Carcinogenic Potential of Phthalic Acid Esters and Related Compounds: Structure–Activity Relationships

by William M. Kluwe*

Chronic toxicity and carcinogenicity studies of several phthalic acid esters (PAEs) and compounds containing a 2-ethylhexyl moiety were conducted in Fischer 344 rats and B6C3F$_1$ (hybrid) mice. The compounds studied were phthalic anhydride, di(2-ethylhexyl) phthalate, butyl benzyl phthalate, diallyl phthalate, di(2-ethylhexyl) adipate, tris(2-ethylhexyl) phosphate, and 2-ethylhexyl sulfate (sodium salt). Estimated maximum tolerable doses and fractionally lower doses of each compound were administered to groups of 50 male and 50 female rats and mice for 2 years, followed by sacrifice, necropsy, and histopathological examination of major organs and tissues. The low toxic potencies of most of the compounds allowed for relatively high doses to be given during the chronic studies. In general, the toxic manifestations of the PAEs were closely correlated with their ester substituents. Although many of the PAEs possessed some carcinogenic activity, target sites for such effects were dissimilar, suggesting the absence of a common mode of action. In contrast, all of the 2-ethylhexyl-containing compounds studied possessed some hepatocarcinogenic activity, indicating that this moiety may have a propensity for causing hepatocarcinogenesis in mice, particularly those of the female sex. The 2-ethylhexyl compound that caused the greatest hepatocarcinogenic response in mice, di(2-ethylhexyl) phthalate, was also hepatocarcinogenic in rats. Similarly, those with a relatively greater effect in female mice were also active in male mice. Thus, sex and species differences in 2-ethylhexyl-induced hepatocarcinogenesis in rodents are probably quantitative rather than qualitative in nature.

Introduction

There is considerable interest in structure–activity relationships in toxicology and carcinogenesis research as they relate to biochemical and pathophysiological mechanisms of toxicant action. However, equally important is the role that such structural correlates with tissue injury could play in predicting chronic toxic response for well-defined classes of chemicals. Recognizing the limitations of technical and financial resources in toxicity testing and the obvious need to estimate chemical hazards in a timely manner, elucidation of structure–activity correlates is a potentially efficient method for predicting chemical toxicity.

Much of the concern over the chronic toxic potential of PAEs was triggered by the report that di(2-ethylhexyl) phthalate (DEHP), the most widely used of the PAE plasticizers, was hepatocarcinogenic in rodents (1). Through its chronic toxicity and carcinogenicity studies in rodents, the National Toxicology Program (NTP) has conducted prechronic and chronic studies in male and female rats and mice of three esters of phthalic acid and the anhydride form of phthalic acid, phthalic anhydride. Careful comparisons of these results may indicate

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whether or not the phthalate moiety *per se* produces a characteristic toxic response. Because of the large number of phthalates used for commercial purposes (2), their high volume of production (3), and their ubiquitous presence in the environment (4) and human tissues (5), an ability to predict toxic potential for phthalic acid esters (PAEs) that have not yet been adequately tested would be advantageous.

The NTP has also conducted prechronic and chronic studies in rodents of three nonphthalate chemicals containing a 2-ethylhexyl moiety, in addition to the 2-ethylhexyl-containing phthalate, DEHP. Comparisons of these data may suggest whether or not the 2-ethylhexyl group is important in the expression of carcinogenic potential, and whether the presence of such a moiety would suggest a potential for causing cancer.

The chemicals studied were phthalic anhydride (PAn), butyl benzyl phthalate (BBP), diallyl phthalate (DAP), di(2-ethylhexyl) phthalate (DEHP), di(2-ethylhexyl) adipate (DEHA), tris(2-ethylhexyl) phosphate (TEHP) and 2-ethylhexyl sulfate (EHS). The structures of these chemicals are shown in Figure 1.

