Maternal–Fetal Transfer of ^{14}C-Di-2-ethylhexyl Phthalate and ^{14}C-Diethyl Phthalate in Rats

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Abstract □ ^{14}C-Di-2-ethylhexyl and ^{14}C-diethyl phthalates were administered intraperitoneally to pregnant rats on either Day 5 or 10 of gestation. Rats were sacrificed at 24-hr intervals starting on Days 8 and 11, respectively; maternal blood, fetal tissue, amniotic fluid, and placentas (whenever possible) were obtained. The ^{14}C-activity of each sample was determined by scintillation counting. It was found that both diesters and/or their metabolic products were present in each of these compartments throughout the gestation period, thus suggesting that the embryo–fetal toxicity and teratogenesis reported previously could be the results of a direct effect of the compound (or its metabolites) upon developing embryonic tissue. Additionally, the reduction in concentration of ^{14}C from these tissues as a function of time was found to fit a first-order excretion curve. From this model curve, the half-life for both compounds was calculated; the average was about 2.33 days for di-2-ethylhexyl phthalate and 2.22 days for diethyl phthalate.

Keyphrases □ Phthalate diesters, radiolabeled—distribution and persistence in maternal blood, fetal tissue, amniotic fluid, and placenta, intraperitoneal administration, rats □ Maternal–fetal transfer—phthalate diesters (radiolabeled), distribution and persistence in maternal blood, fetal tissue, amniotic fluid, and placenta, intraperitoneal administration, rats □ Toxicity—phthalate diesters (radiolabeled), maternal–fetal transfer, rats

Phthalic acid esters are the major plasticizers used by the plastics industry, and di-2-ethylhexyl phthalate (I) is the most widely used plasticizer. Compound I is contained in many plastic products (primarily polyvinyl chloride) used in medical, dental, and paramedical applications as well as in household and vehicular furnishings. Some of these plasticizers also are used as solvents in commercial formulations such as perfumes and insect repellents.

The acute and chronic toxicities of these esters have been found to be rather low by conventional routes of administration (oral, intraperitoneal, intravenous, and inhalation) (1–6); their industrial uses have presented few confirmed toxicological problems, although some adverse effects have been suggested (7–9). Jaeger and Rubin (10) reported finding I in the tissues of two patients who had received transfusions of blood stored in plastic bags. *In vitro* experiments, which they conducted using the perfused rat liver, indicated that this organ would accumulate I but would not hydrolyze it. A related plasticizer, butyl glycolyl-butyl phthalate, was hydrolyzed by this system.

Teratogenic effects of the phthalates were reported in chick embryos when the phthalates were injected into the yolk sac of the developing embryo (11–13). Recently, demonstration of mammalian teratogenicity was reported from intraperitoneal injections of phthalates into pregnant rats (14).

When considering potential teratogenic effects, physicochemical properties of the compound often play a highly significant role. It is generally believed that a compound with a molecular weight of less than 1000 (unless highly bound to plasma proteins or other large molecules) can pass through the placental barrier to reach the developing fetus, where it may exert a direct effect (15). Passage through the placenta, however, is not the sole criterion for teratogenicity, since many drugs are known to reach the fetus without having any adverse effect upon its development. Moreover, a compound that does not pass this barrier may cause metabolic disturbances in the mother which secondarily interfere with development of the fetus (15).

The purposes of this study were to investigate the maternal–fetal transfer in rats of two phthalic acid esters and to determine whether they and/or their metabolites pass through the placental barrier to the developing fetus where they may elicit potential direct embryo–fetal toxicity and/or teratogenic effects.

EXPERIMENTAL

Materials—Carboxy-labeled ^{14}C-di-2-ethylhexyl phthalate (^{14}C-I) and ^{14}C-diethyl phthalate (^{14}C-II)[1] were mixed with unlabeled I and II[2], respectively, to provide the desired level of radioactivity. Test animals were adult virgin female rats of the Sprague–Dawley strain[3], weighing 175–225 g. Male rats of the same strain and age were used as the "stud pool."

Methods—Female rats were selected for experimentation only after observation of at least two complete 4- or 5-day estrus cycles. Females were bred and vaginal smears were obtained each morning. The date of appearance of sperm in the vaginal smear was recorded as Day 0 of pregnancy, as previously described (14).

A group of 13 pregnant rats was injected with 5 ml/kg ip of ^{14}C-I (4.0123×10^7 dpm/ml or 7.16×10^{-3} mCi/mmole) on Day 5 of gestation. A second group of 10 pregnant rats was injected intraperitoneally with the same dosage of ^{14}C-I on Day 10 of gestation. A similar group of pregnant rats was injected with 1.0116 ml/kg ip of ^{14}C-II (1.121×10^8 dpm/ml or 1.00×10^{-2} mCi/mmole) on Day 5 of gestation, and another group was injected on Day 10.

