Embryonic-Fetal Toxicity and Teratogenic Effects of Adipic Acid Esters in Rats

A. R. SINGH, W. H. LAWRENCE, and J. AUTIAN▲

Abstract □ Seven adipates (dimethyl, diethyl, dipropyl, diisobutyl, di-*n*-butyl, di-2-ethylhexyl, and dicyclohexyl) were evaluated for their embryonic-fetal toxicity and teratogenic effects in rats, and the results were compared to those of a previous study with phthalate esters. The data suggest that the adipates are less teratogenic than the comparable phthalates when administered during gestation in equivalent fractions of the respective LD_{50} doses.

Keyphrases □ Adipic acid esters—embryonic fetal toxicity and teratogenic effects in rats □ Embryonic fetal toxicity and teratogenic effects—adipic acid esters, rats □ Teratogenicity and embryonic fetal toxicity—adipic acid esters, rats □ Plasticizers, adipic acid esters—embryonic-fetal toxicity and teratogenic effects, rats

Adipates, esters of adipic acid and various alcohols, are employed as plasticizers for polyvinyl chloride, particularly for low temperature applications. Although no teratological information is available on these materials, there are some reports concerning their acute and subacute toxicities. Data on the acute oral toxicity of some of these adipates have been reported (1–3). A subacute study was conducted on di-2-ethylhexyl adipate, in which rats were fed a diet containing 0.5, 2.0, and 5.0% of the adipate, representing approximately 0.25, 1.0, and 2.5 g./kg./day, respectively. The only abnormality observed was a retardation of growth at the 5.0% level (1). Furthermore, dogs were given 2 g./kg./day of the compound, and only a transient loss of appetite was noted (1). In another study (2), di-2-ethylhexyl adipate was fed to rats; no adverse effect was noted at dietary levels equivalent to 0.16 g./kg./day, but an increased mortality was seen in those receiving 4.74 g./kg./day.

The metabolism and toxicity of adipic acid have also been studied, showing that this compound is oxidized *in vitro* by isolated rat liver mitochondria. However, its *in vivo* metabolism is incomplete, as evidenced by the presence of varying amounts of unchanged acid in the urine (4). When using ^{14}C-labeled adipic acid, it was found that 70% of the radioactivity in a 50-mg. dose administered to rats appeared in the expired carbon dioxide within 24 hr.; such carbon dioxide was produced more rapidly from acid labeled in the first rather than in the second carbon position (5). Low residual activity was confined mainly to the kidneys and liver, and ^{14}C-labeled urea, as well as glutamic, lactic, adipic, β-ketoadipic, and citric acids, was identified in the urine. Further studies showed that the urea and citric acid were not direct metabolites of adipic acid but were formed from the radioactive carbon dioxide. Demonstration that the ^{14}C-label of adipic acid can be incorporated into glycogen, with acetic and succinic acids as intermediates, and the identification of β-ketoadipic acid in the urine indicate that the metabolism of adipic acid is by β-oxidation (5).

Table I—Acute Toxicity of Adipic Acid Esters in Rats

Compound	Acute LD_{50}, ml./kg.	95% Confidence Limits, ml./kg.	Slope (b)[a]
Dimethyl adipate	1.8086	1.5735–2.0789	16.24
Diethyl adipate	2.5119	2.1859–2.8871	16.24
Dipropyl adipate	3.7858	3.3279–4.2087	19.55
Diisobutyl adipate	5.9500	4.6903–7.5482	4.69
Di-*n*-butyl adipate	5.2441	4.9023–5.6096	39.41
Di-2-ethylhexyl adipate	>50	-	—
Dicyclohexyl adipate	5.1007[b]	4.7038–5.5313	16.24

[a] Probit slope (b) = probits/log of dose. [b] Compound warmed to 37–38° to liquefy it for injection.

The acute LD_{50} of adipic acid in mice was found to be 1900 mg./kg. p.o. and 680 mg./kg. i.v. In rats, the intraperitoneal LD_{50} was 275 mg./kg. (6). These workers also fed adipic acid at dietary levels of 0.1–5.0% (representing an intake of 50–2500 mg./kg./day) to rats for 2 years, and the only abnormality they detected was reduced growth at levels of 3.0% (1500 mg./kg./day) or more. In another study, increased mortality, diarrhea, and reduced growth were noted in rats given a dietary level equivalent to 16 or 11.4 g./kg./day for 5 or 33 weeks, respectively (7). These effects were observed during the first 2 weeks, after which surviving animals recovered but histological changes in the liver, kidneys, and intestine were reported. However, none of these effects was evident at a dose level of 5.7 g./kg./day (7)[1].