DEHP is the plasticizer most commonly used for vinyl products (8), comprising up to 50% (by weight) of some plastic materials. DEHA is also used as a plasticizer, particularly in food product containers where other plasticizers would be leached into fatty foods (9). BBP and
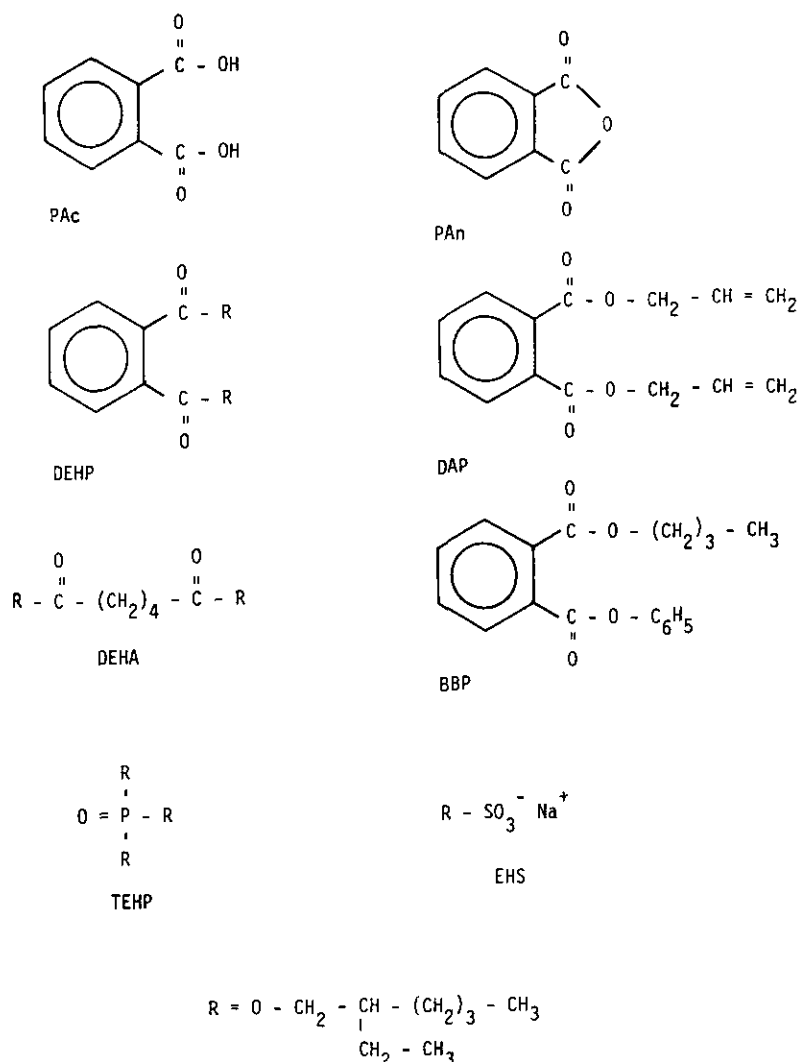
FIGURE 1.

DAP are also used as plasticizers, the former for vinyl products such as flooring and the latter in electrical components. PAn is a precursor in the manufacture of PAEs. It is condensed with the appropriate alcohols to produce the desired PAEs (e.g., PAn + 2-ethylhexanol forms DEHP) (2). EHS is a surfactant used in food processing and textile manufacture (10,11), while TEHP is used both as a plasticizer and as a flame retardant (12).

The data in each of these studies were individually peer-reviewed and approved by a board of independent, nongovernment scientists. More detailed data on each of the individual studies can be obtained from the technical report series (9,13–19).

Methods

Animals

Weanling rats of the inbred Fischer 344 strain and weanling hybrid B6C3F$_1$ mice were evaluated clinically for 10 to 14 days before the starts of the studies to ensure the absence of parasites or disease. The animals were housed throughout the studies in polycarbonate cages containing hardwood bedding, 5 animals of a single sex per cage. Rooms were controlled for temperature 21 ± 2°C), humidity (50 ± 20%) and light-cycle (12 hr fluorescent light; 12 hr dark). Food and water were freely available.

