Retention, Excretion and Metabolism of Di-(2-ethylhexyl) phthalate Administered Orally to the Rat

by

D. T. WILLIAMS and B. J. BLANCHFIELD

Food Research Laboratories, Health Protection Branch
Tunneys' Pasture, Ottawa K1A 0L2

Despite their widespread industrial use and their presence as ubiquitous environmental contaminants there are very few reports on the metabolism of phthalate esters. SCHAFFER et al. (1945) showed urinary excretion of metabolites of di-(2-ethylhexyl) phthalate, measured as phthalic acid, in the dog, rabbit, rat and man. ERICKSON (1945) reported that diphenylphthalate and butylbenzylphthalate were metabolised to a small extent by the beagle dog. CHAMBON et al. (1971) detected small amounts of monobutylphthalate and monoethylphthalate in the urine of rats orally dosed with dibutylphthalate and diethylphthalate. Recently STALLING et al. (1973) have reported that fish metabolise di-(2-ethylhexyl) phthalate-7-^{14}C to five or more metabolic products. SCHULTZ and RUBIN (1973) also found that this compound when administered intravenously in the rat is rapidly metabolised to four unidentified urinary metabolites. ALBRO et al. (1973) have identified five metabolic products in the urine of rats orally dosed with di-(2-ethylhexyl) phthalate. The present experiments were conducted to determine the retention, excretion and metabolism of di-(2-ethylhexyl)phthalate administered orally to the rat.

MATERIALS AND METHODS

Quantitative measurements of radioactivity were made with a liquid scintillation spectrometer (Packard Tri-Carb, Model 3320). Activity in aqueous solutions was measured by adding 1 ml aliquots to 15 ml Aquasol (New England Nuclear). Activity in organic solvents was measured by adding 1 ml aliquots to 15 ml toluene containing 2,5-diphenyloxazole (0.4%) and 1,4-bis(2-(5-phenyloxazolyl)) benzene (0.01%).

Male Wistar strain rats (50 ± 5 g) were used in all experiments and were killed by ether asphyxiation, organs and tissues removed immediately and portions analysed for radioactivity. Urine, feces, blood, spleen, kidney, liver, adipose, skeletal muscle, lungs, testes, heart, brain, skin and carcass were

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usually analysed. Tissue samples were prepared by dissolving 50-100 mg tissue in 1 ml of solubilizer (Soluene) at room temperature and then adding 15 ml of toluene scintillator. 1 ml aliquots of urine were measured in aquasol. Feces samples were homogenised with 1 N aqueous sodium hydroxide and then with ether. The ether and sodium hydroxide extracts were shaken together and 1 ml aliquots of each added to the appropriate scintillator. Quench corrections were made using an automatic external standard.

Thin layer chromatography was carried out on Eastman silica gel chromagrams with fluorescent indicator, in the stated solvent system. Radioactive compounds were detected on these chromagrams by autoradiography using Kodak no-screen X-ray film.

The di-(2-ethylhexyl)phthalate-7-^{14}C was administered either in a single dose as a 10% solution in corn oil or ad libitum in a prepared feed.

Synthesis of di-(2-ethylhexyl)phthalate-7-^{14}C.

Phthalic anhydride-7-^{14}C New England Nuclear (0.0067 mmole) was esterified with 10% boron trichloride in 2-ethylhexanol (0.5 ml) by heating at 90°C for 2 hr. The reaction mixture was then diluted with hexane (50 ml) and the resultant solution washed with methanol-water (1:2, 4 x 50 ml), acetonitrile-water (2:1, 6 x 50 ml) and water (50 ml). The hexane solution was dried with sodium sulphate, filtered and concentrated to dryness. Preparative thin layer chromatography with methylene chloride as solvent gave di-(2-ethylhexyl)phthalate-7-^{14}C (0.0044 m mole), activity 14.8 mCi/mmole. Autoradiography of chromagrams run in three solvent systems (methylene chloride; ethylacetate-isooctane, 15/85; ethyl ether-petroleum ether, 1/4) showed a radiochemical purity greater than 99.9%. Portions of this highly radioactive product were diluted with freshly distilled di-(2-ethylhexyl)phthalate for use in the following experiments.

