listed for the data appearing from left to right in the table; FDA denotes data were obtained from FDA files.

weeks, no adverse effects were reported. Another group of rats was maintained on this regimen for 1½ yr without any adverse effects on the parameters studied, which included hematology, pathology of organ tissues, and organ weights *(5)*. In another study *(4)*, there was no effect on growth or survival when rats were maintained for 1 yr on diets containing 0.2% dibutyl phthalate. At the 1.25% dietary level, although half the rats died during the first week of the study, the survivors did as well as controls. The significance of the first-week deaths was not stated.

A three-generation reproduction study has been reported by Bornmann et al. *(5)*. Female rats were dosed daily with a 50% solution of dibutyl phthalate in oil at 1 ml/kg of body weight. After 6 weeks of treatment the female rats were paired with untreated males. The offspring were bred to produce two additional generations. Details of treatment of offspring are lacking. There was no impairment of reproductive performance. The average weight of endocrine organs of F_1 rats at day 71 of the test was within the range of normal values. In addition, the first incidence of oestrous was normal. Development, growth and fertility throughout the three-generation studies were normal.

Singh et al. (*13*) reported teratogenic studies with dibutyl phthalate given by intraperitoneal (IP) injection to female rats (1/10, 1/5, or 1/3 the LD_{50}/dose) on days 5, 10, and 15 of gestation. Results showed a

partially dose-related increase in resorption (4–37%), no gross abnormalities, and a 20–30% incidence of skeletal malformations, particularly elongated and fused ribs.

Preliminary *in vitro* studies had shown that dibutyl phthalate is hydrolyzed by pancreatic lipase as rapidly as triolein *(4)*, suggesting that phthalates may be transported and metabolized via the same pathways utilized for fat metabolism. However, rats given dibutyl phthalate orally excreted the monobutyl ester as the principal metabolite in the urine, with phthalic acid as the secondary metabolite *(14)*.

Diisodecyl Phthalate

A 14-week feeding study with rats and dogs established a no-effect level of 0.1 g/kg of body weight for both species *(15)*. At the highest levels fed (1%), a slightly elevated liver/body weight ratio was noted in all male dogs, and in 2/3 of the females. Pathological examination revealed swollen and vacuolated hepatocytes in the livers of these animals. In rats, particularly in the males maintained on diets containing 1% of the phthalate ester, livers were markedly heavier than those of controls. No histological changes were observed. Reproduction studies and other special studies have not been reported.

Di-2-ethylhexyl Phthalate (DEHP)

The acute oral LD_{50} values are 30.6 g/kg of body weight for the rat and 33.9 g/kg for the rabbit. Subacute and chronic toxicity studies have been reported *(9–11)*. In general, chronic feeding studies with rats have indicated a no-effect level of 60 mg/kg of body weight/day. At higher dose levels (200–400 mg/kg/day), depressed growth rates as well as enlarged liver and kidneys were reported. In a study with guinea pigs maintained for 1 yr on a diet containing 0.04–0.13% of DEHP, the only effect noted was increased liver weight in females. Since the effect was not dose-related and no histopathological lesions were observed, the significance of this effect is not known. When dogs were fed 0.03 ml/kg of body weight/day of DEHP 5 days a week for a total of 29 doses and then 0.06 ml/kg/day for a total of 240 doses or 77 doses of 0.06 ml/kg day followed by 169 doses at 0.09 ml/kg/day, satisfactory weight gain was observed. Hematologic and biochemical tests, including liver function tests, were normal. The dog maintained on the high dose level showed some histological changes in the liver and kidney. These were reported as congestion in the subcapsular area of the liver and moderate congestion of the kidney with cloudy swelling.

Limited reproduction studies with rats maintained on dietary DEHP at levels of 0.04, 0.13, or 0.4% have been reported *(10)*. The rats were bred, and the F_1 offspring were maintained on the test diet for 1 yr. Comparison of reproductive performance (litters born, total numbers of pups born, mean size of litters, maximum numbers of litters by any female, pups stillborn) showed that the only valid change in the test animals was a decrease in the mean number of litters per female among the F_1 rats at 0.4% dietary level. Both the parental and first filial generations maintained in the 0.4% dietary group showed increased liver and kidney weights. However, no significant histopathologic effects were observed.

