Teratogenicity of Phthalate Esters in Rats

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Abstract □ In the past, it was demonstrated that small quantities of phthalates may be released from polyvinyl chloride items to physiological solutions. To ascertain the effect these plasticizers might have upon the fetus, a teratogenic study was undertaken in rats using eight phthalate esters. Six of the phthalates, whose acute intraperitoneal LD_{50} values were below 10 ml./kg., were administered to rats at doses representing one-tenth, one-fifth, and one-third of the acute LD_{50} dose on the 5th, 10th, and 15th day of gestation; the two less toxic compounds were injected at levels of 5 and 10 ml./kg. Distilled water and normal saline were tested at 10 ml./kg., and cottonseed oil was tested at 5 and 10 ml./kg. Untreated animals were used for control values. The number of corpora lutea ranged from 52 to 65 per group of five rats, with no apparent distribution according to treatment. Embryo-fetal toxicity ranged from 0% to a high of 98.2%. Fetal malformations ranged from 0 to 100%, both for gross and skeletal abnormalities, with skeletal defects generally being more common. Fetuses from all phthalate-treated groups were smaller ($p \leq 0.01$) than the untreated controls. Absence of tail, anophthalmia, and twisted hind legs were the most common gross abnormalities, while elongated and fused ribs and abnormal skull bones were the most common deformities found in stained skeletal specimens.

Keyphrases □ Phthalates—embryo-fetal toxicity, teratogenic effects, rats □ Teratogenicity—phthalates, rats □ Plasticizers—possible leaching of phthalates, teratogenic effects, rats

Numerous devices and materials made from polyvinyl chloride are available for medical, dental, and paramedical applications. To provide the desirable physical properties, particularly flexibility, these materials contain significant quantities of plasticizer, up to 40% of the finished product. Presently, one commonly used group of plasticizers is the esters of phthalic acid, of which di-2-ethylhexyl phthalate (often referred to simply as dioctyl phthalate or DOP[1]) is probably the most widely used. Evidence (1, 2) has been accumulating to indicate that small quantities of plasticizer may be leached from the material by blood or various injectable solutions. However, toxicologic implications were discounted by many based upon animal feeding experiments and the magnitude of difference between the quantities so leached and the acute toxicity of these compounds.

More recently, Jaeger and Rubin (3) reported finding di-2-ethylhexyl phthalate in the tissues of two patients who had received transfusions of blood stored in plastic bags. They found concentrations ranging from 2.5 to 27 mg. % (dry weight) of di-2-ethylhexyl phthalate in the spleen, liver, lung, and abdominal fat, with the spleen containing the least and abdominal fat the most. *In vitro* experiments, which they conducted using the perfused rat liver, indicated this organ would accumulate di-2-ethylhexyl phthalate but would not hydrolyze it, while a related plasticizer, butyl glycolylbutyl phthalate, was hydrolyzed by this system.

Haberman and his coworkers (4–6) reported teratogenic effects (crania bifida, anophthalmia, "cross-beak," exophthalmia, and malrotation of left leg) from some phthalate esters when the compounds were injected into the yolk sac or allantoic cavity or were applied to the chorioallantoic membrane of developing chick embryos. After hatching, the chicks were examined for gross congenital malformations, symptoms of toxicity, and neuromuscular abnormalities. Chicks were kept 3 weeks and observed for adverse effects on development, particularly neurological damage.

In addition to their use in plastics, some of these esters are ingredients in insect repellent formulations. They generally have a low order of oral toxicity, although ingestion can cause GI disturbances, irritation of mucous membranes and eyes, and even CNS depression (7). Toxicity studies on phthalate esters were conducted by a number of investigators (8–15). Some parenteral toxicity data were presented, but the majority of the reports involved oral feeding experiments.

The present study was undertaken to determine the teratogenic potential in mammals of some phthalate esters.

MATERIALS AND METHODS

Materials Eight phthalate esters were included in this study: dimethyl phthalate[2], dimethoxyethyl phthalate[2], diethyl phthalate[2], dibutyl phthalate[2], diisobutyl phthalate[2], butyl carbobutoxymethyl phthalate[3], dioctyl phthalate[3], and di-2-ethylhexyl phthalate[3].

Test animals were adult, virgin female rats of the Sprague-Dawley strain[4], weighing between 200 and 250 g. Male rats of the same strain and age were utilized as the "stud pool."