From groups injected on Day 5 of gestation, one rat was sacrificed with an overdose of ether by inhalation 72 hr after the ^{14}C-I or ^{14}C-II injection and then every 24 hr through Day 20 of gestation to obtain specimens for analysis. From the groups injected on Day 10, one rat was sacrificed every 24 hr through Day 20 of gestation.

About 4–5 ml of maternal blood was collected by cardiac puncture using a heparinized syringe, and amniotic fluid was aspirated from the gravid uterus. The whole fetuses and, whenever possible, the placentas were removed by uterine incision and isolated from the maternal tissue to avoid cross-contamination during subsequent dissection. Whole fetuses (and placentas when collected) were blotted gently with a surgical sponge and transferred to chilled, tared bottles for weighing. The tissues (whole fetuses and placentas) were minced with scissors and diluted three- to fivefold with distilled water. The diluted tissues were then homogenized[4]

[1] Mallinckrodt Nuclear.
[2] Matheson, Coleman and Bell, Norwood, Ohio.
[3] Sprague-Dawley, Inc., Madison, Wis.
[4] Virtis model 23 homogenizer.

Table I—Distribution of Radioactivity in Rats from ^{14}C-Di-2-ethylhexyl Phthalate Injected on Day 5 of Gestation[a]

Gestation Day	Maternal Blood			Placenta			Amniotic Fluid			Fetal Tissue		
	Counts, dpm/g	Total Counts in Tissue[b]	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]
8	6654	95,098	0.240	—	—	—	2577	2577	0.007	2003	470	0.001
9	4338	70,285	0.178	—	—	—	2046	1177	0.003	1392	556	0.001
10	2870	46,297	0.118	—	—	—	1047	200	—[d]	1226	641	0.002
11	1367	22,439	0.057	—	—	—	1321	539	0.001	1058	890	0.002
12	1260	20,326	0.052	1140	1180	0.004	510	375	—[d]	736	176	—[d]
13	1403	27,993	0.072	786	1386	0.004	410	683	0.002	485	579	0.001
14	1593	26,937	0.068	937	1672	0.004	155	225	—[d]	284	437	0.001
15	659	11,656	0.030	484	1215	0.003	167	254	—[d]	149	453	0.001
16	610	10,401	0.026	590	2732	0.007	125	346	—[d]	213	1453	0.003
17	505	8,003	0.021	314	1723	0.004	199	352	—[d]	187	2223	0.005
18	349	6,000	0.015	329	2075	0.005	73	239	—[d]	136	1802	0.004
19	377	6,402	0.017	207	1327	0.003	87	182	—[d]	158	1938	0.005
20	237	4,192	0.011	165	1146	0.003	73	232	—[d]	99	1641	0.003

[a] Mean counts of one to five samples for each tissue. [b] Total blood in the rat was assumed to be 7% of its body weight. [c] Percent of injected dose was based upon quantity of radioactivity administered and total radioactivity estimated for each organ. [d] Less than 0.001% of injected dose.

Table II—Distribution of Radioactivity in Rats from ^{14}C-Di-2-ethylhexyl Phthalate Injected on Day 10 of Gestation[a]

Gestation Day	Maternal Blood			Placenta			Amniotic Fluid			Fetal Tissue		
	Counts, dpm/g	Total Counts in Tissue[b]	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]
11	6002	114,656	0.290	—	—	—	6186	6186	0.016	9416	13,136	0.033
12	6433	118,338	0.300	—	—	—	1601	983	0.002	3301	5,461	0.013
13	3960	67,243	0.170	2679	4447	0.011	507	616	0.002	1454	1,263	0.003
14	1585	30,278	0.076	1259	3023	0.008	378	660	0.002	703	1,224	0.003
15	1858	31,813	0.080	1449	5224	0.013	472	1022	0.003	683	2,550	0.006
16	1481	26,510	0.066	1980	7551	0.019	396	965	0.002	581	2,971	0.008
17	1326	19,139	0.049	851	3940	0.010	231	562	0.001	280	2,519	0.006
18	1221	24,793	0.063	471	3821	0.010	209	733	0.002	299	4,705	0.013
19	755	15,491	0.039	387	2914	0.008	241	458	0.001	132	1,526	0.003
20	304	6,022	0.016	483	3085	0.008	241	275	—[d]	66	726	0.002

[a] Mean counts of one to five samples for each tissue. [b] Total blood in the rat was assumed to be 7% of its body weight. [c] Percent of injected dose was based upon quantity of radioactivity administered and total radioactivity estimated for each organ. [d] Less than 0.001% of injected dose.

at medium speed while the flask was immersed in dry ice.