Teratogenic studies *(13)* showed that although IP administration of DEHP to female rats caused some resorption of fetuses, no teratogenicity was observed. In a previous study, McLaughlin et al. *(16)* reported that the undiluted ester did not have any effect on the development of the chick embryo. Limited metabolic studies have also been reported in the dog, rabbit, and man *(9)*. Dogs dosed with DEHP at approximately 0.2 g/kg of body weight excreted phthalate equivalent to 2.0–4.5% of the dose in the urine in a 72-hr period subsequent to dosing. In a study with rabbits dosed with DEHP at approximately 1–0.6 g/kg of body weight, 26–65.4% of the administered dose was excreted in the urine. No increase in urinary excretion of glycuronic acid, ethereal sulfate, or conjugated amino acids was observed in

rabbits and rats in these studies, although a large increase in the urinary excretion of fatty acids was observed. The significance of the increased urinary fatty acids is not known. Humans administered 5 or 10 g of DEHP excreted approximately 4.5% of the dose in urine in a 24-hr period after dosing. Most was excreted between 5 and 7 hr after the dosing.

o-Phthalic acid is excreted unchanged in dogs, rabbits, and humans. Since the compound is almost quantitatively excreted in the urine following oral administration *(17)*, it has been considered likely that the phthalate content of the urine is a measure of the intestinal absorption of the phthalate ester. Hydrolysis of DEHP may not occur in the liver. When isolated rat livers were perfused with solutions containing DEHP, the DEHP was not metabolized but accumulated in that organ, primarily in the unmetabolized form *(18)*.

Ethylphthalyl Ethyl Glycolate (EPEG)

In studies reported by Hodge et al. *(8)*, rats were maintained on diets containing 0, 0.05, 0.5, or 5.0% EPEG for 2 yr. Rats in the group given 5% showed retardation in growth and longevity, with none of the males surviving the 55th week of feeding and none of the females surviving the 72nd week. Hematological data were normal with the exception of a slight anemia in the group given 5% EPEG. Urine analyses were normal in the groups given 0.05 or 0.5%. Elevated sugar levels were observed only in the group given 5%, and histopathological studies indicated marked changes in the kidneys of these rats. The changes consisted of crystalline masses of calcium oxalate in the renal tubules, and the origin of these crystals was attributed to the ethyl glycolate moiety. Dogs dosed daily with EPEG at dose levels of 0.01, 0.05, or 0.25 g/kg for a period of 1 yr showed no compound-related effects. Specifically, oxalate crystals in the renal tubules or other kidney lesions did not appear. Reproduction and other special studies have not been reported.

Summary

All phthalates studied have a low order of acute toxicity. The no-effect levels of phthalates based on chronic toxicity studies show ranges from 65 to 1625 mg/kg of body weight/day for the rat, with similar values for the dog. In general, no specific lesion has been identified with the feeding of the phthalate esters. Effects may be due to the ester moiety, as in the case of ethylphthalyl ethyl glycolate, where the specific tissue damage observed, namely, crystalline masses of calcium oxalate in the renal tubules, could be attributed to metabolism of the ethyl glycolate moiety. No cases of unusual incidence of carcinogenesis in the chronic feeding studies with phthalates have been reported.

In general, good metabolic data on the phthalate esters are lacking. It would be highly desirable to carry out studies to determine the extent of absorption and subsequent metabolism in a species that is known to metabolize the phthalate moiety in a manner similar to man. Although it has previously been assumed that the phthalate esters would be hydrolyzed to free phthalic acid and the alcohol in the gut and the products of digestion absorbed, the more recent information suggests that some phthalate may be absorbed unchanged and that metabolism in the rat may not proceed greatly beyond the monoester stage *(14)*. The structure of the phthalate ester may be an important factor in determining the site and rate of metabolism, since butyl glycolylbutyl phthalate was metabolized by isolated perfused rat liver, whereas DEHP accumulated in the liver unchanged *(18)*. It is not known if phthalates absorbed via the respiratory route are metabolized in the same way as dietary phthalates. Metabolism via this route needs adequate study, particularly if this is shown to be an important route of exposure.

The significance of the teratogenic effects following IP administration of large doses of phthalate cannot be assessed in terms of the normal dietary exposure. For safety evalua-

tions of substances that may enter the diet, emphasis must be placed on the results of oral toxicity studies, and there is a need to carry out studies with phthalates given orally at levels related to their possible daily intake.

In summary, the available information indicates that the levels of phthalates occurring in the diet from authorized uses do not pose any toxicological hazard. It is recognized that this statement is based in part on the premise that phthalates as a class are metabolized in a similar manner, thus allowing a general approach to the toxicity of these compounds.

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