PEROXISOME INDUCTION STUDIES ON DI(2-ETHYLHEXYL)TEREPHTHALATE

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Groups of five male and five female rats were fed diets containing from 0% to 2.5% di(2-ethylhexyl)terephthalate (DEHT) or 1.2% di(2-ethylhexyl)phthalate (DEHP) for 21 days. Feed consumption and body weight gains were collected and, at study termination, animals were examined for alterations in body weight, differences in serum lipids, changes in the activities of certain enzymes associated with fat metabolism, and proliferation of hepatic peroxisomes. Feed consumption and weight gain were greatly decreased in DEHT-fed animals only at 2.5%. No biologically significant alterations in absolute liver weight occurred with DEHT. Relative liver weights were increased at 2.5% in both sexes and at 1.0% and 1.2% in females. The alterations were due wholly to decreased terminal body weights. Serum triglyceride and cholesterol levels were not found useful in interpreting the effects of DEHT. Cyanide-insensitive palmitoyl CoA oxidation and lauric acid 11- and 12-hydroxylation were increased in animals consuming 2.5%, but no lower levels of DEHT. Induction of hepatic peroxisomes did not occur at 1.2% DEHT. Interpretation of minimal peroxisomal effects with 2.5% DEHT was confounded by reduced feed consumption. Slight decreases in weight gain occurred in males consuming the 1.2% DEHP diet, but differences were minor relative to effects observed at 2.5% DEHT. Results with DEHP contrasted with those obtained with DEHT. Absolute and relative liver

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2. Key words: lipid metabolism, peroxisomes, phthalate esters.

3. Abbreviations: BIBRA, British Industrial Biological Research Association; CMA, Chemical Manufacturers Association; DEHP, di(2-ethylhexyl) phthalate; DEHT, di(2-ethylhexyl) terephthalate; DOTP, dioctylterephthalate.

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weights, activities of enzymes of lipid metabolism, and peroxisome content were all significantly increased at 1.2% DEHP. Reduction of feed intake was implicated in the effects observed at 2.5% DEHT, since the amount of DEHT consumed by 2.5% animals was only 1.4 times as much as by 1.2% animals. A possible explanation for the observed differences between DEHP and DEHT was related to the results of a metabolic fate study on DEHT. Metabolism of DEHT by the rat appears to occur via rapid hydrolysis of both ester linkages to give two moles of 2-ethylhexanol and one mole of terephthalic acid. Although 2-ethylhexanol has been shown to induce peroxisome proliferation, it appears to be less active in this respect than the monoester of DEHP. The relatively smaller amounts of monoester produced during the metabolism of DEHT may explain the differences seen in these experiments.

INTRODUCTION

The suggestion that the possible health effects of DEHT, also known as dioctylterephthalate (DOTP), be examined derives from the results of a study on a structurally similar compound, DEHP (National Toxicology Program, 1982; Kluwe et al., 1982). In that study, DEHP was found to be a putative hepatocellular carcinogen in two species of rodents. DEHP is also a member of a class of materials which elevate the activity of lipid metabolizing enzymes, and, particularly in rodents, induce both liver enlargement and the proliferation of hepatic peroxisomes (Reddy and Lalwani, 1983). Although a relationship between the production of increased numbers of hepatic peroxisomes and liver cancer has been proposed (Reddy et al., 1980), a study commissioned by the Consumer Product Safety Commission did not find a direct or causative association between peroxisome proliferation and the occurrence of liver cancer (CHAP Report, 1985). Nevertheless, the results of the bioassay on DEHP have raised significant questions about the biological properties of chemicals which are structurally related to DEHP. Results of peroxisome proliferation studies sponsored by the Phthalate Esters Panel of the Chemical Manufacturers Association (CMA), and conducted by the British Industrial Biological Research Association (BIBRA), demonstrated that, in general, longer-chain esters were more potent peroxisome inducers than esters with shorter chains, and branched-chain esters were more potent than those with unbranched chains (Barber et al., 1987).

A series of health effects tests have been performed on DEHT by the Health and Environment Laboratories of Eastman Kodak Company under a testing agreement negotiated with the U.S. Environmental Protection Agency. Among the tests which were conducted was a subchronic feeding study in Sprague-Dawley rats. Some of the endpoints which were examined in that study were serum lipid levels, organ weights,

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and the proliferation of hepatic peroxisomes (Topping, 1985). Animals were fed diets containing 0.0%, 0.1%, 0.5%, or 1.0% DEHT for 90 days. Some of the pertinent parameters are summarized in Table 1. Weight gain and feed consumption were not affected by consumption of the test material. Terminal body weights and organ weights were also unaffected by treatment, with the exception of the liver. The only significant increases in liver weights were at the 1.0% dose, and were restricted to liver weights relative to body weight. No compound-related effects were observed following completion of a battery of routine serum clinical chemistry tests, including cholesterol and serum triglycerides, two parameters frequently evaluated in studies on this class of materials. In addition, no compound-related effects were seen at gross necropsy or histopathology examination of a standard set of tissues.