Gaunt *et al.* (9) reported acute toxicity studies of dialkyl 79 adipate (adipate esters in which alcoholic portions contained seven–nine carbon atoms) in mice and rats and short-term feeding studies in rats. They found it to be most toxic to mice by the oral route (LD_{50} = 8–12 g./kg.), while rats tolerated 20 g./kg. without any mortalities. There were no deaths in either mice or rats at intraperitoneal doses up to 20 g./kg. These doses of dialkyl 79 adipate produced piloerection and diarrhea in both species along with reduced spontaneous activity; the diarrhea was more pronounced in the animals receiving the compound orally. In 98-day feeding studies with rats, they found the "no-effect" level to be 0.25% in the diet, representing approximately 125 mg. adipate/kg. rat/day. Some alterations in values obtained for nonlethal parameters (*e.g.*, weight gain, hemoglobin concentration, and relative kidney weight) were noted when the diet contained 0.5 or 1.0% dialkyl 79 adipate; however, no deaths were attributed to the adipate.

As the adipates comprise an important group of

[1] The Joint FAO/WHO Expert Committee on Food Additives allocated a conditional acceptable daily intake for man of 0–5.0 mg./kg. for adipic acid (8).

Table II—Embryonic-Fetal Toxicity of Adipate Esters on Rat Fetuses[a]

Treatment Groups	Volume Injected, ml./kg.	Number of Corpora Lutea	Number of Resorptions	Dead Fetuses	Live Fetuses	Mean Weight of Fetuses, g.[b]
Blunt needle (injection)	—	69	4 (6.0)	0	63 (94.0)	3.91 ± 0.02
Distilled water	10.00	59	4 (6.8)	0	55 (93.2)	4.40 ± 0.33
Normal saline	10.00	62	7 (11.5)	0	54 (88.5)	4.10 ± 0.13
Cottonseed oil	10.00	71	5 (7.5)	0	62 (92.5)	3.89 ± 0.09
Dimethyl adipate	0.0603	60	4 (6.8)	0	55 (93.2)	3.94 ± 0.05
	0.1809	71	9 (14.1)	0	55 (85.9)	3.79 ± 0.08
	0.3617	60	1 (1.8)	0	56 (98.2)	3.90 ± 0.08
	0.6028	62	3 (5.7)	0	50 (94.3)	3.85 ± 0.08
Diethyl adipate	0.0837	55	1 (1.9)	0	51 (98.1)	4.12 ± 0.12
	0.2512	58	4 (7.0)	0	53 (93.0)	4.01 ± 0.08
	0.5024	59	5 (9.3)	0	49 (90.7)	3.93 ± 0.04
	0.8373	58	6 (10.7)	2 (3.6)	48 (85.7)	3.95 ± 0.07
Dipropyl adipate	0.1262	66	2 (3.2)	0	61 (96.8)	3.90 ± 0.09
	0.3786	61	6 (10.9)	0	49 (89.1)	3.77 ± 0.17
	0.7572	64	6 (9.8)	0	55 (90.2)	3.63 ± 0.11*
	1.2619	56	9 (20.0)	0	36 (80.0)	3.61 ± 0.22
Diisobutyl adipate	0.1983	62	2 (3.6)	0	54 (96.4)	3.91 ± 0.07
	0.5950	64	1 (1.6)	0	62 (98.4)	3.93 ± 0.05
	1.1900	65	3 (4.8)	1 (1.6)	59 (93.6)	3.69 ± 0.03**
	1.9833	63	2 (3.2)	1 (1.6)	60 (95.2)	3.65 ± 0.08*
Di-*n*-butyl adipate	0.1748	52	2 (3.8)	0	50 (96.2)	3.91 ± 0.09
	0.5244	61	3 (4.9)	0	58 (95.1)	3.88 ± 0.05
	1.0488	72	2 (2.9)	1 (1.4)	66 (95.7)	3.71 ± 0.07*
	1.7480	65	6 (9.4)	2 (3.1)	56 (87.5)	3.72 ± 0.20
Di-2-ethylhexyl adipate	1.00	62	3 (5.3)	0	54 (94.7)	3.90 ± 0.09
	5.00	65	2 (3.1)	0	63 (96.9)	3.83 ± 0.03*
	10.00	60	4 (7.0)	0	53 (93.0)	3.49 ± 0.14*
Dicyclohexyl adipate	0.1700	68	9 (14.5)	2 (3.2)	51 (82.3)	3.70 ± 0.22
	0.5100	59	10 (19.6)	1 (2.0)	40 (78.4)	3.50 ± 0.22
	1.0201	63	12 (19.4)	2 (3.2)	48 (77.4)	3.50 ± 0.34
	1.7002	66	13 (20.0)	5 (7.7)	47 (72.3)	3.06 ± 0.23**