Chemicals

PAn (> 98% pure) was obtained from Koppers Co. (Pittsburgh, PA), BBP (> 98% pure) from Missouri Solvents and Chemicals (Kansas City, MO), DAP (> 99% pure) from Hardwicke Chemical Co. (Elgin, SC), DEHP (> 99.5% pure) and DEHA (> 98% pure) from W.R. Grace and Co. (Fords, NJ), and TEHP (> 98% pure) from the United States Army Chemical Systems Laboratory (Aberdeen, MD). EHS was obtained as a 40% aqueous solution (38.8% EHS, 0.7% 2-ethylhexanol, 55.1% water) from Alcolac Inc. (Baltimore, MD).

PAn, BBP, DEHP, DEHA, or EHS were each mixed directly into the feed, while DAP and TEHP were mixed

with corn oil and administered by gavage. Gavage solutions and feed were analyzed periodically to ensure appropriate dosing.

Experimental Design

Animals certified healthy were assigned to cages, and the cages were then assigned to treatment groups, using tables of random numbers. Groups of 10 animals of each species and sex per dose were exposed to each (generally five) chemical dose and vehicle or untreated control (vehicle for gavage studies; untreated for feed studies) for a period of 13 weeks. Chemical-containing food was available throughout the studies, while gavage treatments were administered once daily, 5 days/week. All animals that died on test or were sacrificed in a moribund condition, and survivors at the end of the 13 weeks were given a complete necropsy. Major organs from the controls and highest dose were examined histologically for chemically induced morphological changes. Specific target tissues were examined histologically in lower dose groups to reach a no-observed effect level.

Estimated maximum tolerable doses (the highest doses estimated not to reduce lifespan from other than carcinogenic effects) for the chronic studies were determined from the prechronic (13 week) data and used as the higher doses in the chronic studies. Groups of 50 animals of each species and sex were exposed for two years (~ 104 weeks) to one of two chemical doses or control (the PAn control group consisted of only 20 animals/sex/species; all other studies contained 50 control animals/sex/species). All animals that died on test or were sacrificed in a moribund condition, and those killed at termination were given a complete necropsy. Histological examinations were performed on the major organs of all animals. Both neoplastic and nonneoplastic lesions were recorded.

Tumor occurrence data were analyzed both for dose-related trends and for pairwise comparison of treated groups with controls by three alternate methods: life-table analysis (most appropriate for fatal tumors), incidental tumor test (most appropriate for nonfatal tumors observed at necropsy), and Fisher's exact test and Cochran-Armitage trend test (based on the overall proportions of tumor-bearing animals) (20,21). Survival probabilities in the chronic studies were estimated by the product-limit procedure (22), and analyzed for chemical effects by the methods of Cox (23) and Tarone (24).

Results

Prechronic Studies

Dose ranges for the 13-week studies were as follows: BBP, 1600–25,000 ppm; DAP, 25–400 mg/kg; PAn, 6200–50,000 ppm; DEHP, 1600–25,000 ppm for rats, 800–12,500 ppm for mice; DEHA, 1600–25,000 ppm; TEHP, 250–4000 mg/kg for rats, 500–8000 mg/kg for mice; EHS,

10,000–40,000 ppm. In general, the highest doses used in the subchronic studies caused mild-moderate retardations of body weight gain. Compound-related deaths in these studies occurred only at the 400 mg/kg dose of DAP in male rats. DAP also caused hepatocellular necrosis and hepatic fibrosis or cirrhosis in both sexes of rats at 200 and 400 mg/kg; no liver lesions were observed in the DAP-treated mice. Testicular atrophy was observed in rats receiving the higher doses of BBP or DEHP. This lesion was characterized by a loss of the germinating epithelium of the seminiferous tubules; Sertoli cells and interstitium appeared to be unaffected. No lesions were observed in rats receiving PAn, DEHA, TEHP, or EHS, and no lesions were observed in mice receiving any of the seven compounds. Based on these data, the doses shown in Table 1 were chosen for the chronic studies. The two doses chosen per chemical, separated by a factor of two, will hereafter be referred to as the "higher" and the "lower" doses.