Tissues from five animals administered 1.0% DEHT or the control diet in the 90-day feeding study were examined for hepatocyte peroxisome content using computerized morphometric techniques applied to electron microscopy photographs of diaminobenzidine-stained peroxisomes. The results of that analysis (Table 2) showed no differences between control and treated animals in some of the commonly measured peroxisome parameters, and neither peroxisome size nor number was increased by consumption of 1.0% DEHT for 90 days. Thus, DEHT appeared to be significantly less active than DEHP in producing general hepatic effects or hepatic peroxisome proliferation. To study the potential effects of DEHT on peroxisomal enzymes as well as structure, an additional study was initiated according to the protocol used by the CMA Phthalate Esters Panel.

METHODS

The materials and methods utilized in this study were similar to others conducted within the BIBRA Laboratories. These have been described in detail in the final reports delivered to CMA. The complete reports are available from CMA or can be found in the public record for phthalate esters at the U.S. Environment Protection Agency. They have previously been presented at the 25th Annual Meeting of the Society of Toxicology in 1986 (Gray et al., 1986). A brief outline of the methodology is presented below.

Groups of five male and five female rats were fed diets containing DEHT at concentrations of 0%, 0.1%, 0.5%, 1.0%, 1.2%, or 2.5% by weight. Additional groups of five male and five female rats were fed DEHP at a dietary concentration of 1.2%. These latter two groups served as a study control. After an appropriate acclimatization period, animals were fed the test diets for 21 days. During the course of the study, feed consumption and body weight changes were measured. At the end of the study, animals were fasted overnight, killed by exsanguination under ether anesthesia, and examined for abnormalities. Livers, kidneys, and testes were weighed and sections were preserved for light and electron microscopy examination. The portions of the

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TABLE 1
Terminal Body Weights, Liver Weights and Serum Lipids in Rats Fed DEHT at Levels of 0 (Control), 0.1, 0.5 or 1.0% for 90 days

		Dietary Level of DEHT			
Parameter	**Sex**	**0.0%**	**0.1%**	**0.5%**	**1.0%**
Terminal Body Wt (g)	Males	530 ± 8	528 ± 11	517 ± 9	519 ± 12
	Females	303 ± 6	297 ± 4	293 ± 6	296 ± 6
Liver Wt (g)	Males	14.71 ± 0.37	14.61 ± 0.47	14.83 ± 0.46	15.99 ± 0.52
	Females	8.66 ± 0.19	8.64 ± 0.17	8.48 ± 0.16	9.20 ± 0.28
Relative Liver Wt	Males	2.77 ± 0.05	2.76 ± 0.05	2.86 ± 0.06	3.08* ± 0.06
(g/100 g body weight)	Females	2.86 ± 0.04	2.92 ± 0.04	2.91 ± 0.04	3.11* ± 0.06
Triglycerides	Males	64.49 ± 4.86	60.80 ± 7.79	67.82 ± 9.28	78.28 ± 10.25
(mg/dL)	Females	66.34 ± 5.82	53.72 ± 3.33	52.16 ± 3.19	58.58 ± 7.44
Cholesterol	Males	50.18 ± 3.36	47.22 ± 4.56	50.20 ± 4.08	57.09 ± 4.55
(mg/dL)	Females	59.54 ± 3.36	57.81 ± 5.34	58.10 ± 2.28	63.42 ± 5.35

Each value represents the mean of values from 20 animals (Terminal Body Weight, Liver Weight, Relative Liver Weight) or 10 animals (Triglycerides, Cholesterol). Those marked with asterisks differ significantly from the control by one way ANOVA and Duncan's multiple range test using a P value of $P \leq 0.05$.

Figures are means ± standard error.

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TABLE 2
Morphometric Analysis of Hepatic Peroxisomes in Rats Fed DEHT at Levels of 0.0 (Control) or 1.0% for 90 Days

Dietary Level (%)	Peroxisome Area (μm^2)	Peroxisome Diameter (μm)	Peroxisome Cell Fraction (%)	Peroxisome Density (No./100 (μm^2)
0.0	0.142 ± 0.008	0.239 ± 0.007	1.45 ± 0.15	10.22 ± 1.24
1.0	0.125 ± 0.007	0.225 ± 0.006	1.18 ± 0.12	9.45 ± 0.61

Each value represents the mean of values obtained from 5 animals. For each animal five sections of liver were examined. Figures are means ± standard error.

livers not fixed for histological examination were used for biochemical analysis. Serum was analyzed for triglycerides and cholesterol.