Procedure—Acute toxicity of the phthalates was determined by administering graded doses of these specific samples by intraperitoneal injection in rats and observing the animals 7 days for mortality. LD_{50} values of 95% confidence limits were calculated by Cornfield and Mantel's modification (16) of Karber's method or by the method of Weil (17).

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. Vaginal smears were 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 microscope slide. The slide was examined microscopically while fresh, and the stages of the estrus cycle were recorded according to cell types found in the vaginal smear.

Five female rats were housed with one male in a large cage and kept in a room at 22–27°. Laboratory chow (Purina) and fresh, clean tap water were provided *ad libitum*. The onset of gestation was established by the presence of sperm in the vaginal smear and was designated as Day 0. The following day was recorded as Day 1 of the gestation period. At this time, the female rats were moved to

[1] Common use of the term DOP or dioctyl phthalate in referring to the compound with the branched chain octyl group, 2-ethylhexyl, often creates confusion concerning the exact chemical identity between the straight-chain plasticizer (dioctyl phthalate) and its branched-chain isomer (di-2-ethylhexyl phthalate).

[2] Eastman Chemical Products, Kingsport, Tenn.
[3] Matheson, Coleman and Bell, Cincinnati, Ohio.
[4] Sprague-Dawley Inc., Madison, Wis.

Table I— Acute Toxicity in Rats

Compound	LD_{50}, ml./kg.	95% Confidence Limits, ml./kg.
Dimethyl phthalate	3.3751	2.5338 4.4955
Dimethoxyethyl phthalate	3.7355	3.0496-4.5744
Diethyl phthalate	5.0579	3.7973 6.7370
Dibutyl phthalate	3.0496	2.0688 4.4957
Diisobutyl phthalate	3.7498	2.5000-5.6249
Butyl carbobutoxymethyl phthalate	6.8892	5.1720-9.1766
Dioctyl phthalate	>50 ml./kg.	
Di-2-ethylhexyl phthalate	>50 ml./kg.	

individual cages where they were kept undisturbed except for specified injections. Each group was composed of five female rats.

All treatments were administerd by intraperitoneal injections on the 5th, 10th, and 15th day of gestation. The six more toxic phthalates were administered at three dosage levels: one-tenth, one-fifth, and one-third of the acute LD_{50} in each of the three injections[5]. The other two phthalates (dioctyl phthalate and di-2-ethylhexyl phthalate) and cottonseed oil were administered at two-dosage levels of 5 and 10 ml./kg., while normal saline and distilled water were used only at the 10-ml./kg. level. An untreated control was also included.

On the 20th day of gestation, 1 day prior to expected parturition, each rat was placed in an inhalation chamber and killed with an overdose of ether. 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 to the nearest tenth of a milligram[6]. All fetuses, both viable and nonviable, were examined for gross malformations.

A randomly selected number of fetuses (30–50% of the total), excluding whenever possible those showing gross evidence of malformations, were prepared as transparent specimens to permit visualization of their skeletal system for evaluation of skeletal malformations. This was accomplished by the procedure of Staples and Schnell (18). Briefly, this procedure involves fixing in 70% ethanol, dehydrating with acetone, clearing with 1% potassium hydroxide, and selective staining of bone with alizarin red S stain. Then the specimens are rinsed in cold water, gently blotted, and immersed in a well-stirred mixture of 70% aqueous ethanol, glycerol, and benzyl alcohol. The cleared specimen may be preserved, if desired, in pure glycerol with the addition of a crystal of thymol to inhibit bacterial and mold growth.

RESULTS

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

The study encompassed 27 groups, each containing five female rats. The number of corpora lutea observed ranged from 52 to 65 per group, with a mean of 58.15. Of the 1570 corpora lutea observed in this study, 1535 (97.8%) were accounted for as live fetuses, dead fetuses, or resorption sites.

The acute intraperitoneal LD_{50} values and 95% confidence limits for the phthalate esters are presented in Table I. As shown, LD_{50} values were obtained for six of the phthalates employed in this study, but the specific samples of dioctyl phthalate and di-2-ethylhexyl phthalate used failed to produce 50% mortality at doses considerably in excess of 50 ml./kg.