Aliquots of blood, amniotic fluid, placentas, or fetal tissues (usually 100–150 mg) were solubilized[5]. The tissues (only) were warmed for about 2 hr at 50° in a water bath to minimize chemiluminescence. The samples were bleached when necessary with benzoyl peroxide[6] (up to 3 ml) and adjusted to pH 6.4 with acetic acid (67%). The samples treated with benzoyl peroxide exhibited significant chemiluminescence, which was minimized by storing the prepared samples in the dark for 24 hr. Ten milliliters of preblended liquid scintillation cocktail[7] was added to the samples of blood, amniotic fluid, or homogenized placentas or fetal tissues. Finally, these prepared samples were counted in a liquid scintillation spectrometer[8].

After the samples were counted, a standard aliquot of ^{14}C-toluene[9] (5000 dpm) was added to each, and these samples were recounted to obtain an estimate of the counting efficiency. The absolute radioactivity of the samples was calculated based upon the specific activity of the internal standard, ^{14}C-toluene.

Counts, counting efficiency, and original weight of sample were used to calculate the radioactivity of each specimen in terms of disintegrations per minute per gram or disintegrations per minute per organ. These values were used in conjunction with the specific activity of the injected ^{14}C-I and ^{14}C-II to estimate their molar concentrations (assuming all radioactivity was still present as the diesters) in the maternal blood, embryonic fluid, placentas, and whole fetuses. For this purpose, the total blood in the rat was assumed to be 7% of its body weight.

[5] With 2 ml of TS-1 solubilizer (0.6 *N* solution in toluene), Research Products International Corp., Elk Grove Village, Ill.
[6] Research Products International Corp., Elk Grove Village, Ill.
[7] 3a40, Research Products International Corp., Elk Grove Village, Ill.
[8] Model LS-100C, Beckman Instruments, Fullerton, Calif.
[9] Beckman Instruments, Fullerton, Calif.

RESULTS

A total of 46 pregnant rats and their fetuses were studied following ^{14}C-I and ^{14}C-II administration on Days 5 or 10 of gestation. Radioactivity was detected in maternal blood, placentas, amniotic fluid, and developing fetuses at all gestational stages investigated. No grossly obvious toxic effects were seen in the pregnant females during the gestation period after injection of either radioactive phthalate. Tables I and II present a summary of the mean counts per gram of tissue, estimated counts per organ, and percent of injected dose taken up by various tissues after ^{14}C-I was injected on Day 5 or 10 of gestation. Similar data following ^{14}C-II injection are shown in Tables III and IV.

Both radioactive phthalates and/or their metabolites were widely distributed and were found in maternal blood, placentas, amniotic fluid, and fetal tissues. Less than 1% of the injected dose was present in these tissues at any of the measured times. Generally, the level of radioactivity in the placenta, amniotic fluid, and whole fetus seemed to be related to the concentration in the maternal blood, but none of these tissues exhibited a consistently higher level than that found in the maternal blood for either compound.

Radioactivity in the maternal blood increased, reaching a peak during the first 48 hr following injection of ^{14}C-I and during the first 24 hr after radioactive II injection. The concentration of radioactivity then diminished quickly for both compounds. A similar pattern was observed in the amniotic fluid and fetal tissues. Some radioactivity was detected in all tissues examined throughout the experimental period (10–15 days postinjection).

Table III—Distribution of Radioactivity in Rats from ^{14}C-Diethyl Phthalate Injected on Day 5 of Gestation[a]

Gestation Day	Maternal Blood			Placenta			Amniotic Fluid			Fetal Tissue		
	Counts, dpm/g	Total Counts in Tissue[b]	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]
8	1584	29,811	0.100	—	—	—	421	11	—[d]	1403	333	0.001
9	1372	27,180	0.085	—	—	—	416	21	—[d]	1108	562	0.002
10	1064	19,422	0.066	—	—	—	621	61	—[d]	724	372	0.001
11	644	9,204	0.040	668	447	0.002	285	131	—[d]	1375	145	—[d]
12	747	14,376	0.046	465	390	0.001	263	198	—[d]	694	124	—[d]
13	361	6,258	0.028	301	412	0.002	63	128	—[d]	170	107	—[d]
14	385	7,491	0.023	274	572	0.002	77	159	—[d]	121	210	—[d]
15	275	4,806	0.017	157	613	0.002	82	231	—[d]	70	228	0.001
16	224	3,804	0.014	184	1245	0.005	46	167	—[d]	68	507	0.002
17	234	3,444	0.015	164	1313	0.006	74	176	—[d]	68	672	0.003
18	207	3,661	0.012	146	1347	0.005	52	166	—[d]	65	947	0.003
19	146	2,479	0.008	108	831	0.003	36	122	—[d]	56	916	0.003
20	185	3,848	0.012	87	965	0.003	47	116	—[d]	69	1185	0.003

