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Carcinogenicity Testing of Phthalate Esters and Related Compounds by the National Toxicology Program and the National Cancer Institute

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Five phthalate esters and related compounds (phthalic anhydride, phthalamide, di(2-ethylhexyl) phthalate, di(2-ethylhexyl) adipate and butyl benzyl phthalate) have been tested for carcinogenic effects in standard lifetime rodent feeding studies. Groups of 50 male and female rats and mice were fed diets containing various concentrations of the test chemicals for 102–106 consecutive weeks. The dietary concentrations were estimated to be maximally tolerated doses and half maximally tolerated doses. All animals that died during the study and all survivors at the end of two years were examined grossly and microscopically for the presence of tumors. The incidences of animals with tumors at a specific anatomic site in the treated groups and the controls were compared statistically.

Neither phthalamide nor phthalic anhydride increased tumor incidences in rats or mice. Di(2-ethylhexyl) phthalate increased the incidences of liver tumors in rats and mice of both sexes, while di(2-ethylhexyl) adipate caused liver tumors in male and female mice, only. Butyl benzyl phthalate did not cause tumors in male or female mice, but the incidence of myelomonocytic leukemia in butyl benzyl phthalate-treated female rats was significantly greater than that in the controls. Chemically induced early deaths in the butyl benzyl phthalate-treated male rats precluded an evaluation of carcinogenic potential in this sex.

Under the conditions of these tests, di(2-ethylhexyl) phthalate was considered to be carcinogenic in both rats and mice and di(2-ethylhexyl) adipate was considered to be carcinogenic in mice. The evidence for carcinogenic effects of butyl benzyl phthalate in female rats was judged to be equivocal because of the variable nature of the incidence of myelomonocytic leukemia in Fischer 344 rats. Phthalamide and phthalic anhydride did not exhibit carcinogenic effects in these studies.

Introduction

Di(2-ethylhexyl) phthalate (DEHP), di(2-ethylhexyl) adipate (DEHA) and butyl benzyl phthalate (BBP) are chemicals frequently added to plastic formulations to impart flexibility or other characteristics that determine product use. Such plasticizers do not become part of the polymer matrix and, under certain use or disposal conditions, can migrate from the plastic to the external environment. Although they are not components of the plastic polymer, plasticizers such as DEHP may comprise as much as 50% (by weight) of highly flexible poly(vinyl chloride) products. DEHP is also used as a hydraulic fluid and as a dielectric fluid. Phthalate esters, not surprisingly, have become ubiquitous environmental pollutants.

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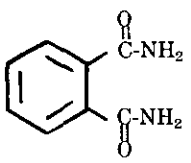
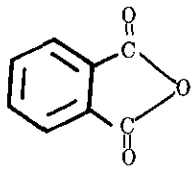
Phthalate esters are generally synthesized by the condensation of alcohols with phthalic anhydride. Phthalamide is not used as a plasticizer or in the synthesis of phthalate esters. Rather, it is an intermediate in the commercial synthesis of organic amines. All five of these chemicals are being compared, however, because of similarities in chemical structure (Fig. 1).

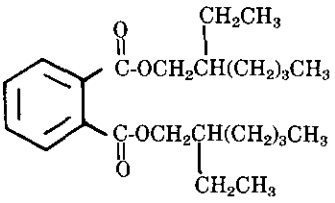
The National Toxicology Program (NTP) and its predecessor, the National Cancer Institute Carcinogenicity Bioassay Program, tests chemicals for carcinogenic potential as part of a broad initiative to aid governmental regulatory agencies in protecting the public health. Among the criteria used in choosing chemicals for testing are volume of production, number of persons exposed and frequency and level of anticipated human exposure. Phthalate esters and related compounds were tested because of their presence in high concentrations in plastics, the ubiquity of phthalate ester-plasticized products in the home, work and out-of-doors environments and because of a general lack of previous chronic toxicity testing with these agents.