The results of this investigation are presented in Tables II and III for the untreated controls and the groups treated with distilled water, normal saline, cottonseed oil, and the eight phthalate esters. Table II presents the data for the number of corpora lutea observed, resorption sites, dead fetuses, live fetuses, and fetal weights according to treatment groups. Table III presents a comparison of the number and percent of resorptions, gross abnormalities, and skeletal abnormalities.

[5] See Table I for LD_{50} values.
[6] Using a Mettler H6T balance.

DISCUSSION

Compound-dependent and dose-related effects upon embryonic and fetal growth and development were generally observed with these phthalates. The effects produced by normal saline should not be too surprising, since Nishimura (19) indicated that abnormalities can be produced in animals by hypertonic saline (1.9–3.75 g./kg.) when given at a critical time of fetal development.

Resorptions There were no resorptions, dead fetuses, and gross or skeletal abnormalities in the untreated control group. The intermediate doses of diethyl phthalate and dimethyl phthalate did not produce any resorption sites. The reason for this finding is not clear, since both higher and lower doses of both compounds produced some resorptions. The intermediate dose of dimethyl phthalate, however, did show one fetal death. All other substances tested showed some resorptions, with the highest incidences occurring with the two highest doses of dimethoxyethyl phthalate: 96.5 and 89.7%, respectively. The lowest incidences of resorptions occurred in the groups treated with diethyl phthalate (high dose), dibutyl phthalate (middle dose), and dioctyl phthalate (low dose). With each of these last three compounds, two resorption sites were observed, representing 3.6, 3.6, and 3.8%, respectively. In this category, di-2-ethylhexyl phthalate showed greater activity than dioctyl phthalate; this trend, however, was reversed when one considers fetal abnormalities.

Fetal Deaths Fetal deaths were observed with less frequency and in smaller number than the other criteria considered. Although most of the treated groups revealed some resorption sites, dead fetuses were found in groups treated with only three of the compounds (dimethyl, dimethoxyethyl, and diisobutyl phthalates); of these, only dimethoxyethyl phthalate showed a high level of fetal toxicity at all dose levels tested. A comparison of fetal deaths for these three compounds is presented in Table IV. As shown, the low dose of dimethoxyethyl phthalate produced the greatest number of fetal deaths; however, this finding reflects the more potent early toxicity of the higher doses of the compound as seen in the large numbers of resorptions produced. For instance, the high dose produced only one fetal death, but only two of the 57 fertilized ova developed into fetuses (50% fetal death). The intermediate dose produced four fetal deaths (66.7%), but only six of the 58 fertilized ova developed into fetuses. Thus, the final column of Table IV presents percent of fetal deaths plus resorptions. This comparison exhibits a dose-response relationship for the high, medium, and low doses of dimethoxyethyl phthalate of 98.2, 96.6, and 56.9%, respectively.

Teratogenicity—*Gross Abnormalities* –At the time of sacrifice, all fetuses, both viable and nonviable, were removed and examined grossly for structural abnormalities. A significant number of malformations were observed. They were not uniformly distributed throughout the treatment groups but tended to cluster around certain phthalates and to show a dose-related response pattern when considered as percentage of fetuses showing malformations. One malformed fetus was seen in each of the following groups: normal saline, cottonseed oil (10 ml./kg.), dimethoxyethyl phthalate (0.374 ml./kg.), and butyl carbobutoxymethyl phthalate (2.296 and 1.378 ml./kg.); this result represented from 1.8 to 2.4% of the fetuses from the various groups. Two malformations were noted for dimethoxyethyl phthalate (1.245 ml./kg.) and diisobutyl phthalate (0.750 ml./kg.). Due to the high incidence of resorptions for this dose of dimethoxyethyl phthalate, this result represented 100% of fetuses, whereas it represented only 3.9% for the diisobutyl phthalate group. The greatest number of gross malformations (15 fetuses or 27.3%) was seen with dioctyl phthalate (10 ml./kg.). A complete tabulation of the number of fetuses in which gross abnormalities were noted, as well as percentage of the total, is presented in Table III.

The most common gross abnormalities found in the phthalate-treated animals were absence of tail, anophthalmia, twisted hind legs[7], and hematomas (hemangiomas). Thus, there appears to be some similarity between the responses observed in rats in this study and the observations reported by Bower *et al.* (6) who used chicks to study the response to phthalates when injected into the incubating egg; they observed anophthalmia along with other abnormalities.