[a] Mean counts of one to five samples for each tissue. [b] Total blood in the rat was assumed to be 7% of its body weight. [c] Percent of injected dose was based upon quantity of radioactivity administered and total radioactivity estimated for each organ. [d] Less than 0.001% of injected dose.

Table IV—Distribution of Radioactivity in Rats from ^{14}C-Diethyl Phthalate Injected on Day 10 of Gestation[a]

Gestation Day	Maternal Blood			Placenta			Amniotic Fluid			Fetal Tissue		
	Counts, dpm/g	Total Counts in Tissue[b]	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]	Counts, dpm/g	Total Counts in Tissue	Percent of Injected Dose[c]
11	2625	50,145	0.164	1575	871	0.003	1077	471	0.002	3685	317	0.001
12	1892	41,497	0.118	1516	1434	0.004	904	824	0.002	905	216	—[d]
13	1815	33,645	0.113	1033	1549	0.005	448	428	0.001	509	332	0.001
14	1532	27,423	0.095	682	1424	0.005	225	390	0.001	249	333	0.001
15	1343	27,081	0.085	234	824	0.002	283	898	0.003	202	887	0.003
16	1032	18,546	0.065	216	799	0.003	253	526	0.002	150	664	0.002
17	543	11,257	0.033	151	800	0.003	169	583	0.002	162	1808	0.006
18	302	6,068	0.018	112	1775	0.005	98	364	0.001	112	1820	0.005
19	193	3,892	0.012	200	1473	0.004	84	257	—[d]	117	1695	0.006
20	134	2,674	0.008	125	856	0.003	51	101	—[d]	70	1176	0.003

[a] Mean counts of one to five samples for each tissue. [b] Total blood in the rat was assumed to be 7% of its body weight. [c] Percent of injected dose was based upon quantity of radioactivity administered and total radioactivity estimated for each organ. [d] Less than 0.001% of injected dose.

A first-order rate equation was fitted to that portion of the experimental data where radioactive counts were declining with respect to time. Least-square slopes were obtained from the logarithm of radioactivity *versus* time in days for maternal blood, amniotic fluid, and fetal tissue compartments. For three of the four cases (administration of ^{14}C-I at Days 5 and 10 and of ^{14}C-II at Day 10), the 95% confidence intervals of the least-square slopes were overlapping for all of these compartments. In the other case (^{14}C-II administration on Day 5), the confidence intervals of the least-square slopes failed to overlap, but the gap was present only in the third decimal place. From this treatment of the experimental data, it is not possible to determine that the slopes (or rate constants) are significantly different from one another.

DISCUSSION

Previous studies (14) revealed embryo–fetal toxicity and teratogenicity in rats from intraperitoneal injections of these phthalate esters. This study clearly demonstrated the presence of phthalates and/or their metabolites in the developing fetus from early embryogenesis to birth, including the critical period of teratogenic sensitivity. The embryo–fetal toxicity and fetal abnormalities produced by these phthalates, together with the demonstration of their presence in the early embryo, suggest that phthalate esters and/or their metabolites may act directly on embryonic tissues in the induction of teratogenicity.

Using ^{14}C-radioactivity data, a half-life was calculated for each compound. The half-life of II was 2.22 days; for I, it was 2.33 days. These values are in reasonably good agreement with those reported by Daniel and Bratt (18), who gave a half-life of 1–2 days for I in rat liver and of 3–5 days for fat.

These studies showed that radioactivity from ^{14}C-II and ^{14}C-I is transmitted across the placenta from mother to fetus in pregnant rats and that the radioactivity is detectable for at least 15 days postinjection. Although the exact chemical nature of the radioactive compound(s) transmitted to the fetus was not determined, some of them probably were mixtures of parent compounds, monoesters, and phthalic acid.

Studies on absorption, distribution, metabolism, and excretion of carboxy-labeled ^{14}C-I in rats were reported previously (16–18). The three studies found that increased water-soluble products were formed and excreted primarily in the urine and feces. Albro *et al.* (17) suggested there is probably an initial hydrolysis of the compound to the monoester, mono-2-ethylhexyl phthalate (III), with subsequent side-chain oxidation to the alcohol, ketone, and acids or hydrolysis to phthalic acid. Daniel and Bratt (18) reported finding only the diester in the liver of treated animals, while Schultz and Rubin (16) found both water-soluble and organic-extractable fractions in the liver at 1 and 24 hr after intravenous administration of ^{14}C-I.