Chronic Studies: Survival

With the exception of BBP and, possibly DEHP, the chemical treatments did not clearly reduce survival in the chronic studies. Survival of the lower dose DEHP female mice only (not the higher dose) was significantly less than that in the controls. This single statistically significant survival difference in eight comparisons (two species, two sexes, two doses), occurring as it did only in the lower dose group and in the absence of life-threatening toxic lesions, was considered consistent with a chance occurrence and not a chemical effect. In contrast, male rats at both doses of BBP began to die after ~ 15 weeks of treatment and were all terminated at 30 weeks due to poor group survival. Although no degenerative lesions were observed histologically in the BBP-treated male rats, internal hemorrhaging appeared to have occurred as evidenced by the observance of clotted blood at necropsy.

Chronic Studies: Neoplasia

Increased occurrence of tumors were not observed for either sex or species of animal in the PAn study, or for BBP-treated mice or DEHA-treated rats. One each of the lower dose male and female mice and two each of the higher dose male and female mice in the DAP study exhibited papillomas of the forestomach. (DAP also caused gastric hyperplasia and chronic gastric inflammation in the mice.)

BBP increased the occurrence of mononuclear cell leukemia, a common tumor in aged Fischer 344 rats, in the higher dose female rats (Table 2). DAP treatment was related to an increased occurrence of this same tumor in female rats, as well as with an equivocal increase in the occurrence of lymphomas in male mice (Table 2).

DEHP increased the occurrences of hepatocellular tumors in both male and female rats and mice (Table 3). This hepatocarcinogenic effect was evident in the higher

Table 1. Doses used for the chronic studies.

Compound	Species	Sex	Control[a]	Dose	
				Lower	Higher
Phthalic anhydride	Rat	M, F	U	7,500 ppm	15,000 ppm
	Mouse	M	U	16,000 ppm	33,000 ppm
		F	U	12,000 ppm	24,000 ppm
Diallyl phthalate	Rat	M, F	V	50 mg/kg	100 mg/kg
	Mouse	M, F	V	150 mg/kg	300 mg/kg
Butyl benzyl phthalate	Rat	F	U	6,000 ppm	12,000 ppm
	Mouse	M, F	U	6,000 ppm	12,000 ppm
Di(2-ethylhexyl) phthalate	Rat	M, F	U	6,000 ppm	12,000 ppm
	Mouse	M, F	U	3,000 ppm	6,000 ppm
Di(2-ethylhexyl) adipate	Rat	M, F	U	12,000 ppm	25,000 ppm
	Mouse	M, F	U	12,000 ppm	25,000 ppm
Tris(2-ethylhexyl) phosphate	Rat	M	V	2,000 mg/kg	4,000 mg/kg
		F	V	1,000 mg/kg	2,000 mg/kg
	Mouse	M, F	V	500 mg/kg	1,000 mg/kg
2-Ethylhexyl sulfate (Na⁺)	Rat	M	U	10,000 ppm	20,000 ppm
	Mouse	M	U	5,000 ppm	10,000 ppm
		F	U	10,000 ppm	20,000 ppm

[a] U, untreated; V, vehicle.

dose male rats and in both the lower and higher dose female rats and male and female mice. DEHP appeared to increase the occurrence of both those neoplasms categorized as carcinomas (malignant) and those characterized as adenomas or neoplastic nodules (benign).