[a] Five pregnant female rats were injected in each group on Days 5, 10, and 15 of pregnancy. [b] Numbers represent the average values (grams) ± the standard error of the mean for each group. *$p \leq 0.05$, **$p \leq 0.01$, significantly different from blunt needle-injected controls.

plasticizers, it was decided to initiate embryonic-fetal toxicity and teratogenic tests in rats with seven adipic acid esters. A parallel study (10) on some phthalic acid esters provided data with which to make a comparison between certain members of the two series.

EXPERIMENTAL

Materials—The adipates included in this study were: dimethyl[2], diisobutyl[2], diethyl[3], dipropyl[3], di-2-ethylhexyl[3], di-*n*-butyl[3], and dicyclohexyl[3]. Test animals were adult, virgin female, Sprague-Dawley rats[4], weighing 175–225 g. Adult, male rats of this strain were utilized as the "stud pool."

Procedure—Acute toxicity of the adipate esters was determined by administering graded doses of these specific samples by intraperitoneal injection to rats and observing the animals for mortality for 7 days. Acute LD_{50} values, 95% confidence limits, and slopes were calculated by Cornfield and Mantel's modification of Karber's method (11).

Female rats were selected for experimentation only after observation of at least two complete 4- or 5-day estrus cycles. Occurrence of estrus was determined by daily vaginal smears, obtained by introducing 0.2 ml. of fresh, clean tap water into the vagina with a smooth, clean, sterile medicine dropper, withdrawing a part of the liquid, and transferring it to a clean slide. The slide was examined microscopically while fresh, and the stage of estrus (proestrus, estrus, or diestrus) was determined according to cell types found in the vaginal smear.

Five female rats were housed with one male in a large cage at room temperature (22–27°) with food[5] and fresh tap water provided *ad libitum*. The onset of gestation was established by the presence of sperm in the vaginal smear and was designated as Day 0, with the following day being Day 1 of the gestation period. At this time, the female rats were moved to individual cages, where they were kept undisturbed except for specified injections. There were 31 groups, each composed of five female rats.

All treatments were administered by intraperitoneal injection on the 5th, 10th, and 15th days of gestation. The six more toxic adipates were injected intraperitoneally at four dosage levels: one-thirtieth, one-tenth, one-fifth, and one-third of their acute LD_{50} values in each injection. Di-2-ethylhexyl adipate was administered at three dosage levels of 1, 5, and 10 ml./kg. Distilled water, normal saline, and cottonseed oil were used at a dose of 10 ml./kg., corresponding to the largest volume of adipate injected (di-2-ethylhexyl adipate), although no diluent was used with the adipates. A "blunt-needle-injected" group[6] was also included for comparative purposes.

On the 20th day of gestation, 1 day prior to expected parturition, the rats were sacrificed by ether inhalation. The uterine horns and ovaries were surgically exposed to permit counting and recording of the numbers of corpora lutea, resorption sites, and viable and dead fetuses. Fetuses were removed, blotted dry, and weighed[7] to the nearest tenth of a milligram. All fetuses, both viable and nonviable, were examined for gross abnormalities. A randomly selected number (usually 50% of the total, excluding whenever possible those showing gross evidence of deformities) were prepared as transparent specimens to permit skeletal visualization for evaluating malformations. In a few instances, in which the incidence of resorption was very high, all fetuses were included. Skeletal visualization was accomplished by the procedure of Staples and Schnell (12). The remaining fetuses were examined for visceral abnormalities.