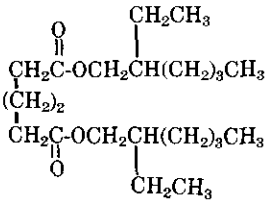
Phthalamide



Phthalic Anhydride



Di(2-ethylhexyl) Phthalate



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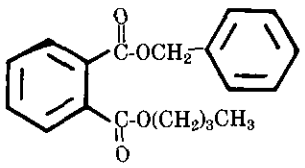

Butyl Benzyl Phthalate

FIGURE 1. Chemical structures of the five compounds tested.

Methods

Male and female rats of the inbred Fischer 344 strain and male and female mice of the hybrid $B6C3F_1$ strain, obtained from Frederick Cancer Research Center (Frederick, Md.), were used in all of the studies. The test chemicals were incorporated into the feed and supplied to the animals for 102–106 consecutive weeks beginning at 4–6 weeks of age. In general, each treatment group consisted of 50 animals of each sex and species at the estimated maximally tolerated dose (referred to as the high dose), one-half of the estimated maximally tolerated dose (referred to as the low dose) and untreated controls (control groups for phthalamide and phthalic anhydride consisted of 20 animals, control groups for other chemicals consisted of 50 animals). Estimated maximally tolerated doses were determined by preceeding 90-day subchronic feeding studies.

All animals that died during the study (and were not excessively cannibalized or autolyzed) and all survivors at the end of the study were subjected to a gross necropsy and a complete micropathological examination. Statistical comparisons of the incidences of animals with tumors at a specific anatomical site and of survival and body weight gain were made using both pairwise comparisons and trend tests. The basic designs of the studies conformed to the recommendations of the National Cancer Institute (*1*). Complete technical details of each of the bioassays are available in the form of technical reports from the NTP (*2-6*). Test chemical source, composition and doses for each of the studies were as follows.

Phthalamide

Phthalamide was obtained from Sherwin-Williams, Inc. (Pittsburgh, Pa.) and demonstrated by HPLC to be greater than 99% pure. Concentrations of phthalamide in the feed were 0 (control), 15,000 and 30,000 ppm for male rats; 0, 5,000 and 10,000 ppm for female rats; 0, 25,000 and 50,000 ppm for male mice; and 0, 6,200, 12,500 and 25,000 ppm for female mice.

Phthalic Anhydride

Phthalic anhydride was obtained from Koppers Co. (Pittsburgh, Pa.) and demonstrated by HPLC to be 98.8% pure. Concentrations of phthalic anhydride in the feed were 0 (control), 7,500 and 15,000 ppm for male and female rats. Male mice were fed diets containing 0, 25,000 and 50,000 ppm for the initial 32 weeks and 0, 12,500 and 25,000 ppm for

the remainder of the study; female mice received 0, 25,000 and 50,000 ppm for 32 weeks and then 0, 6,250 and 12,500 ppm for the rest of the study.

Di(2-ethylhexyl) Phthalate

DEHP was obtained from W. R. Grace and Co. (Fords, N.J.) and demonstrated by thin-layer and vapor-phase chromatography to be greater than 99.5% pure. Concentrations of di(2-ethylhexyl) phthalate in the feed were 0 (control), 6,000 and 12,000 ppm for male and female rats and 0, 3,000 and 6,000 ppm for male and female mice.

Di(2-ethylhexyl) Adipate

Di(2-ethylhexyl)adipate was obtained from W. R. Grace and Co. (Fords, N.J.) and demonstrated by thin-layer and vapor-phase chromatography to be greater than 98% pure. Concentrations of di(2-ethylhexyl) adipate in the feed were 0 (control), 12,500 and 25,000 ppm for both male and female rats and mice.

Butyl Benzyl Phthalate

Butyl benzyl phthalate was obtained from Missouri Solvents and Chemicals (Kansas City, Mo.) and demonstrated by thin-layer and vapor-phase chromatography to be greater than 98% pure. Concentrations of butyl benzyl phthalate in the feed were 0 (control), 6,000 and 12,000 ppm for both male and female rats and mice.

Results

Phthalamide

Phthalamide administration did not decrease body weight gain nor lessen the probability of survival in either sex of rats or male mice. Survival for female mice at the end of two years, however, was only 36% in the high dose group in comparison to 80% for the controls. The incidences of phthalamide-treated animals with tumors at a specific anatomical site did not differ significantly from those of the controls.