[7] Twisted hind legs are considered an abnormality here since all fetuses were delivered surgically, thus avoiding the possible trauma of natural birth.

Table II—Teratogenic Effects of a Group of Phthalate Esters on Rat Fetus[a]

Treatment Groups	Volume Injected, ml./kg.	Number of Corpora Lutea	Number of Resorptions[b]	Number of Dead Fetuses[b,c]	Number of Live Fetuses[b]	Average Weight of Fetus[d]
Untreated controls	None	60	0	0	59 (100.0%)	4.83 ± 0.01
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[e]
Cottonseed oil	10.00	59	4 (6.8%)	0	55 (93.2%)	4.45 ± 0.17
	5.00	54	3 (6.4%)	0	44 (93.6%)	4.65 ± 0.19
Dimethyl	1.125	55	17 (32.1%)	5 (9.4%)	31 (58.5%)	2.20 ± 0.18[e]
phthalate	0.675	55	0	1 (1.9%)	52 (98.1%)	2.60 ± 0.01[e]
	0.338	65	21 (33.3%)	0	42 (66.7%)	2.38 ± 0.13[e]
Dimethoxyethyl	1.245	58	55 (96.5%)	1 (1.8%)	1 (1.8%)	1.75 ± 0.00[e]
phthalate	0.747	58	52 (89.7%)	4 (6.9%)	2 (3.4%)	1.88 ± 0.38[e]
	0.374	59	16 (27.6%)	17 (29.3%)	25 (43.1%)	2.19 ± 0.25[e]
Diethyl	1.686	57	2 (3.6%)	0	54 (96.4%)	2.85 ± 0.27[e]
phthalate	1.012	59	0	0	57 (100.0%)	2.85 ± 0.19[e]
	0.506	65	28 (44.4%)	0	35 (55.6%)	2.63 ± 0.24[e]
Dibutyl	1.017	64	23 (36.5%)	0	40 (63.5%)	3.60 ± 0.10[e]
phthalate	0.610	56	2 (3.6%)	0	53 (96.4%)	3.65 ± 0.10[e]
	0.305	56	4 (7.3%)	0	51 (92.7%)	3.70 ± 0.09[e]
Diisobutyl	1.250	64	16 (25.8%)	0	46 (74.2%)	2.00 ± 0.46[e]
phthalate	0.750	55	3 (5.5%)	2 (3.6%)	50 (90.9%)	3.50 ± 0.21[e]
	0.375	52	5 (9.6%)	0	47 (90.4%)	3.60 ± 0.13[e]
Butyl carbobutoxy-	2.296	57	13 (24.1%)	0	41 (75.9%)	3.05 ± 0.30[e]
methyl phthalate	1.378	56	8 (14.5%)	0	47 (85.5%)	3.30 ± 0.05[e]
	0.689	55	4 (7.8%)	0	47 (92.2%)	3.95 ± 0.09[e]
Dioctyl	10.00	60	5 (8.3%)	0	55 (91.7%)	3.40 ± 0.13[e]
phthalate	5.00	53	2 (3.8%)	0	51 (96.2%)	4.00 ± 0.18[e]
Di-2-ethylhexyl	10.00	56	15 (26.8%)	0	41 (73.2%)	3.50 ± 0.18[e]
phthalate	5.00	61	5 (8.2%)	0	56 (91.8%)	3.55 ± 0.17[e]

[a] Five pregnant female rats were injected in each group on Days 5, 10, and 15 of pregnancy. [b] Numbers in parentheses indicate percent values based upon total number of implantations. [c] Also see Table IV, where fetal deaths are expressed as a percentage of total fetuses. [d] Numbers represent the average values (grams) ± *SE* for each group. [e] Significantly different from untreated controls at 99% level ($p \leq 0.01$) by Student's *t* test (20).

Skeletal Abnormalities—Generally, 30–50% of the fetuses were prepared for visualization of the skeletal structure. When possible, fetuses were selected that did not show gross abnormalities. However, in a few instances, in which the resorption rate was very high, all fetuses were used. Even with this type of specimen selection, there was generally a higher incidence of skeletal abnormalities than gross abnormalities.