Biochemical analyses of the liver included assays of cyanide-insensitive palmitoyl CoA oxidation and lauric acid 11- and 12-hydroxylase activity. Cyanide-insensitive palmitoyl CoA oxidation was measured in homogenates of whole liver according to Gray et al. (1983b), but with the addition of 10 μmolar flavin adenine dinucleotide to each cuvette. Microsomal fractions were assayed for lauric acid hydroxylation as described previously (Lake et al., 1984), with the products separated by high pressure liquid chromatography (Orton and Parker, 1982).

Transmission electron microscopy was conducted on osmium-fixed sections of liver tissue. Sections were taken from two rats of each sex from the controls, the groups fed 1.2% or 2.5% DEHT, and the group fed DEHP at 1.2%. Portal and centrilobular regions of each section were examined and scored separately on a scale of 1 to 5, with 1 representing few peroxisomes, 2 to 3 representing slight to moderate increases, and 4 and 5 representing marked and very marked increases in peroxisomes, respectively.

RESULTS

The most obvious result of the feeding of diets containing DEHT was that it became unpalatable or intolerable when fed at a dose level of 2.5%. Feed consumption in this group was substantially reduced as illustrated in Figure 1. There was less difference in feed consumption between the control animals and those consuming the 1.2% diets in either sex. In animals treated with DEHP at a dietary level of 1.2%, feed intake was reduced for three days in males and for ten days in females, but was statistically similar to controls in both sexes for the remainder of the study. The reduction in feed intake significantly affected weight gain (Figure 2). Body weight gain was greatly reduced in both sexes in animals fed the diets containing 2.5% DEHT. The females lost weight and did not regain initial body weights at any time during the course of the study. Animals consuming 1.2% or less DEHT demonstrated body weight gain similar to

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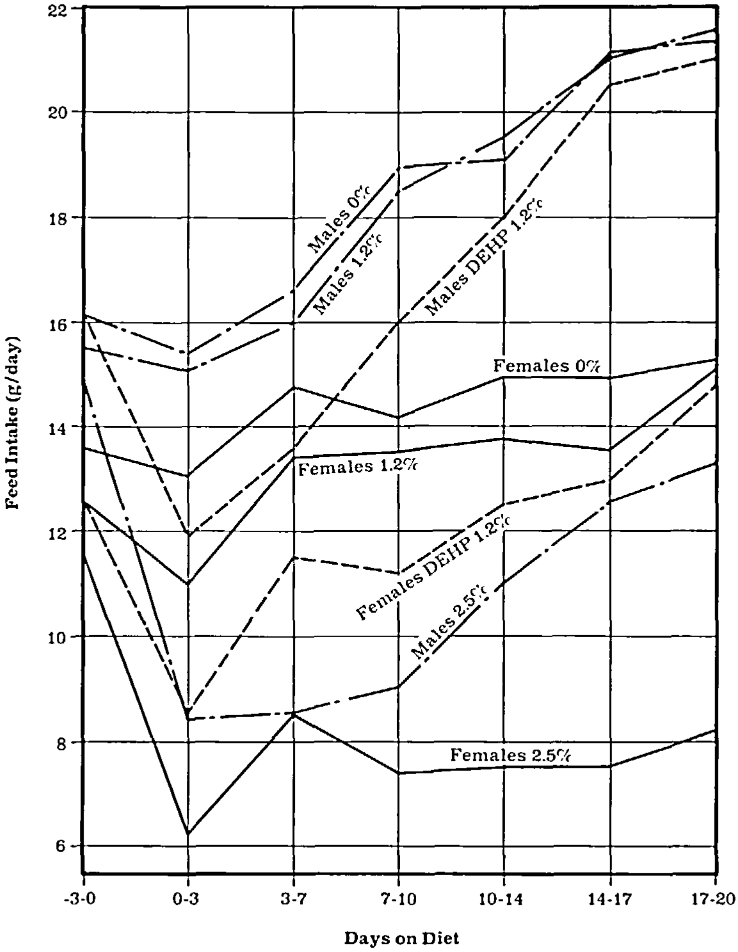

FIGURE 1. Feed intake in male and female rats consuming diets containing 0%, 1.2% or 2.5% DEHT or 1.2% DEHP. ——, females consuming 0, 1.2% or 2.5% DEHT; —·——, males consuming 0, 1.2% or 2.5% DEHT; – – –, males and females consuming 1.2% DEHP.

that of the control animals in both sexes. Animals consuming DEHP at a level of 1.2% showed slightly reduced weight gain in males, but not in females. Some of the difference observed in the males may be due to a slightly lower initial body weight among animals within the group.