[2] Organic Chemical Division, Monsanto, St. Louis, Mo.
[3] Eastman Chemical Products, Kingsport, Tenn.
[4] Sprague-Dawley, Inc., Madison, Wis.
[5] Purina laboratory chow.
[6] In this group of controls, a dull needle was inserted in the same manner as used for intraperitoneal injections, but no substance was actually injected.
[7] Using a Mettler H6T balance.

Table III—Gross, Skeletal, and Visceral Malformations Produced by Adipic Acid Esters

Treatment Groups	Volume Injected, ml./kg.	Resorptions[a] (%)	Abnormalities— Gross[b]	Skeletal[c]	Visceral[d]
Blunt needle (injection)	—	4 (6.0)	0	1 (3.0%)	0
Distilled water	10.00	4 (6.8)	0	0	—
Normal saline	10.00	7 (11.5)	1 (1.9%)	4 (14.3%)	—
Cottonseed oil	10.00	5 (7.5)	1 (1.6%)	2 (6.3%)	0
Dimethyl adipate	0.0603	4 (6.8)	0	0	0
	0.1809	9 (14.1)[e]	1 (1.8%)	2 (7.4%)	0
	0.3617	1 (1.8)	2 (3.6%)[e]	4 (13.8%)[e]	0
	0.6028	3 (5.7)	4 (8.0%)[e]	5 (19.2%)[e]	2 (8.3%)
Diethyl adipate	0.0837	1 (1.9)	0	0	0
	0.2512	4 (7.0)	1 (1.9%)	0	1 (4.0%)
	0.5024	5 (9.3)	1 (2.0%)	1 (4.0%)	0
	0.8373	6 (10.7)	2 (4.2%)[e]	2 (8.0%)	0
Dipropyl adipate	0.1262	2 (3.2)	0	0	0
	0.3786	6 (10.9)	1 (2.0%)	0	0
	0.7572	6 (9.8)	2 (3.6%)	0	0
	1.2619	9 (20.0)[e]	2 (5.6%)[e]	1 (5.3%)	0
Diisobutyl adipate	0.1983	2 (3.6)	0	0	0
	0.5950	1 (1.6)	2 (3.2%)[e]	2 (6.3%)	0
	1.1900	3 (4.8)	1 (1.7%)	3 (10.0%)	0
	1.9833	2 (3.2)	5 (8.3%)[e]	3 (9.7%)	1 (3.4%)
Di-*n*-butyl adipate	0.1748	2 (3.8)	0	0	0
	0.5244	3 (4.9)	1 (1.7%)	0	1 (3.7%)
	1.0488	2 (2.9)	2 (3.0%)	0	0
	1.7480	6 (9.4)	3 (5.4%)[e]	2 (6.7%)	1 (3.8%)
Di-2-ethylhexyl adipate	1.00	3 (5.3)	0	1 (3.6%)	0
	5.00	2 (3.1)	1 (1.6%)	3 (9.4%)	1 (3.2%)
	10.00	4 (7.0)	2 (3.8%)[e]	2 (7.1%)	1 (4.0%)
Dicyclohexyl adipate	0.1700	9 (14.5)[e]	0	0	0
	0.5100	10 (19.6)[e]	4 (10.0%)[e]	0	0
	1.0201	12 (19.4)[e]	3 (6.3%)[e]	1 (3.8%)	0
	1.7002	13 (20.0)[e]	4 (8.5%)[e]	1 (4.0%)	0

[a] Percent resorptions are based on total number of resorptions and dead and live fetuses. [b] Percent gross abnormalities are based on total number of fetuses. [c] Percent skeletal abnormalities are based on total number of stained fetuses (50% of total fetuses). [d] Percent visceral abnormalities are based on total number of unstained fetuses. [e] Values greater than the 95% confidence interval of the "pooled volume control."

The following parameters of adverse effects were investigated: (*a*) embryonic–fetal toxicity, as evidenced by resorptions and stillbirths, (*b*) gross (external) malformations of fetuses, (*c*) skeletal malformations, (*d*) visceral abnormalities, and (*e*) fetal size (weight). In all evaluations, both viable and nonviable fetuses were considered.