Except for DEHP, none of the seven chemicals increased the occurrence of liver tumors in rats. However, the other three chemicals with a 2-ethylhexyl moiety exhibited varying degrees of hepatocarcinogenic activity in mice, ranging from clear causal effect (DEHA) to equivocal causal effect (EHS). As shown in Table 4, DEHA increased the occurrence of liver tumors in both male and female mice (although the response was stronger in females), and TEHP caused a small but significant ($p <$ 0.05, $p <$ 0.01) increase in hepatocellular carcinomas in female mice, while EHS equivocally increased hepatocellular tumors in female mice only. As with DEHP, the increases appeared to occur in both carcinomas and adenomas, although somewhat more strongly in the former.

Chronic Studies: Nonneoplastic Lesions.

Nonneoplastic, chemically induced lesions were not observed in animals treated chronically with PAn, BBP (excluding male rats), DEHA or TEHP. Dose-dependent necrosis and fibrosis of the liver, periportal in distribution, and severe bile duct hyperplasia were observed in DAP-treated rats. DAP-treated mice did not develop liver lesions, but gastric inflammation and hyperplasia were evident. EHS also caused papillary hyperplasia of the forestomach in male and female mice. Chronic inflammation of the kidney was observed in the higher dose male DEHP mice, and EHS appeared to be nephrotoxic to rats in the chronic study, causing focal calcification (males and females) and chronic inflammation (males only). In addition, a rare renal tubular adenoma occurred in a female rat at the higher dose of EHS, and rare tumors of the renal pelvis occurred in one each of the higher dose EHS male and female rats.

Table 2. Effects of dially phthalate (DAP) or butyl benzyl phthalate (BBP) on the occurrence of hematopoietic system tumors.

Compound	Species	Sex	Tumor[a]	Overall incidence (%) and level of statistical significance[b]		
				Control	Lower dose	Higher dose
BBP	Rat	Female	Leukemia (MNC)	7/49 (14%) p < 0.01	7/49 (14%) —	18/50 (36%) p ≅ 0.01
DAP	Rat	Female	Leukemia (MNC)	15/50 (30%) p < 0.05	15/43 (35%) —	25/49 (51%) p < 0.05
	Mouse	Male	Lymphoma	6/50 (12%) p < 0.05	5/50 (10%) —	12/50 (24%) p ≅ 0.05

[a] MNC, mononuclear cell (leukemia type).
[b] Values from the trend test are listed below the control group incidences, while values from pairwise comparisons are listed below the treated group incidences. Only those values where both survival-adjusted tests (life table and incidental tumor tests) indicated $p \leq 0.05$ are shown. Historical incidences in control animals ($\bar{x} \pm$ SD; $N >$1000 female rats, 650 male mice): leukemias, 16.1 ± 8.9% in female rats; lymphomas, 10.7 ± 6.4% in male mice. These data are derived from 14 independent studies in mice and 23 independent studies in rats.

Table 3. Effects of di(2-ethylhexyl) phthalate on the occurrences of hepatocellular tumors.

Species	Sex	Type of hepatocellular tumor	Overall incidence (%) and level of statistical significance[a]			
			Control	Lower dose	Higher dose	Historical controls[b]
Rat	Male	All (combined)[c]	3/50 (6%)	6/49 (12%)	12/49 (24%)	4.2 ± 3.9%
			$p<0.1$	—	$p<0.05$	
		Carcinoma	1/50 (2%)	1/49 (2%)	5/49 (10%)	0.8 ± 1.1%
			$p<0.05$	—	—	
	Female	All (combined)[c]	0/50 (0%)	6/49 (12%)	13/50 (26%)	3.1 ± 3.2%
			$p<0.001$	$p<0.05$	$p<0.001$	
		Carcinoma	0/50 (0%)	2/49 (4%)	8/50 (16%)	0.2 ± 0.7%
			$p<0.005$	—	$p<0.01$	
Mouse	Male	All (combined)[d]	14/50 (28%)	25/48 (52%)	29/50 (58%)	31.1 ± 7.5%
			$p<0.01$	$p<0.05$	$p<0.01$	
		Carcinoma	9/50 (18%)	14/48 (29%)	19/50 (38%)	21.3 ± 6.9%
			$p<0.05$	—	$p<0.05$	
	Female	All (combined)[d]	1/50 (2%)	12/50 (24%)	18/50 (36%)	7.9 ± 4.6%
			$p<0.0001$	$p<0.001$	$p<0.0001$	
		Carcinoma	0/50 (0%)	7/50 (14%)	17/50 (34%)	4.1 ± 3.0%
			$p<0.0001$	$p<0.01$	$p<0.0001$	