Terminal body weights and liver weights are presented in Table 3. No significant alterations in absolute liver weight were apparent in animals consuming up to 2.5% dietary DEHT, with the exception of a slight increase at 1.2% in females. The effect was not dose-dependent and was not present in females consuming the 2.5% diets. No significant effect was seen in relative liver weights in males except at 2.5%, the level at

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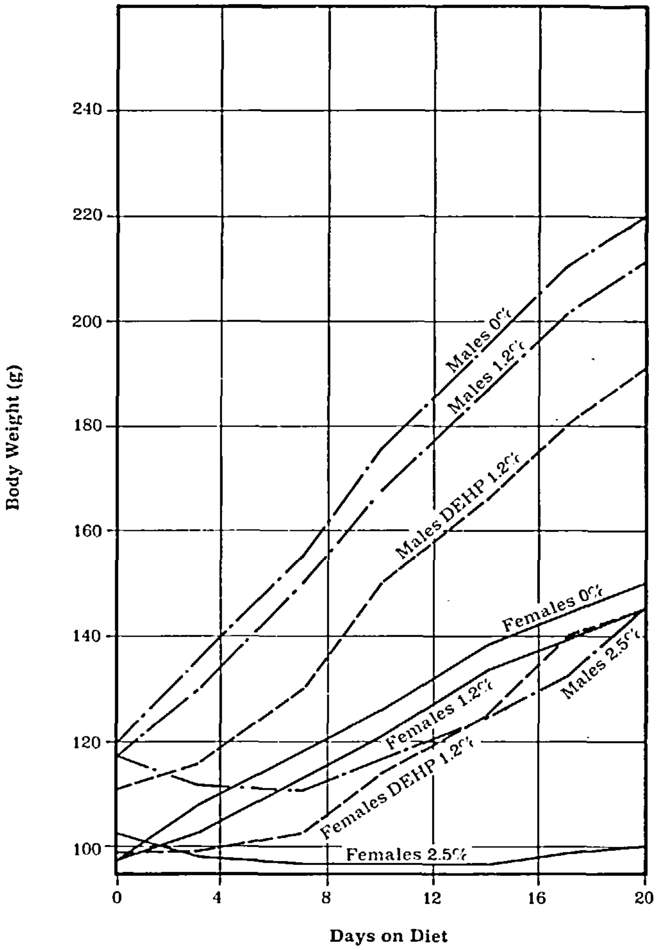

FIGURE 2. Body weight in male and female rats consuming diets containing 0%, 1.2% or 2.5% DEHT or 1.2% DEHP.——, females consuming 0, 1.2% or 2.5% DEHT;- - —; males consuming 0, 1.2% or 2.5% DEHT; — — -, males and females consuming 1.2% DEHP.

which body weight gain was greatly depressed. Statistically significant increases in liver weight relative to body weight were seen in females at 1.0% and 1.2%, but a major increase was present at only 2.5% in the diet. The changes in relative liver weights observed at 2.5% were due almost wholly to reduced body weights, rather than to increased absolute liver weight. DEHP at 1.2% was much more potent at increasing liver weights than any dose of DEHT which was tested. When compared with the effects seen with the phthalate esters (Barber et al., 1987), the effect of DEHT approximates that observed in animals consuming diets containing the short-chain and unbranched long-chain esters, rather than what might be expected in animals fed branched long-chain esters that are structurally similar to DEHP, diisononyl phthal-

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TABLE 3

Terminal Body Weights, Liver Weights, and Liver Weights Relative to Body Weight in Rats Fed DEHT at Levels of 0 (Control), 0.1, 0.5, 1.0, 1.2 or 2.5% or DEHP at 1.2% for 21 Days