RESULTS

The observed number of corpora lutea ranged from 52 to 72 per group, with a mean of 62.5. Of the 1938 corpora lutea observed in this study, 1840 or 94.9% were accounted for as live fetuses, dead fetuses, or resorption sites.

The acute intraperitoneal LD_{50} values and the 95% confidence limits for the adipate esters are presented in Table I. As shown, LD_{50} values were obtained for six of the adipates used in this study, but the sample of di-2-ethylhexyl adipate used failed to produce 50% mortality at doses considerably in excess of 50 ml./kg. Dimethyl adipate is the most acutely toxic, while the other adipate esters showed a decreased toxicity, generally as the size of the alcoholic portion increased.

Compound-dependent and dose-related effects upon embryonic and fetal growth and development were generally observed with these adipates. The results of developmental changes are presented in Tables II and III for the distilled water, normal saline, cottonseed oil, blunt-needle-injected, and all experimental groups. Table II presents data concerning the number of corpora lutea, resorption sites, dead fetuses, live fetuses, and fetal weights according to treatment groups. Table III shows a comparison of the number and percent of resorptions and gross, skeletal, and visceral abnormalities.

Resorptions—All control and treatment groups exhibited one or more resorptions. These ranged from 1.6% for one-tenth of the acute LD_{50} of diisobutyl adipate to 20.0% for one-third of the LD_{50} dose level of dicyclohexyl and dipropyl adipates. The highest incidences were seen with the four dose levels of dicyclohexyl adipate (20.0, 19.4, 19.6, and 14.5%), and the highest dose of dipropyl adipate (20.0%). The lowest incidences of resorptions occurred in the groups treated with one-tenth of the LD_{50} of diisobutyl adipate (1.6%), one-fifth of the LD_{50} of dimethyl adipate (1.8%), and one-thirtieth of the LD_{50} of diethyl adipate (1.9%). Only one resorption site was observed in each of these last three experimental groups.

Fetal Deaths—Fetal deaths were observed with much less frequency and in smaller numbers than the other criteria considered. Although most experimental groups revealed some resorption sites, dead fetuses were found only in groups treated with four of the compounds: diisobutyl, diethyl, di-*n*-butyl, and dicyclohexyl adipates. The two high doses of diisobutyl adipate produced one dead fetus (1.6%) for each dose level; two dead fetuses (3.6%) were found in the group receiving the high dose of diethyl adipate; and two (3.1%) dead fetuses and one (1.4%) dead fetus were found in groups treated with one-third and one-fifth of the acute LD_{50} dose levels of di-*n*-butyl adipate, respectively. Dicyclohexyl adipate produced fetal toxicity at all four dose levels employed. It revealed the largest number of fetal deaths in this study. Two (3.2%), one (2.0%), two (3.2%), and five (7.7%) fetal deaths were noted with one-thirtieth, one-tenth, one-fifth, and one-third of the acute LD_{50} dose, respectively.

Teratogenicity—*Gross Abnormalities*—A significant number of malformations were observed throughout the treatment groups, and these tended to show a dose-related incidence when considered as percentage of malformations. No gross abnormalities were seen in the blunt-needle (without injection) or distilled water control groups; neither were there any with one-thirtieth of the acute LD_{50} dose of any adipates employed nor with 1 ml./kg. of the di-2-ethylhexyl adipate. One malformed fetus was seen in each group treated with normal saline, cottonseed oil, dimethyl adipate (0.1809 ml./kg.), diethyl adipate (0.5024 and 0.2512 ml./kg.), di-*n*-butyl adipate (0.5244 ml./kg.), diisobutyl adipate (1.1900 ml./kg.), dipropyl adipate (0.3786 ml./kg.), and di-2-ethylhexyl adipate (5 ml./kg.), representing from 1.6 to 2.0% of the fetuses within the various groups. The greatest number of gross malformations seen was five per group, which occurred with the high dose of diisobutyl adipate; this represented 8.3% of the total fetuses. A complete tabulation of the

number and percentage of fetuses in which gross abnormalities were noted is presented in Table III.

The most common gross abnormalities observed were hemangiomas on various parts of the fetus, twisted hindlegs, and compact head and neck (some were monsters). One-fifth the acute LD_{50} of dimethyl and dipropyl adipates produced one fetus each without a tail. Most adipates studied produced a number of poorly developed and very small fetuses.