Skeletal abnormalities were seen with saline, cottonseed oil (10 ml./kg. but not with 5 ml./kg.), and all three dose levels of dimethyl, dimethoxyethyl, diethyl, dibutyl, diisobutyl, and butyl carbobutoxymethyl phthalates. No skeletal abnormalities were observed with either dose level of dioctyl and di-2-ethylhexyl phthalates. Also, there were no abnormal bone structures in the untreated controls or the distilled water-treated animals. The percentage of skeletal mal-

Table III—Gross and Skeletal Malformations Produced by Phthalate Esters

Treatment Groups	Volume Injected, ml./kg.	Number of Resorptions[a]	Number of Gross Abnormalities[b]	Number of Skeletal Abnormalities[c]
Untreated controls	None	0	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	4 (6.8%)	1 (1.8%)	3 (10.7%)
	5.00	3 (6.4%)	0	0
Dimethyl phthalate	1.125	17 (32.1%)	4 (11.1%)	9 (75.0%)
	0.675	0	4 (7.5%)	6 (35.3%)
	0.338	21 (33.3%)	4 (9.5%)	4 (25.0%)
Dimethoxyethyl phthalate	1.245	55 (96.5%)	2 (100.0%)	2 (100.0%)
	0.747	52 (89.7%)	5 (83.3%)	4 (100.0%)
	0.374	16 (27.6%)	1 (2.4%)	13 (92.9%)
Diethyl phthalate	1.686	2 (3.6%)	0	13 (81.3%)
	1.012	0	0	8 (47.1%)
	0.506	28 (44.4%)	0	5 (26.3%)
Dibutyl phthalate	1.017	23 (36.5%)	0	8 (33.3%)
	0.610	2 (3.6%)	0	7 (24.1%)
	0.305	4 (7.3%)	0	6 (20.7%)
Diisobutyl phthalate	1.250	16 (25.8%)	0	8 (33.3%)
	0.750	3 (5.5%)	2 (3.9%)	5 (17.2%)
	0.375	5 (9.6%)	0	4 (14.8%)
Butyl carbobutoxy-	2.296	13 (24.1%)	1 (2.4%)	5 (21.7%)
methyl phthalate	1.378	8 (14.5%)	1 (2.1%)	4 (16.0%)
	0.689	4 (7.8%)	0	4 (13.8%)
Dioctyl phthalate	10.00	5 (8.3%)	15 (27.3%)	0
	5.00	2 (3.8%)	8 (15.7%)	0
Di-2-ethylhexyl phthalate	10.00	15 (26.8%)	9 (22.0%)	0
	5.00	5 (8.2%)	0	0

[a] Numbers in parentheses represent percent resorption based on total number of implantations. [b] Numbers in parentheses indicate percent gross abnormalities based on total number of fetuses. [c] Numbers in parentheses represent percent skeletal abnormalities based on total number of stained fetuses.

Table IV– Comparison of Fetal Deaths

Compound and Dose Level, ml./kg.	Fetal Deaths[a] Number	Fetal Deaths[a] Percent	Fetal Deaths + Resorptions[b], %
Dimethyl phthalate:			
1.125	5	13.9	41.5
0.675	1	1.9	1.9
0.338	0	0	33.3
Dimethoxyethyl phthalate:			
1.245	1	50.0	98.2
0.747	4	66.7	96.6
0.374	17	40.5	56.9
Diisobutyl phthalate:			
1.250	0	0	25.8
0.750	2	3.8	9.1
0.375	0	0	9.6

[a] Percent fetal deaths = (dead fetuses/total fetuses) × 100. [b] Fetal deaths + resorptions = (dead fetuses + resorption sites/total fetuses + resorption sites) × 100.

formations ranged from 13.8% for the low dose of butyl carbobutoxymethyl phthalate to 100% for the two highest doses of dimethoxyethyl phthalate and 92.9% for the low dose of this compound. The next most active phthalates in this regard were dimethyl and diethyl phthalates. Percentage of skeletal malformations produced by the three doses of dimethyl phthalate were 75.0, 35.3, and 25.0%, respectively; for diethyl phthalate, they were 81.3, 47.1, and 26.3%, respectively.