[a] Values from the trend test are listed below the control group incidences, while values for pairwise comparisons are listed below the treated group incidences. Only those values where both survival-adjusted tests (Life Table and Incidental Tumor Tests) indicated $p<0.05$ are shown.

[b] Incidences for historical controls are given as $x \pm$ SD, $N>2000$ rats, 1000 mice of each sex. (These data are based on 22 independent studies in mice and 42 independent studies in rats.)

[c] Combined = hepatocellular carcinomas or neoplastic nodules.

[d] Combined = hepatocellular carcinomas or adenomas.

Discussion

Comparative evaluation of several studies using a common protocol allows for limited conclusions to be drawn about the relationship of molecular structure to the biological activity of the subject chemicals. The generally low order of acute toxic potency of the phthalate esters (and the 2-ethylhexyl compounds tested) led to the use of relatively high doses for the chronic studies, thus increasing the probability of detecting toxic effects should such occur. Despite the large doses, little nonneoplastic toxicity or survival reduction occurred, reinforcing the adequacy of these studies for carcinogenicity evaluation.

Several potential target sites for carcinogenic activity of phthalates were found, including liver (DEHP), hematopoietic system (BBP, DAP) and forestomach (DAP). However, these sites were generally affected by only one of the PAEs, and the increased tumor occurrences were

Table 4. Effects of di(2-ethylhexyl) adipate (DEHA), tris(2-ethylhexyl) phosphate (TEHP), or 2-ethylhexyl sulfate (EHS) on the occurrences of hepatocellular tumors in mice.

Compound	Sex	Type of hepatocellular tumor	Overall incidence (%) and level of statistical significance[a]		
			Control	Lower dose	Higher dose
DEHA	Male	All (combined)	13/50 (26%)	20/49 (41%)	27/49 (55%)
			$p<0.01$	—	$p<0.01$
		Carcinoma	7/50 (14%)	12/49 (24%)	12/49 (24%)
			—		
	Female	All (combined)	3/50 (6%)	19/50 (38%)	18/49 (37%)
			$p<0.001$	$p<0.001$	$p<0.001$
		Carcinoma	1/50 (2%)	14/50 (28%)	12/49 (24%)
			$p<0.01$	$p<0.001$	$p<0.005$
TEHP	Male	All (combined)	15/50 (30%)	21/50 (42%)	18/49 (37%)
			—	—	—
		Carcinoma	9/50 (18%)	12/50 (24%)	12/50 (24%)
			—	—	—
	Female	All (combined)	2/48 (4%)	8/50 (16%)	10/50 (20%)
			$p<0.05$	—	$p<0.05$
		Carcinoma	0/48 (0%)	4/50 (8%)	7/50 (14%)
			$p<0.01$	—	$p<0.05$
EHS	Male	All (combined)	10/50 (22%)	8/45 (18%)	8/44 (18%)
			—	—	—
		Carcinoma	8/45 (18%)	8/45 (18%)	6/44 (14%)
			—	—	—
	Female	All (combined)	1/45 (2%)	3/46 (7%)	6/45 (13%)
			$p<0.05$	—	$p \approx 0.05$
		Carcinoma	0/45 (0%)	1/46 (2%)	3/45 (7%)
			$p \approx 0.05$	—	$p \approx 0.05$

[a] See Table 3 for historical incidences.

generally restricted to one sex and species. Therefore, the comparative data do not suggest that PAEs per se, or the phthalate moiety, possess innate carcinogenic potential.