Phthalic Anhydride

Body weight gains in male rats and in both sexes of mice were reduced by phthalic anhydride, but survival in all of the treated groups of animals was unaffected. The incidences of phthalic anhydride-treated animals with tumors at a specific anatomical site did not differ significantly from those of the controls.

Di(2-ethylhexyl) Phthalate (DEHP)

DEHP treatment decreased body weight gains for female mice and for male and female rats. Animal survival, however, was not compromised by dietary ingestion of DEHP. Liver tumors occurred in both DEHP-treated rats and mice at incidences significantly greater than in the respective controls (Table 1). The incidences of animals with hepatocellular carcinomas was significantly greater for female rats and for male and female mice treated with DEHP than for the controls.

Table 1. Incidences of animals with liver tumors in the carcinogenicity bioassay of di(2-ethylhexyl) phthalate.[a]

Species	Sex	Liver tumor	Incidence		
			Control	Low dose	High dose
Rat	Male	Hepatocellular carcinoma	1/50	1/49	5/49
		Neoplastic nodule	2/50	5/49	7/49
		Hepatocellular carcinoma or neoplastic nodule	3/50	6/49	12/49[b]
Rat	Female	Hepatocellular carcinoma	0/50	2/49	8/50[c]
		Neoplastic nodule	0/50	4/49	5/50[b]
		Hepatocellular carcinoma or neoplastic nodule	0/50	6/49[b]	13/50[d]
Mouse	Male	Hepatocellular carcinoma	9/50	14/48	19/50[b]
		Hepatocellular adenoma	6/50	11/48	10/50
		Hepatocellular carcinoma or adenoma	14/50	25/48[b]	29/50[c]
Mouse	Male	Hepatocellular carcinoma	0/50	7/50[b]	17/50[d]
		Hepatocellular adenoma	1/50	5/50	1/50
		Hepatocellular carcinoma or adenoma	1/50	12/50[b]	18/50[d]

[a]Fischer 344 rats and B6C3F$_1$ mice were fed diets containing 0 (control), 3,000 (low dose mice), 6,000 (high dose mice, low dose rats) or 12,000 ppm (high dose rats) of DEHP for approximately two years. The ratios of animals bearing liver tumors to the total number of animals examined microscopically are depicted.

[b]Significantly greater than controls, $p < 0.05$.

[c]Significantly greater than controls, $p < 0.005$.

[d]Significantly greater than controls, $p < 0.001$.

Di(2-ethylhexyl) Adipate (DEHA)

Ingestion of DEHA decreased body weight gains in male and female rats and mice. Animal survival, however, was not compromised. The incidences of tumors at specific anatomical sites in DEHA-treated rats were not increased relative to the controls, but liver tumors (hepatocellular carcinomas in female mice, hepatocellular adenomas in male mice) occurred more frequently in DEHA-treated mice than in the controls (Table 2).

Butyl Benzyl Phthalate (BBP)

Body weight gains in male and female mice and in female rats were reduced by the ingestion of BBP, but survival was unaffected. In contrast, excessive numbers of BBP-treated male rats died from apparent internal hemorrhaging after approximately 3 months of exposure. The study in male rats was terminated early, therefore, and no evaluation of tumorigenic response in the male rats could be made. The incidences of tumors at specific anatomical sites in BBP-treated male and female mice did not differ significantly from those in controls. The incidence of animals with myelomonocytic leukemia, however, was greater for the high-dose female rats than for the controls (Table 3).

Discussion

Neither phthalamide nor phthalic anhydride was demonstrated to be carcinogenic for Fischer 344 rats or $B6C3F_1$ hybrid mice, under the conditions of the standard bioassay, since the incidences of chemically treated animals with tumors at a specific anatomical site did not differ significantly from those in controls.