The skeletal abnormalities most commonly encountered were elongated and fused ribs (bilateral and unilateral[8]), absence of tail bones, abnormal or incomplete skull bones, and incomplete or missing leg bones. The missing leg bones were most often the ulna in the fore legs and the fibula in the hind legs and, less often, the radius in the fore legs and the tibia in the hind legs. Observation of incomplete skull bones may merely represent a developmental defect in which delayed ossification may be secondary to general retardation of growth and development of the fetus.

Fetal Size—Fetuses from all groups of phthalate-treated rats were smaller than the untreated controls. This difference was significant at the 99% level ($p \leq 0.01$). Although fetuses from treatments other than phthalates were slightly smaller than the controls, the difference was not significant at this level except for the saline-treated group. The control fetuses averaged 4.83 g. each, while those from the high and middle doses of dimethoxyethyl phthalate-treated rats had a mean fetal weight of 1.75 and 1.88 g., or 36.2 and 38.9% of the controls. Only a small number of fetuses were obtained from these groups (two and six, respectively). The mean fetal weight was 2.00 g. (41.4% of control) for those animals treated with the high dose of diisobutyl phthalate; this finding, however, was based upon 46 fetuses. Although fetuses from all phthalate-treated rats were significantly smaller than from the controls, there was a progressive increase in fetal size up to maximum mean of 4.00 g. (82.8% of controls) for the low dose of dioctyl phthalate. These data are presented in Table II.

Teratology, Summary Comments—A significant number of malformed fetuses were observed in most phthalate-treated animals. A few were also found in the groups treated with normal saline and cottonseed oil (10 ml./kg.). However, none was found in the untreated, distilled water-treated, cottonseed oil-treated (5 ml./kg.), or di-2-ethylhexyl phthalate-treated (5 ml./kg.) groups.

Dimethoxyethyl phthalate was an interesting member of the phthalate series because of the high level of activity exhibited by this compound on the developing embryo and fetus. Most of the fertilized ova were detected only as resorption sites in the two higher dose levels. One dead fetus and one live fetus were delivered in the high dose group, and both of these had a short or compressed head and neck. At the middle dose level, two fetuses had no tail or eyes, another was missing a tail, and two dead fetuses had twisted hind legs. After visualizing the skeleton, the bones of the hind legs appeared to be very weak and delicate and were poorly formed. In the low dose group, one fetus was without a tail and seven of 14 visualized fetuses did not have tail bones. Six of these 14 had elongated and fused ribs.

Another compound of this series, dimethyl phthalate, produced marked teratogenic effects. In the group that received the high dose of this phthalate, three fetuses were observed that had neither tails nor eyes, and one fetus had a normal tail but was without eyes. Examination of skeletal structure revealed four of 12 fetuses had elongated and fused ribs, two did not have tail bones, and three had abnormal and incomplete skull bones. In the middle dose group, four fetuses had abnormally small tails and six of the 17 stained fetuses had complete or incomplete elongated and fused ribs (four complete and two incomplete, *i.e.*, only on one side). At the low dose, two fetuses were observed with short tails and two were without eyes. Of the 16 stained fetuses in this group, two had elongated and fused ribs on both sides, one had unilateral elongation of the ribs, and one was without tail bones.

Similar gross and skeletal abnormalities were observed in groups treated with other phthalate esters. Diethyl and dibutyl phthalates did not produce any gross malformations or dead fetuses; however, a number of skeletal abnormalities were noted. In the high dose group treated with diethyl phthalate, 12 of 16 stained fetuses showed complete or incomplete elongated and fused ribs and one fetus had curved and elongated upper and lower jaw bones. In the middle dose group, eight of 17 fetuses revealed abnormal skeletal structures, especially elongated and fused ribs and incomplete skull bone formation, while five of 19 fetuses in the low dose group had elongated and fused ribs. Dibutyl phthalate-treated animals produced fetuses with elongation and fusion of the ribs with a frequency of eight of 24, seven of 29, and six of 29 in the high, medium, and low dose groups, respectively.

There were no gross abnormalities or dead fetuses in the groups treated with diisobutyl phthalate except for the middle dose, in which two dead fetuses were found; both were without eyes due to incomplete formation of the head. After visualization and staining, eight of 24, five of 29, and four of 27 were seen to have partially elongated and fused ribs in the high, medium, and low dose groups, respectively.