Parameter	Sex	Dietary Levels of DEHT 0.0%	0.1%	0.5%	1.0%	1.2%	2.5%	DEHP 1.2%
Terminal Body Wt (g)	Males	205 ± 5.7	199 ± 5.6	198 ± 4.7	184* ± 7.3	193 ± 4.0	123*** ± 9.1	173** ± 6.6
	Females	133 ± 4.5	133 ± 3.0	132 ± 4.3	129 ± 2.3	127 ± 1.6	87*** ± 6.3	124 ± 3.2
Liver Wt (g)	Males	6.78 ± 0.21	6.73 ± 0.23	6.85 ± 0.24	6.82 ± 0.29	6.88 ± 0.66	6.69 ± 0.64	12.41*** ± 0.48
	Females	3.94 ± 0.12	3.93 ± 0.07	4.04 ± 0.12	4.29 ± 0.09	4.61** ± 0.08	3.98 ± 0.26	7.55*** ± 0.06
Relative Liver Wt (g/100 g body wt)	Males	3.31 ± 0.08	3.37 ± 0.03	3.45 ± 0.08	3.71 ± 0.04	3.55 ± 0.31	5.39*** ± 0.14	7.17*** ± 0.16
	Females	2.97 ± 0.04	2.96 ± 0.06	3.07 ± 0.08	3.34** ± 0.02	3.62** ± 0.04	4.60*** ± 0.12	6.09*** ± 0.19

Each value represents the mean of values for 5 animals except for those marked with a superscript indicating a missing value. Those marked with asterisks differ significantly from the control by one way ANOVA or Student's t-test in the case of the control and the DEHP treated group.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Figures are means ± standard error.

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ate and diisodecyl phthalate. This is illustrated in Figure 3, which gives the relative liver weights from male rats consuming a series of phthalate esters. Results similar to those seen in males were also obtained in female rats. No effects related to consumption of the test article were found in kidneys or testes.

The effects of DEHT on the biochemical parameters which were examined are shown in Table 4. There was a significant reduction in triglycerides in sera from males fed DEHT at the 2.5% level. A slight increase was present in females, but also only at the high dose. Similar changes in this parameter were seen in both sexes in animals consuming 1.2% DEHT. Very little difference was seen among cholesterol values from treated and control animals. The lack of effect on cholesterol and the opposing trends in triglyceride levels with an increasing dose of DEHT in the two sexes renders the interpretation of the effect of this material on serum lipids difficult.

Measurement of palmitoyl CoA oxidation shows that, at dietary concentrations of up to 1.2%, DEHT is a very weak inducer of this enzyme system. A statistically significant

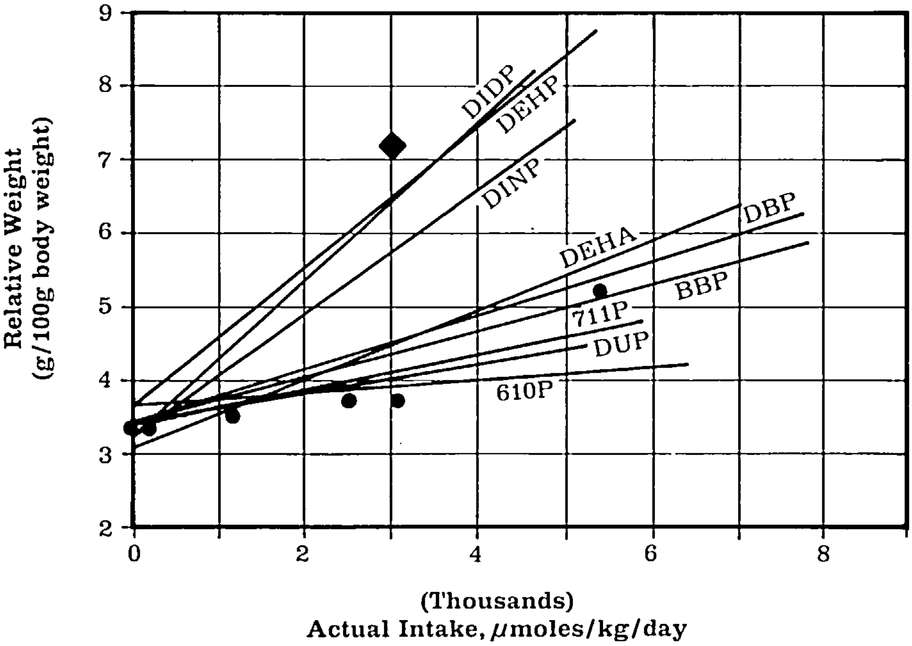

FIGURE 3. Relative liver weights in male rats consuming diets containing 0 to 2.5% DEHT or 1.2% DEHP, compared with linear regressions from relative liver weight data for nine related esters. Relative liver weights from animals consuming: ●, DEHT or ◆, 1.2% DEHP. Abbreviations: DIDP, diisodecyl phthalate; DEHP, di(ethylhexyl) phthalate; DINP, diisononyl phthalate; DEHA, diethylhexyl adipate; DBP, dibenzyl phthalate; BBP, butyl benzyl phthalate; DUP, diundecyl phthalate; 711P, 7-11 phthalate; 610P, 6-10 phthalate. Data on related esters are from Barber et al. (1987).