DEHP was considered to be carcinogenic in both Fischer 344 rats and $B6C3F_1$ mice because of the significantly increased incidences in test chemical-treated animals of hepatocellular carcinomas in male and female mice and in female rats. The incidence of male rats with either hepatocellular carcinomas or neoplastic nodules (total liver tumors) was also significantly greater in the high-dose group than in the controls. The absence of nonneoplastic lesions in the liver and the failure of DEHP to reduce survival suggests that the liver tumors were not caused by an indirect hepatotoxic effect.

DEHA was considered to be carcinogenic in $B6C3F_1$ mice because of the greater incidence in test chemical-treated animals of hepatocellular carcinomas in female mice. Since only the incidence of hepatocellular adenomas (benign liver tumors), not that of hepatocellular carcinomas (malignant liver tumors), was significantly increased in the male

Table 2. Incidences of mice with liver tumors in the carcinogenicity bioassay of di(2-ethylhexyl) adipate.[a]

Species	Liver tumor	Incidence		
		Control	Low dose	High dose
Male	Hepatocellular carcinoma	7/50	12/49	12/49
	Hepatocellular adenoma	6/50	8/49	15/49[b]
	Hepatocellular carcinoma or adenoma	13/50	20/49	27/49[c]
Female	Hepatocellular carcinoma	1/50	14/50[d]	12/49[c]
	Hepatocellular adenoma	2/50	5/50	6/49
	Hepatocellular carcinoma or adenoma	3/50	19/50[d]	18/49[d]

[a]$B6C3F_1$ mice were fed diets containing 0 (control), 12,000 (low dose) or 25,000 ppm (high dose) of DEHA for approximately two years. The ratios of mice bearing liver tumors to the total number of mice examined microscopically are depicted.
[b]Significantly greater than controls, $p < 0.05$.
[c]Significantly greater than controls, $p < 0.005$.
[d]Significantly greater than controls, $p < 0.001$.

Table 3. Incidences of female rats with tumors of the hematopoietic system in the carcinogenicity bioassay of butyl benzyl phthlate.[a]

Hematopoietic system tumor	Incidence		
	Control	Low dose	High dose
Myelomonocytic leukemia	7/49	7/49	18/50[b]
Lymphoma	0/49	0/49	1/50
Myelomonocytic leukemia or lymphoma	7/49	7/49	19/50[b]

[a]Female Fischer 344 rats were fed diets containing 0 (controls), 6,000 (low dose) or 12,000 ppm (high dose) of BBP for approximately two years. The ratios of female rats bearing tumors of the hematopoietic system to the total number of female rats examined microscopically are depicted.
[b]Significantly greater than controls, $p < 0.05$.

mice, the data were judged to be evidence of probable carcinogenicity of DEHA in male $B6C3F_1$ mice. Carcinogenic effects of DEHA in Fischer 344 rats was not demonstrated.

Although of statistical significance, the increased incidence of myelomonocytic leukemia in female rats receiving the high dose of BBP was considered to be of equivocal biological significance due to considerable variation in the background incidence of myelomonocytic leukemia in Fischer 344 rats. Moreover, the organ distribution and cytological characteristics of the leukemia occurring in the high-dose BBP-treated female rats did not differ from that in the controls, indicating that the form of leukemia in the treated rats was the same as that occurring naturally in this strain of rat. The data, therefore, were considered to be evidence of probable carcinogenicity of BBP in female Fischer 344 rats. The study was judged to be inadequate in male rats because of chemically induced early deaths. No evidence of BBP carcinogenicity was observed in $B6C3F_1$ mice.

In summary, two of the five phthalate esters and related compounds tested by the National Toxicology Program and the National Cancer Institute for carcinogenic potential in rodent feeding studies, DEHP and DEHA, were judged to be carcinogenic. DEHP caused liver tumors in both rats and mice, while DEHA caused liver tumors in mice, only. Because of the similarities in chemical structure and the site of induced tumor formation, it can be speculated that DEHP and DEHA act through similar mechanisms. BBP was causally associated with an increased frequency of myelomonocytic leukemia in female Fischer 344 rats, but the characteristics of the induced tumor precluded an unequivocal evaluation of carcinogenic potential. The final two chemicals, phthalamide and phthalic anhydride, exhibited no evidence of carcinogenicity in these studies.

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