Skeletal Abnormalities—The number of skeletal abnormalities observed for each group and its percentage of visualized fetuses are presented in Table III. The abnormalities most often seen were elongated anterior ribs, often fused to the sternebrae, as well as elongated and fused posterior ribs (bilateral or unilateral); less frequently, one or two ribs were missing.

The incidences of skeletal abnormalities ranged from 0% for the distilled water, the lowest dose of dimethyl and diisobutyl adipates, the two lower doses of diethyl and dicyclohexyl adipates, and the three lower doses of di-*n*-butyl and dipropyl adipates up to a high of 19.2% for the high dose of dimethyl adipate.

Visceral Abnormalities—Visceral abnormalities were observed with much less frequency and in smaller numbers than were the gross and skeletal abnormalities. No visceral abnormalities were seen in the blunt-needle control group, cottonseed oil group, or any groups treated with dipropyl and dicyclohexyl adipates. The highest incidence of visceral abnormalities seen was two per group, which occurred with the high dose of dimethyl adipate. The most common visceral abnormalities observed were umbilical hernia, angulated anal opening, and absence of one kidney.

Fetal Size—Fetuses from most of the treated groups of rats were smaller, based upon weight, than those from the blunt needle-injected control group. However, these differences were not statistically significant except with the two higher dose levels of diisobutyl, the two higher dose levels of di-2-ethylhexyl, the one-fifth LD_{50} dose levels of dipropyl and di-*n*-butyl, and the highest dose level of dicyclohexyl adipates. The control fetuses averaged 3.91 g. each, while those from the adipate ester-treated rats had a mean fetal weight from 3.06 to 4.12 g. (Table II).

DISCUSSION

Administration of these adipate esters to pregnant rats on the 5th, 10th, and 15th days of gestation generally produced a compound-dependent and dose-related increase in resorptions and gross and skeletal abnormalities and a reduction in fetal weight at birth. The types of abnormalities found with these compounds were quite similar to those reported by Singh *et al.* (10) for a group of phthalate esters; however, the incidences of abnormalities were less in the adipate-treated animals. Although visceral abnormalities were observed less frequently than other types, when they occurred they were generally in the groups receiving the highest dose or doses. The two exceptions seen were with 0.2512 ml./kg. of diethyl adipate and 0.5244 ml./kg. of di-*n*-butyl adipate. In the former group, one fetus had an umbilical hernia and, in the latter group, one fetus exhibited an incomplete vascular development, particularly in the area of the shoulders.

As shown in Tables II and III, the blunt-needle-injected rats produced no dead fetuses or gross or visceral abnormalities, and there were only four (6%) resorption sites, one (3%) skeletal abnormality, and 63 (94%) live fetuses from 69 corpora lutea. Thus, if these findings are used as the baseline, most treatments produced an effect in one or more of the parameters studied except the lowest dose level, one-thirtieth of the acute LD_{50} of each adipate ester.

A more conservative evaluation could be made between the effects produced by the adipic acid esters when compared to the results of a "volume control" to compensate for any effects resulting from the physical or mechanical trauma of injection and handling. Since no diluent was used when these compounds were injected and the injected volume ranged from 0.0603 to 10.00 ml./kg., it was decided to employ distilled water, normal saline, and cottonseed oil in a quantity equivalent to the largest dose of the test compound (10.00 ml./kg.). Since the results obtained from these three "controls" were not uniform and there was no justification for selecting one of these in preference to the others, the data were combined to calculate a 95% confidence interval (13) for the "pooled volume control" in regard to resorptions and gross and skeletal abnormalities. The confidence intervals thus obtained were as follows: resorptions, 3.7–12.3%; gross abnormalities, 0.7–3.1%; and skeletal malformations, 1.0–12.6%. A significant increase in resorptions was observed for the 0.1809-ml./kg. level of dimethyl adipate, for 1.2619 ml./kg. of dipropyl adipate, and for all four dose levels of dicyclohexyl adipate. The incidence of gross abnormalities was increased significantly in groups receiving the following treatments: 0.3617 and 0.6028 ml./kg. of dimethyl adipate, 0.8373 ml./kg. of diethyl adipate, 0.7572 and 1.2619 ml./kg. of dipropyl adipate, 0.5950 and 1.9833 ml./kg. of diisobutyl adipate, 1.7480 ml./kg. of di-*n*-butyl adipate, the high dose (10.00 ml./kg.) of di-2-ethylhexyl adipate, and the three highest doses of dicyclohexyl adipate. Concerning skeletal malformations, only the two highest dose levels of dimethyl adipate showed an incidence greater than the 95% confidence interval (Table III).