In contrast, all four chemicals with a 2-ethylhexyl group (DEHP, DEHA, TEHP, EHS) exhibited some evidence of hepatocarcinogenic potential in rodents, ranging from very strong (DEHP) to equivocal (EHS). The data for these compounds are compared in Table 5, wherein the level of statistical significance of increased liver tumor incidence is used as a rough estimate of the degree of hepatocarcinogenic effect (ignoring molar differences in dose).

The data in Table 5 indicate a greater relative susceptibility of mice than rats, and of females than males to the hepatocarcinogenic activity of the 2-ethylhexyl-containing chemicals. The reason(s) for such differences is unknown. Although liver tumors occur with a higher spontaneous frequency in $B6C3F_1$ mice than in Fischer 344 rats, the male $B6C3F_1$ mice, which have a much higher spontaneous liver tumor rate than do the females of this strain (31% vs. 8%), were less susceptible than the females. Thus, the apparent species and sex differences in response to DEHP, DEHA, TEHP, or EHS are not easily equated with spontaneous liver tumor frequencies. DEHP treatment has been reported to promote growth and malignant transformation of diethylnitrosamine-induced hepatocellular foci in $B6C3F_1$ mice (25), but another laboratory has indicated a lack of such activity for DEHP in Fischer 344 rats (J. Popp, Chemical Industry Institute of Toxicology, personal communication). Such species differences in the promotional capabilities of DEHP towards diethylnitrosamine-induced hepatocellular foci, despite hepatocarcinogenic activity in both test species, suggest the possibility of species differences in the mechanisms of DEHP-stimulated tumor formation. Since those compounds with the strongest activity in female mice (DEHP, DEHA) were also effective in male mice, and the strongest carcinogen (strength of evidence) in mice (DEHP) was also carcinogenic in rats, these sex and species differences in response would appear to be of a quantitative rather than of a qualitative nature.

The four 2-ethylhexyl-containing compounds, despite their shared moiety, differ in chemical and physical characteristics. DEHP and DEHA are lipophilic plasticizers, EHS is a water-soluble anionic surfactant (detergent), while TEHP is a phosphate flame retardant with plasticizing properties. Both DEHP and DEHA are known to be rapidly metabolized to 2-ethylhexanol and their corresponding monoesters in the gut (26,27). Some 2-ethylhexanol is also likely on structural grounds to be released from TEHP in vivo, although confirming data are not available. Most of an oral dose of EHS was reportedly excreted in an unchanged form, but some (~ 28%) was converted to 2-ethylhexanol and 2-ethylhexyl metabolites (28). Thus, all four compounds are probably metabolized to 2-ethylhexanol, and the rank order of 2-ethylhexanol generation would be DEHP, DEHA >> EHS (TEHP unknown). Therefore, it would be tempting to conclude that the released 2-ethylhexanol is responsible for the hepatocarcinogenic effect, but insufficient data are available to confirm or refute such speculation.

The PAEs and the 2-ethylhexyl-containing compounds compared in this communication have been found to lack mutagenic activity in Salmonella typhimurium testing strains (14,15,29–31; also E. Zeiger, National Toxicology Program, personal communication). While some chromosomal changes have been reported to be induced by DEHP or the monoeasters mono(2-ethylhexyl) phthalate or mono(2-ethylhexyl) adipate in vitro (32), such effects have not been confirmed in vivo. An alternate mechanism of hepatocarcinogenic action for the 2-ethylhexyl-containing compounds may be via peroxisome proliferation. Reddy and co-workers, based on a positive correlation between the abilities of several chemicals to cause hepatic peroxisome proliferation and to produce liver tumors in animals, have proposed peroxisome proliferators as a distinct class of chemical carcinogens (33–35). Treatment of rats or mice with DEHP, DEHA, 2-ethylhexanol or 2-ethylhexanoic acid (a metabolite of 2-ethylhexanol), caused hepatic peroxisome proliferation, while structurally similar compounds lacking a 2-ethylhexyl group (e.g., adipic acid, hexanol) did not (36). DEHP-induced peroxisomes contained much higher specific activities of H_2O_2-generating oxidases than did normal peroxisomes,