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TABLE 4

Serum Triglycerides and Cholesterol, Cyanide-Insensitive Palmitoyl-CoA (PCoA) Oxidation Levels in Livers, and Lauric Acid 11-(LA 11-OH) and 12-Hydroxylase (LA 12-OH) Activities in Livers from Rats Fed DEHT at Levels of 0 (Control), 0.1, 0.5, 1.0, 1.2 or 2.5% or DEHP at 1.2% for 21 Days

		Dietary Level of DEHT						DEHP
Parameter	Sex	0.0%	0.1%	0.5%	1.0%	1.2%	2.5%	1.2%
Triglycerides (mmol/L)	Males	0.09 ± 0.04	0.07 ± 0.14	1.00 ± 0.16	0.73 ± 0.04	0.76 ± 0.08	0.47** ± 0.04	0.60*** ± 0.04
	Females	0.35 ± 0.01	0.34 ± 0.01	0.37 ± 0.03	0.32 ± 0.02	0.40 ± 0.03	0.46* ± 0.04	0.49* ± 0.05
Cholesterol (mmol/L)	Males	1.8 ± 0.1	1.9 ± 0.1	2.3 ± 0.4	1.9 ± 0.0	1.8 ± 0.1	2.1** ± 0.2	1.5 ± 0.1
	Females	2.2 ± 0.0	2.1 ± 0.1	2.1 ± 0.1	1.9** ± 0.1	2.1 ± 0.1	2.7*** ± 0.2	1.9* ± 0.1
PCoA (nmol/min/mg protein)	Males	3.8 ± 0.4	3.4 ± 0.3	3.8 ± 0.2	3.4 ± 0.2	4.7 ± 0.6	16.1*** ± 1.9	31.1*** ± 2.3
	Females	3.5 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	3.3 ± 0.4	3.5 ± 0.3	7.9*** ± 1.3	24.2*** ± 3.7
LA 11-OH (nmol/min/mg protein)	Males	0.7 ± 0.09	0.7 ± 0.09	1.1 ± 0.09	0.8 ± 0.03	1.2 ± 0.06	3.2*** ± 0.41	4.2* ± 1.16
	Females	0.2 ± 0.02	0.2 ± 0.02	0.3 ± 0.01	0.3 ± 0.03	0.3 ± 0.02	0.6*** ± 0.13	1.0[1]** ± 0.20
LA 12-OH (nmol/min/mg protein)	Males	1.1 ± 0.15	1.3 ± 0.21	2.0 ± 0.23	2.3 ± 0.10	3.4* ± 0.32	11.5* ± 1.85	20.0** ± 4.84
	Females	0.5 ± 0.06	0.5 ± 0.04	0.6 ± 0.05	0.5 ± 0.03	0.7 ± 0.04	1.8*** ± 0.49	3.4[1]* ± 0.98

Each value represents the mean of values for 5 animals except for those marked with a superscript indicating a missing value. Those marked with asterisks differ significantly from the control by one way ANOVA or Student's t-test in the case of the control and the DEHP treated group.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Figures are means ± standard error.

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increase over control levels was seen only at the 2.5% dose in both males and females. DEHP, at 1.2%, caused a much higher elevation of palmitoyl CoA oxidation than was seen in animals consuming 2.5% DEHT. Compared to results for the nine related materials examined by BIBRA for CMA (Figure 4), the activity in DEHT-fed rats is similar to, or less than, that observed with short- or unbranched-chain esters, and lower than that seen with branched, long-chain esters. Hence, the effect of DEHT on palmitoyl CoA oxidation parallels the effect seen in relative liver weights; that is, there was little effect with increasing dose except at a dietary concentration of 2.5%.

Lauric acid 11-hydroxylase activity was significantly increased only at the 2.5% level. No alteration of activity occurred at any lower dose. The non-linearity of the response for this enzyme, as well as for palmitoyl CoA oxidation and relative liver weight, indicates that the effect seen at the highest dose may have been due to low feed intake and/or mobilization of fat stores, rather than to the consumption of DEHT. This is in contrast to the effect obtained with 1.2% DEHP, where much higher levels of enzymatic activity were recorded, but under conditions where feed intake was only initially and transiently reduced.