Dicyclohexyl adipate was interesting due to the marked effect this compound had upon the developing embryo and fetus. Quite a number of fertilized ova were detected only as resorption sites in all four dose levels, indicative of an early embryotoxic effect; dead fetuses were found with all four dose levels employed, indicating a later toxic action as well. In spite of its embryonic-fetal toxicity, a number of gross abnormalities were noted in the three higher dose groups. Most abnormalities produced by this compound were hemangiomas of different parts of the body, and twisted hindlegs were often observed. After visualizing and staining the skeleton, one of the 25 visualized fetuses of the high dose level had two ribs less than normal; one of the 26 in the next highest dose group had elongated and fused posterior ribs, while those in the two lower dose groups did not show any skeletal malformation. No visceral abnormalities were seen in the groups receiving this adipate.

Another compound, dimethyl adipate, produced considerable embryotoxic and teratogenic effects. In the high dose group, one fetus had hemangiomas of the neck, one on the shoulder, one of the foreleg, and one on the right thigh. Skeletal examinations of this group revealed five of 26 fetuses had elongated frontal ribs fused to the sternebrae. This group also contained two visceral abnormalities: one fetus did not have the left kidney and one fetus had an angulated anal opening. In the second highest dose group, one fetus did not have a tail; two of the 29 stained fetuses had a few elongated and fused posterior ribs, and two fetuses had elongated anterior ribs fused to the sternebrae. The 0.1809-ml./kg. dose level of this compound produced only one fetus with hemangiomas of the right hindquarter, while two of the 27 stained fetuses had elongated anterior ribs fused to the sternebrae. The lowest dose level did not produce any gross, skeletal, or visceral abnormalities.

Diisobutyl adipate produced a number of gross and skeletal abnormalities in offspring of treated rats; however, only one fetus demonstrated a visceral abnormality (perforated anal opening) in the high dose group. Also within this group, one fetus had a hemangioma of the chin and another had one on the back; three had twisted hindlegs. Skeletal examinations of this group revealed three of 31 fetuses had elongated and fused posterior ribs. In the second highest dose group, one fetus had a hemangioma of the chin; two of 30 stained fetuses had elongated and fused posterior ribs, and one fetus had elongated anterior ribs fused to the sternebrae. In the group receiving 0.5950 ml./kg., one fetus had a hemangioma of the shoulder, and another fetus had a hemangioma of the neck. One of 32 stained fetuses had elongated and fused posterior ribs, and one had elongated anterior ribs fused to the sternebrae. The lowest dose group of this compound did not show any gross, skeletal, or visceral abnormalities.

Diethyl, dipropyl, di-*n*-butyl, and di-2-ethylhexyl adipates produced few gross, skeletal, or visceral abnormalities, and these occurred mainly at the higher dose levels. In very few of these groups were the incidences greater than the pooled volume control. Two dead fetuses were found in the high dose group of diethyl adipate, and two dead fetuses were observed in the high dose group and one dead fetus in the second highest dose group of di-*n*-butyl adipate.

One-thirtieth of the acute LD_{50} dose of the six adipic acid esters used in this study for which there was an acute LD_{50} (dimethyl, diethyl, dipropyl, diisobutyl, di-*n*-butyl, and dicyclohexyl adipates) were completely tolerated by rat fetuses after intraperitoneal administration on the 5th, 10th, and 15th days of pregnancy, since this dose level of each compound did not produce any gross, skeletal, or visceral abnormalities. Therefore, these compounds may be considered not teratogenic under these conditions at this dose level. Therefore, from the data obtained, it would appear that the "no effect" level for these adipates under the conditions of this test would be equal to or greater than one-thirtieth but less than one-tenth

Table IV—Comparison of Embryonic–Fetal Toxicity and Teratogenicity of Adipates and Phthalates[a]