Table 5. Comparative effects of compounds with a 2-ethylhexyl moiety on the occurrences of hepatocellular tumors in rats and mice.[a]

| | | Rats | | | | Mice | | | |
| | | Male | | Female | | Male | | Female | |
Type of hepatocellular tumor	Test compound	Lower dose	Higher dose	Lower dose	Higher dose	Lower dose	Higher dose	Lower dose	Higher dose
All (combined)[b]	DEHP	−	+	+	+ + +	+	+ +	+ + +	+ + + +
	DEHA	−	−	−	−	−	+ +	+ + +	+ + +
	TEHP	−	−	−	−	−	−	−	+
	2EHS	−	−	−	−	−	−	−	+
Carcinomas	DEHP	−	±	−	+ +	−	+	+ +	+ + + +
	DEHA	−	−	−	−	−	−	+ + +	+ +
	TEHP	−	−	−	−	−	−	−	+
	2EHS	−	−	−	−	−	−	−	±

[a] Legend (level of statistical significance): −, $p \geqslant 0.05$; ±, $p \approx 0.05$; +, $0.01 < p < 0.05$; + +, $0.001 < p < 0.01$; + + +, $0.0001 < p < 0.001$; + + + +, $p < 0.0001$.

[b] Combined = Hepatocellular carcinomas or adenomas (mice) or neoplastic nodules (rats).

but nearly the same activity as the H_2O_2-detoxifying exzyme catalase (36). More recently, Melnick and co-workers have demonstrated an increased steady state level of H_2O_2 in liver homogenates from DEHP-treated rats (37). Whether or not such an increased capability of peroxisomal generation of H_2O_2 could upset cellular oxygen metabolism and lead to cellular damage via excessive generation of reactive oxygen species is at present unknown. There is no data available to indicate whether or not TEHP or EHS induce hepatic peroxisomes in rodents, but metabolic release of 2-ethylhexanol from these compounds would be expected to do so.

The nonneoplastic effects of the PAEs seem to be closely related to metabolism to the monoesters and the alcohols. Both BBP and DEHP caused testicular atrophy, effects that can be reproduced by phthalate monoesters with linear alkyl chain lengths of approximately 3–7 (38–40). Recent studies have shown that BBP is extensively metabolized in rats to monobutyl phthalate (41). Similarly, rats extensively metabolize DAP to allyl alcohol and acrolein, and the hepatotoxic effects of DAP in rats (periportal hepatocellular necrosis, fibrosis and cirrhosis) are consistent with those of allyl alcohol and acrolein (19). Mice are better able to detoxify the allyl alcohol product of DAP and do not manifest with a hepatotoxic response (19).

In conclusion, comparative evaluations of chronic toxic response to several PAEs did not reveal common neoplastic or nonneoplastic lesions suggestive of structural correlates of toxic activity. However, the 2-ethylhexyl group appeared to be related to hepatocarcinogenic activity in rodents, particularly in female mice. The major objectives of chronic animal carcinogenicity studies are to detect chemicals with oncogenic activity in experimental animals and to estimate the hazards of such chemicals to man. The limitations of this type of study include the required use of relatively high doses (generally much higher than anticipated human exposure), surrogate species (generally rodents) and subjective criteria of tumorigenic activity. Such animal data are of irreplaceable value in predicting toxic effects (or lack thereof) in humans, but additional studies are needed to confirm or refute the validity of extrapolation from the response of rodents to the response of humans. In particular, more study is needed on the speculated causal relationship between peroxisomes and neoplasia, and of DEHP-induced peroxisomes to DEHP-induced hepatocellular tumors. This type of information, along with the recent data on species differences in chemically induced peroxisomal proliferation (42,43), will strengthen confidence in our ability to predict the hazards of DEHP, 2-ethylhexyl-containing compounds, and peroxisome proliferators in general to human health.

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