Studies[10] with ^{14}C-I in mice revealed the presence of ^{14}C-III and other metabolites in tissues of the treated animals as well as in urine and feces. Dillingham and Autian (19) reported that approximately 83% of an intraperitoneal dose of ^{14}C-I was excreted in the urine of mice in 14 days and that no significant radioactivity was found in the mice after 35 days. In another study[10], both mice and rabbits metabolized and excreted ^{14}C-II in a fashion somewhat similar to that for I.

Autian (20) reported inhibition of mammalian cell growth

[10] Unpublished data, Materials Science Toxicology Laboratories.

(mouse fibroblasts or L-cells) in culture from both I and II. Using a protein assay system for quantitation of cell growth, 3×10^{-3} mole/liter of II reduced growth by 50%; a similar effect was observed for I at a concentration of 5×10^{-5} mole/liter. These original data also revealed that approximately 10% growth inhibition was produced by II at a level of 5×10^{-4} mole/liter and that a similar effect was elicited by 4.7×10^{-5} mole/liter of I. In the present study, fetal concentrations of II (based upon ^{14}C counts) ranged from 1.5×10^{-4} to 2.8×10^{-6} mole/kg; similar ranges of I were from 5.9×10^{-4} to 4×10^{-6} mole/kg. Thus, these data suggest the phthalates may be reaching developing fetal tissues in concentrations comparable to those that have been demonstrated to affect growth of mammalian cells in culture.

It was found that maternal blood levels reached a peak radioactive concentration in 24 hr or less after intraperitoneal administration of ^{14}C-II and within 48 hr after ^{14}C-I. Counts (disintegrations per minute per gram) obtained 48 hr or more after ^{14}C-I injection were invariably higher in maternal blood than in fetal tissues; concentrations in maternal blood also appeared to influence the concentrations in the amniotic fluid and placenta.

Radioactivity following a single injection of the ^{14}C-phthalate was observed in maternal blood and fetal tissues throughout the observation period (10–15 days). The relatively long *in vivo* half-life (about 2.2–2.3 days) for these compounds suggests that repetitive maternal exposures during pregnancy might lead to accumulation of toxicologically significant quantities of such compounds within the major organs of the fetus. To explore this potential problem further, a comparative study should be conducted to quantitate the phthalate and/or its metabolites in various major maternal and fetal organs. Concurrently, investigations should be conducted to determine the effects of varying levels of the phthalate esters (and/or metabolites) upon developmental structures and functions of the various organs.

REFERENCES

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

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

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

(4) D. W. Fassett, in "Industrial Hygiene and Toxicology," vol. II, F. A. Patty, Ed., Interscience, New York, N.Y., 1963, pp. 1908–1910.

(5) R. Lefaux, "Practical Toxicology of Plastics," CRC Press, Cleveland, Ohio, 1968, pp. 138, 139.

(6) W. H. Lawrence, M. Malik, J. E. Turner, A. R. Singh, and J. Autian, *Environ. Res.*, **9**, 1(1975).

(7) W. N. Sokol, Y. Aelony, and G. N. Beall, *J. Amer. Med. Ass.*, **226**, 639(1973).

(8) J. Neergaard, B. Nielsen, V. Faurby, D. H. Christensen, and O. F. Nielsen, *Scand. J. Urol. Nephrol.*, **5**, 141(1971).

(9) *Chem. Eng. News*, **Feb. 15, 1971**, 12, 13.

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

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

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

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

(14) A. R. Singh, W. H. Lawrence, and J. Autian, *J. Pharm. Sci.*, **61**, 51(1972).

(15) M. L. Murphy, in "Ciba Foundation Symposium on Congenital Malformations," G. E. W. Wolstenholme and C. M. O'Connor, Eds., Little, Brown, Boston, Mass., 1960, pp. 78–114.

(16) C. O. Schultz and R. J. Rubin, *Environ. Health Perspec.*, **3**, 123(Jan. 1973).

(17) P. W. Albro, R. Thomas, and L. Fishbein, *J. Chromatogr.*, **76**, 321(1973).

(18) J. W. Daniel and H. Bratt, *Toxicology*, **2**, 51(1974).

(19) E. O. Dillingham and J. Autian, *Environ. Health Perspec.*, **3**, 81 (Jan. 1973).

(20) J. Autian, *ibid.*, **4**, 3(June 1973).

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