Fraction of Acute LD_{50} Dose of Ester Administered	Dead Fetuses, %		Resorptions, %		Abnormalities, %: Gross		Abnormalities, %: Skeletal	
	Adipate	Phthalate	Adipate	Phthalate	Adipate	Phthalate	Adipate	Phthalate
Dimethyl								
0.10	0	0	14.1	33.3	1.8	9.5	7.4	25.0
0.20	0	1.9	1.8	0	3.6	7.5	13.8	35.3
0.33	0	9.4	5.7	32.1	8.0	11.1	19.2	75.0
Diethyl								
0.10	0	0	7.0	3.6	1.9	0	0	26.3
0.20	0	0	9.3	0	2.0	0	4.0	47.1
0.33	3.6	0	10.7	44.4	4.2	0	8.0	81.3
Diisobutyl								
0.10	0	0	1.6	9.6	3.2	0	6.3	14.8
0.20	1.6	3.6	4.8	5.5	1.7	3.9	10.0	17.2
0.33	1.6	0	3.2	25.8	8.3	0	9.7	33.3

[a] Phthalate ester data taken from Singh *et al.* (10).

of their respective acute LD_{50} values. Di-2-ethylhexyl adipate, which failed to produce 50% mortality at doses in excess of 50 ml./kg. i.p., did not produce gross or visceral abnormalities at the 1-ml./kg. dose level; only one skeletal abnormality was noted in this group.

A previous study with phthalic acid esters (10) indicated a range of embryonic–fetal toxicity from 0 to 98.2% in rats treated with one-tenth, one-fifth, and one-third of the acute LD_{50} doses of the phthalates, whereas similar dose levels of adipates resulted in an embryonic fetal toxicity of 1.6–27.7%. It would appear, therefore, that the adipic acid esters possess a lower degree of toxicity to the developing embryo and fetus than was seen with the phthalic acid esters.

Fetal malformations varied from 0 to 100%, both for gross and skeletal abnormalities, with skeletal defects generally being more common with phthalate ester-treated rats; fetal malformations in adipate-treated rats ranged from 1.6 to 10% for gross abnormalities and from 0 to 19.2% for skeletal deformities. Thus, the comparison suggests the phthalates exert a more potent teratogenic effect than that noted for the adipates.

Three adipates included in this study were comparable analogs to phthalates reported earlier (10). When comparable doses, based upon the fraction of an acute LD_{50} dose of each compound, were administered to rats at the same time intervals, marked differences were noted in the abnormalities seen. Examination of Table IV reveals differences between the esters; in general, the phthalates tended to produce the greatest effect. This is especially noticeable for skeletal abnormalities.

The mean fetal weight on the 20th day of gestation ranged from 1.75 to 4.0 g. in rats treated with phthalic acid esters; in those treated with adipic acid esters, the mean fetal weight was 3.06–4.12 g. This indicates that adipic acid esters exert less growth-retarding effect than the phthalic acid esters. Overall, the data suggest that the embryonic–fetal toxicity and teratogenic effects of adipic acid esters were less than those produced by comparable doses of the phthalic acid esters.

SUMMARY AND CONCLUSIONS

All of the adipic acid esters studied exerted some degree of deleterious effect upon the developing embryo and fetus. At one or more of the dose levels employed, each compound produced some or all of the following effects: resorptions; gross, skeletal, and visceral abnormalities; fetal death; and decreased fetal size. Blunt-needle-injected animals were used as controls to determine the "normal" fetal size and a baseline of normal values; within this group there were no gross or visceral abnormalities or fetal deaths, and only 6% resorptions and one (3%) skeletal deformity were observed. However, seven experimental groups contained fetuses of significantly lighter weights than these controls. A secondary comparison was made using a 95% confidence interval obtained by pooling data from volume controls of distilled water, normal saline, and cottonseed oil. When a comparison was made using the 95% confidence limits of the pooled volume controls, 12 of the adipate-treated groups showed a significant incidence of gross abnormalities, six groups had a significant increase in resorptions, and two showed a definite increase in skeletal malformations.

Although this regimen of adipic acid ester administration did not elicit as great an effect as did the phthalates upon embryonic and fetal development, the incidences of adverse effects noted were generally dose related and compound dependent, perhaps with the more soluble compounds tending to be more active. The "no effect" level established in this study for adipic acid esters was approximately one-thirtieth of the acute LD_{50} dose.

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▲ To whom inquiries should be directed.