RESULTS

Experiment 1

To determine if DEHP-7-^{14}C was metabolised to $^{14}CO_2$ and expired via the lungs two rats were intubated with 1.16g/Kg(1μCi) and 1.9g/Kg(2μCi) DEHP-7-^{14}C respectively. Each rat was individually maintained in

a controlled ventillation glass cage for eight days with water and rat chow freely available. Expired carbon dioxide was collected in ethanolamine-monomethyl ethylene glycol which was replaced and analysed for radioactivity every 24 hr. No activity significantly above background activity could be detected. This indicated a level of $^{14}CO_2$, if present, of less than 0.1% of the dose.

Experiment 2

To evaluate excretion in urine and feces two rats (A,B) were each intubated with 1 g/Kg DEHP-7-^{14}C (1 μCi) and given rat chow and water ad libitum. Urine and feces were collected every 24 hr and analysed for radioactivity (Table I).

TABLE I

^{14}C Activity detected in urine and feces expressed as percentage of dose

	0-24 hr		24-28 hr		48-72 hr		72-192hr		Total	
	A	B	A	B	A	B	A	B	A	B
Urine	49.3	60.5	2.2	6.7	0.8	1.1	1.2	1.9	53.5	70.2
Feces Metab	8.0	5.5	2.5	0.8	0	0.2	0	0.05	10.5	6.5
Feces DEHP	38.7	22.8	0.03	0.3	0.05	0.8	0	0.07	38.8	24.0

The rats were killed after 8 days and organs and tissues analysed for radioactivity. Activity was detected only in the adipose tissue, 56 (A) and 105 (B) cpm/g tissue, equivalent to 1.7 and 3.2 ppm of phthalate ester.

Experiment 3

In an attempt to ascertain whether other organs had transient levels of phthalate five rats (C,D,E,F,G) were intubated with 0.8 g/Kg DEHP-7-^{14}C (1 μCi) and then permitted rat chow and water ad

libitum. Urine samples were collected every 24 hr and analysed for radioactivity (Table II).

TABLE II

^{14}C Activity in Urine expressed as percentage of dose

	0 - 24 hr	24 - 48 hr	48 - 72 hr	72 - 96 hr
C	49.3	-	-	-
D	49.8	2.0	-	-
E	59.9	3.1	-	-
F	78.6	7.3	0.9	0.5
G	67.7	2.3	0.9	0.8

One rat was killed at 24 hr (C) and two rats killed at 48 (D,E) and 96 hr (F,G) after dosing. Organs and tissues were removed immediately and analysed (Table III)

TABLE III

^{14}C Activity expressed as cpm/g tissue

	C	D	E	F	G
Kidney	481	0	58	0	0
Liver	599	38	65	13	0
Adipose	497	275	496	700	236
Skeletal Muscle	113	0	22	0	0
Testes	113	0	9	0	0

Experiment 4

This was a short term feeding study to determine if there was any build up of ^{14}C activity in tissues or organs. Ground rat chow was added to 500 mg DEHP-7-^{14}C (13μCi) dissolved in corn oil (10 ml) to give a total weight of 500 g. The feed was then intimately mixed and fed ad libitum to two rats (H,J) for 15 days. Weight of feed consumed (H - 212 g, J - 204 g) and weight of rats was noted periodically. After 15 days the rats were killed and tissues and organs analysed (Table IV).

TABLE IV

^{14}C Activity expressed as cpm/g tissue

	H	J	K	L
Kidney	681	1123	376	880
Liver	2055	1866	607	1524
Adipose	385	294	0	80
Skeletal Muscle	0	57	0	37
Lungs	0	68	0	47
Testes	67	7	2	30
Heart	0	120	0	70

To allow for activity which might be due only to feed consumed in the last 24 - 48 hr two rats (K,L) were fed an identical diet to rats H and J except that it contained non-radioactive DEHP. The weight of the rats was periodically noted and when their weight was approximately equal to that of rats H and J on day 13 the feed was replaced with radioactive feed for 2 days. Radioactive feed consumed on the last 2 days for rats K and L was 23.4 g (K) and 25.9 g (L) as compared to 28.6 (H) and 36.5 g (J) for the last 2 days of rats H and J.