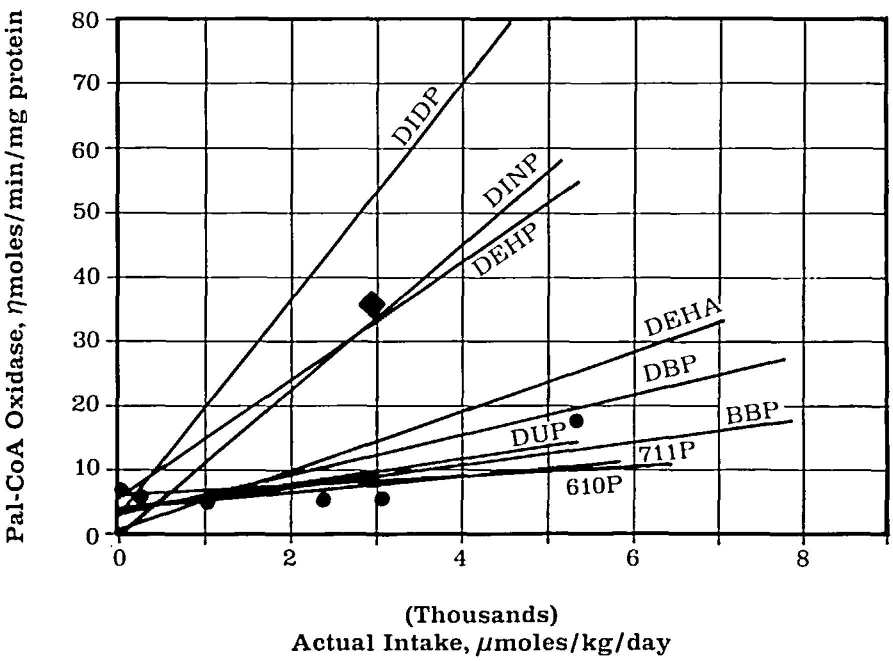

FIGURE 4. Palmitoyl CoA oxidation activity in livers from male rats consuming diets containing 0 to 2.5% DEHT or 1.2% DEHP, compared with linear regressions from palmitoyl CoA oxidation activity data from nine related esters. Palmitoyl CoA oxidation activity in livers from animals consuming: ●, DEHT or ◆, 1.2% DEHP. For abbreviations, see Fig. 3. Data on related esters are from Barber et al. (1987).

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The response in lauric acid 12-hydroxylase activity paralleled the effects of DEHT on lauric acid 11-hydroxylase activity. The pattern in enzymatic activity was non-linear, with enzyme levels not markedly higher than controls except in the 2.5% DEHT and 1.2% DEHP groups.

The induction of centrilobular peroxisomes in females by DEHT is presented graphically in Figure 5. This figure represents the mean subjective peroxisome score obtained from examination of liver sections with electron microscopy. It contains data from the negative control group, the 1.2% and 2.5% DEHT groups, and the group administered the positive reference material (1.2% DEHP). For comparison, the peroxisome scores obtained from the BIBRA assays (Barber et al., 1987) on structurally similar materials are also included. All of the scores from the related materials are from animals consuming 2.5% test material in the diet. Similar effects were seen in males and females. In all cases, dietary administration of 1.2% DEHT caused no increase in the number of hepatic peroxisomes compared to control animals. This result substantiates the peroxisome results obtained in our subchronic study (Topping, 1985). In that study, no peroxisome induction was detected, even