The high and medium dose groups of butyl carbobutoxymethyl phthalate each contained one very small, abnormal fetus, weighing only 1 g., but there were no dead fetuses. No gross abnormalities were seen in the low dose group. After staining, however, elongated and fused ribs were seen in five of 23, four of 25, and four of 29 fetuses in the high, medium, and low dose groups, respectively.

As a result of their low acute toxicity, dioctyl and di-2-ethylhexyl phthalates were injected at two fixed dosage levels of 5 and 10 ml./kg. each. Twenty-three grossly malformed fetuses were seen with dioctyl phthalate, 15 of 55 at the high dose and eight of 51 at the low dose. Twisted hind legs was the most commonly seen feature. No abnormalities were noted in the animals treated with 5 ml./kg. of di-2-ethylhexyl phthalate, but nine of the 41 fetuses in the 10-ml./kg. dose group exhibited hemangiomas of the legs and one had twisted hind legs. Although resorptions were seen in all four groups treated with these two phthalates, no dead fetuses were present.

SUMMARY AND CONCLUSION

As indicated previously, all phthalate esters utilized in this study exert a deleterious effect upon the developing embryo and/or fetus. At one or more of the dose levels employed, each compound produced some or all of the following effects: resorptions, gross abnormalities, skeletal malformations, fetal death, or decreased fetal size. Untreated control animals were used to determine "normal" fetal size; within this group, there were no resorptions, gross or skeletal abnormalities, or fetal deaths.

The regimen of phthalate administration did not interfere with fertility, as reflected by the ratio of corpora lutea to implantation sites, but there were significant effects upon embryonic and/or fetal development. The observed incidence of adverse effects was generally dose related and compound dependent, with the more water-soluble compounds tending to be most active. Of the phthalate esters included, dioctyl and di-2-ethylhexyl were the least soluble and tended to exert the least deleterious effect on embryo-fetal development.

In view of the widespread use of di-2-ethylhexyl phthalate and, to a lesser extent, butyl carbobutoxymethyl phthalate (butyl glycolyl-

[8] Bilateral or complete fused ribs were noted spread throughout the rib cage, whereas unilateral or incompletely fused ribs were generally observed in the lower ribs.

butyl phthalate) as plasticizers in medically used devices, the question arises as to the potential danger to the human reproductive process from plasticizers leached from these devices by blood or parenteral solutions. While the doses of di-2-ethylhexyl phthalate employed in this study were greatly in excess of the 5–7 mg. % which have been reported in whole blood stored in di-2-ethylhexyl phthalate-plasticized blood bags (3), the possible cumulative nature of the plasticizer in the body must also be considered. Jaeger and Rubin (3) found from 2.5 to 27 mg. % (dry weight) of this plasticizer in tissues of patients who were known to have received blood transfusions; in addition, they found that while the perfused rat liver could hydrolyze the plasticizer butyl glycolylbutyl phthalate, it did not hydrolyze di-2-ethylhexyl phthalate but tended to sequester and accumulate it.

Further investigations are needed to determine the fate of these small, but possibly repeated, quantities of plasticizers that may enter the patient as a result of medical or surgical treatment and to determine what, if any, effect on reproduction or other physiological processes may result.

REFERENCES

(1) A. S. Trimble, B. S. Goldman, J. K. Yao, L. K. Kovats, and W. G. Bigelow, *Surgery*, **59**, 857(1966).

(2) W. L. Guess, J. Jacob, and J. Autian, *Drug Intel.*, **1**, 120 (1967).

(3) R. J. Jaeger and R. J. Rubin, *Science*, **170**, 460(1970).

(4) W. L. Guess, S. Haberman, D. F. Rowan, R. K. Bower, and J. Autian, *Amer. J. Hosp. Pharm.*, **24**, 494(1967).

(5) S. Haberman, W. L. Guess, D. F. Rowan, R. O. Bowman, and R. K. Bower, *SPE (Soc. Plast. Eng.) J.*, **24**, 62(1968).

(6) R. K. Bower, S. Haberman, and P. D. Minton, *J. Pharmacol. Exp. Ther.*, **171**, 314(1970).

(7) "The Merck Index," 8th ed., Merck & Co., Inc., Rahway, N. J., 1968.

(8) H. C. Hodge, *Proc. Soc. Exp. Biol. Med.*, **53**, 20(1943).

(9) C. B. Shaffer, C. P. Carpenter, and H. F. Smyth, Jr., *J. Ind. Hyg. Toxicol.*, **27**, 130(1945).