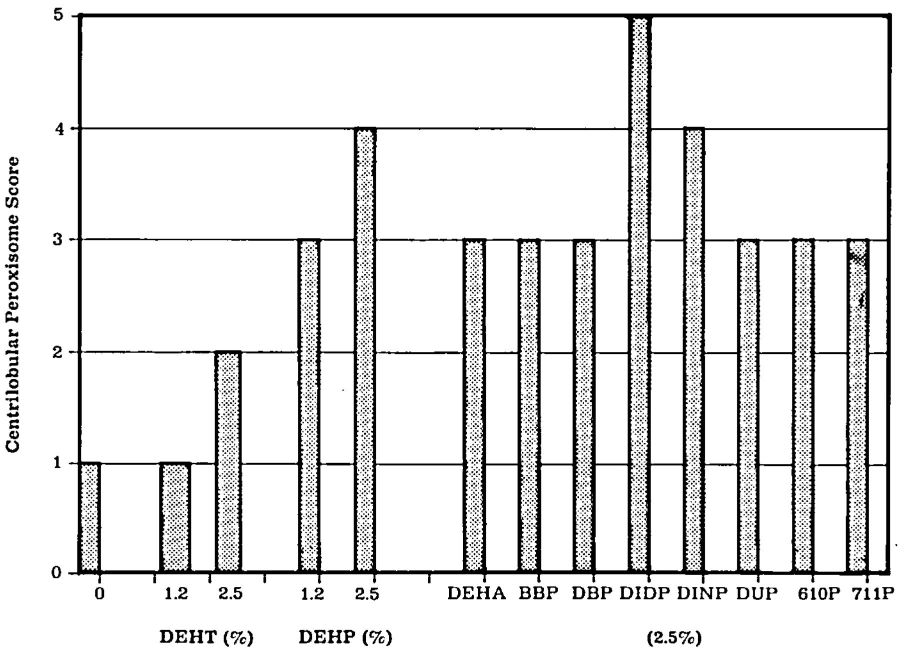

FIGURE 5. Mean hepatic centrilobular peroxisome proliferation scores from female rats consuming diets containing 0, 1.2% or 2.5% DEHT, 1.2% DEHP or related esters. Peroxisome scores: 1, few; 2, slight increase; 3, moderate increase; 4, marked increase; 5, very marked increase. Numbers represent concentration of the test material in the diet. For abbreviations, see Fig. 3. Data on related esters are from Barber et al. (1987).

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after 90 days of dietary exposure to doses of DEHT as high as 1.0%. In the present study, increases in peroxisomes occurred only after consuming the 2.5% diet of DEHT. The response to 2.5% DEHT was significantly less than that seen with a comparable dose of DEHP, and was less than the peroxisomal response seen with the other esters tested in the study of Barber et al. (1987).

DISCUSSION

Almost without exception, the inclusion of DEHT in the diet caused significant effects only at a level which severely decreased food consumption and weight gain. All of the findings on animals fed 2.5% DEHT are of doubtful significance with respect to systemic toxicity of the material, since fasting alone has been implicated in altered lipid metabolism and the formation of hepatic peroxisomes (Ishii et al., 1980). The effect of reduced feed intake is implicated in the causation of the effects which were seen, since the actual dose of DEHT to animals fed the 2.5% diet was only approximately 40% greater than that to animals consuming the 1.2% diet, yet the magnitude of the difference in response was much greater in nearly every instance. Statistically significant increases in relative liver weights were found with 1.0% DEHT in the diet in the 90-day study, and with 1.2% DEHT in the present 28-day study. Significant effects on peroxisomal enzymes were seen, but they were not of the magnitude obtained with DEHP, and the number of peroxisomes was not increased after DEHT as it was after DEHP.

To understand the difference in hepatic response to DEHP and DEHT, data were reviewed from a study of the metabolic fate of DEHT in rats (Enriquez et al., 1986). The disposition of radiolabeled DEHT administered by gavage was evaluated 48 hours after dosing. After 48 hr, 36.6% of the material was found unchanged in the feces, and 51% was found in the urine as free terephthalic acid. Only 2.5% of the radioactive label was identified in the feces as mono(ethylhexyl)terephthalate, with the remainder appearing as further metabolites of 2-ethylhexanol or the monoester. The presence of large amounts of free terephthalic acid, together with the small amount of monoester, suggests that the major metabolic pathway of DEHT is via complete hydrolysis of the ester linkages, yielding two moles of 2-ethylhexanol and one mole of terephthalic acid. Thus, the metabolic pathway for DEHT appears to be different from the one described for metabolism of DEHP (Albro et al., 1973). DEHP is metabolized primarily to equimolar amounts of mono(ethylhexyl)phthalate and 2-ethylhexanol. Although 2-ethylhexanol or its metabolites has been shown to induce peroxisome proliferation (Moody and Reddy, 1978), the most active metabolites of DEHP in the induction of peroxisomes has been postulated to be the monoester or products of its metabolism. This has been demonstrated in *in vitro* studies with rat

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hepatocytes (Gray et al., 1983a; Mitchell et al., 1985). The difference in production of the monoester after metabolism of DEHP and DEHT in rodents may account for the differences in liver effects seen with these two compounds.

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DISCUSSION

SPEAKER FROM THE FLOOR: Is terephthalate a plasticizer similar to DEHP?

DR. TOPPING: It has some of the same applications because it is an isomer of DEHP. But it is not identical, and there are some areas in which it can't be used.

SPEAKER FROM THE FLOOR: I wanted to mention that one of my earlier questions really has not been answered. It referred to any plasticizer made with 2-ethylhexanol. Has anything been done with some other acid and alcohol besides those?

DR. TOPPING: Not to my knowledge.

SPEAKER FROM THE FLOOR: On some of your slides, you had columns of palmitoyl-CoA oxidase which refer specifically to the first out of four enzymes in the pathway. Are you measuring just that first enzyme by increased lipid production or are you measuring the overall beta oxidation pathway?

I would like to make a small semantic suggestion. Since the term "oxidase" refers only to the first enzyme, if you are using it as an overall pathway, it would perhaps be wise to call it palmitoyl-CoA oxidation in order to distinguish it in the minds of us biochemists.

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