(10) J. H. Draize, E. Alverez, M. F. Whitesell, G. Woodard, E. C. Hagan, and A. A. Nelson, *J. Pharmacol. Exp. Ther.*, **93**, 26 (1948).

(11) C. P. Carpenter, C. S. Weil, and H. F. Smyth, *Arch. Ind. Hyg. Occup. Med.*, **8**, 219(1953).

(12) A. J. Lehman, *Ass. Food Drug Offic., U. S. Quart. Bull.*, **19**, 87(1955).

(13) R. S. Harris, H. C. Hodge, E. A. Maynard, and H. J. Blanchet, *Arch. Ind. Health*, **13**, 259(1956).

(14) J. McLaughlin, Jr., J. P. Marliac, M. J. Verrett, M. K. Mutchler, and O. G. Fitzhugh, *Toxicol. Appl. Pharmacol.*, **5**, 760 (1963).

(15) D. Calley, J. Autian, and W. L. Guess, *J. Pharm. Sci.*, **55**, 158(1966).

(16) J. Cornfield and N. Mantel, *Amer. Statist. Ass. J.*, **45**, 181 (1950).

(17) C. S. Weil, *Biometrics*, **8**, 249(1952).

(18) R. E. Staples and V. L. Schnell, *Stain Technol.*, **39**, 61(1964).

(19) H. Nishimura, "Chemistry and Prevention of Congenital Abnormalities," Charles C Thomas, Springfield, Ill., 1964.

(20) H. Bancroft, "Introduction to Biostatistics," Harper & Row, New York, N. Y., 1963, chap. 15.

ACKNOWLEDGMENTS AND ADDRESSES

Received March 29, 1971, from the *Materials Science Toxicology Laboratories, College of Dentistry and College of Pharmacy, University of Tennessee Medical Units, Memphis, TN 38103*

Accepted for publication August 27, 1971.

Presented at The Society of Toxicology Meeting, Washington, D. C., March 1971.

Supported in part by Research Grant DE02944-02 from the National Institute of Dental Research, Bethesda, MD 20014

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Synthesis and NMR Spectral Characteristics of Bisnorargemonine Isomers

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Abstract □ Two structural isomers of bisnorargemonine, 3,9-dihydroxy-2,8-dimethoxy-*N*-methylpavinane (I*a*) and 2,8-dihydroxy-3,9-dimethoxy-*N*-methylpavinane (I*b*), were synthesized employing the Pictet–Gams modification of the Bischler–Napieralski cyclization to construct the required isoquinoline intermediates. These were quaternized to the methiodides, followed by partial reduction to the *N*-methyl-1,2-dihydroisoquinolines, which were then cyclized under acidic conditions to provide I*a* and I*b*. Comparison of the NMR spectra of I*a* and I*b* with similar compounds revealed interesting aspects of the spectral properties pertaining to this ring system.

Keyphrases □ Bisnorargemonine isomers—synthesis, NMR characteristics □ 3,9-Dihydroxy-2,8-dimethoxy-*N*-methylpavinane—synthesis, NMR characteristics □ 2,8-Dihydroxy-3,9-dimethoxy-*N*-methylpavinane—synthesis, NMR characteristics □ NMR spectroscopy—characteristics of bisnorargemonine isomers

The recent synthesis of (±)-bisnorargemonine (1, 2) confirmed Structure I*c* assigned to it previously on the basis of its unique NMR spectrum (3). Two alternate structures, I*a* and I*b*, could have been considered but were rejected due to a predictable nonconformity in their NMR spectra. However, it was of interest to synthesize I*a* and I*b*, not only to verify the predicted NMR patterns but also to seek a practical synthetic route to provide these phenolic bases for projected oxidative coupling studies. The incidental provision of spectral and other data could also be of value in the future in assigning structures in the event that either or both of these diphenols are found to be naturally occurring, a not unlikely possibility.

Pursuant to a suggestion by Stermitz[1], it is believed appropriate to introduce a new type of nomenclature for this ring system since further extension of the "argemonine" nomenclature seems inadvisable if not impossible. Accordingly, it is proposed that the term "pav-

[1] Private communication from Dr. F. Stermitz, Colorado State University, Fort Collins, Colo., which forms the basis for the proposed nomenclature.