Rats H and J appeared to accumulate activity in the adipose tissue equivalent to 9.0 (H) and 6.9 (J) ppm of phthalate. 95% of the activity was excreted via the urine for rats H and J.

Experiment 5

Since experiment 4 indicated the percentage of excretion of activity via the urine increased with decreasing phthalate ingestion a further experiment was run to evaluate this. Two rats (M,N) were fed a diet containing 2 mg DEHP-7-^{14}C/g feed and four rats (O,P,Q,R) were fed a diet containing 0.01 mg DEHP-7-^{14}C/g feed. Excretion of activity in the urine and feces was determined and is given in Table V.

TABLE V

^{14}C Activity in urine and feces as percentage of total activity

	M	N	O	P	Q	R
Urine	92.0	90.8	96.6	96.8	95.1	97.8
Feces (Metabolites)	5.0	5.8	0	0	0	0
Feces (DEHP)	2.9	3.3	3.4	3.2	4.9	2.2

Although a small percentage of the DEHP remains unmetabolised at both dose levels at the low dose level all of the metabolic products are excreted in the urine.

Investigation of excreted products

Feces. The ether portion of the feces extract contained only DEHP-7-^{14}C, identified by TLC-autoradiography. The aqueous alkaline extract was acidified (pH1) and extracted six times with an equal volume of ether (95% of activity extracted). Methylation of a portion of this ether extract with diazomethane and analysis by TLC-autoradiography, methylene chloride as solvent, showed only one radioactive compound with the same R_f as dimethylphthalate.

Urine. The urine of rats C,D,E,F,G from experiment 3 were combined and extracted, at neutral pH, six times with an equal volume of ether (4% of activity extracted). A portion of this extract was methylated and analysed by TLC-autoradiography, methylene chloride as solvent. The single radioactive compound was identified as monomethylmonoethylhexylphthalate and the presence of this compound was confirmed by GLC-mass spectrometry.

The residual urine was acidified (pH1) and extracted six times with an equal volume of ether (90% activity extracted). TLC-autoradiography, chloroform-ethanol-ammonium hydroxide-methanol (60/35/10/1) as solvent, showed at least seven radioactive compounds in approximately equal concentrations. A portion of the extract was hydrolysed with 1N sodium hydroxide for 16 hrs at 60°, acidified (pH1) and extracted with ether (95% activity extracted). Methylation with diazomethane followed by TLC-autoradiography showed only one spot due to dimethyl phthalate.

The urine of rats H,J from experiment 4 were combined and submitted to the same extraction procedure as above. 0.7% of the activity was extracted into ether from the urine at neutral pH but this extract did not contain any monomethylmonoethylhexylphthalate. 94% of the activity was extracted from acidified urine and TLC-autoradiography showed the same compounds found in the urine of rats in experiment 3 with some slight changes in the ratios of these compounds. Base hydrolysis, acidification, ether extraction and methylation again gave only dimethylphthalate when examined by TLC-autoradiography.

DISCUSSION AND CONCLUSIONS

There is only limited retention and accumulation of DEHP-7-^{14}C by the male rat whether it is given as a single dose or fed as part of a diet. The adipose tissue was the only tissue or organ which consistently contained radioactivity, presumably as unchanged DEHP-7-^{14}C although the levels of activity were too low to confirm this by autoradiography. In a fifteen day feeding study, with a diet containing 0.1% DEHP-7-^{14}C (Experiment 4), the adipose tissue accumulated radioactivity equivalent to 6 - 9 ppm of DEHP. Activity was also detected in kidney, liver testes, skeletal muscle, lungs and heart but much of this is probably due to DEHP-7-^{14}C consumed during the last 24 - 48 hr of the study (Experiment 3, 4). STEIN et al. (1973) have reported that rats fed six weeks on a diet containing 0.1% non-radioactive DEHP accumulated this compound in the heart and epididymal fat pad.

Virtually all of the ingested DEHP-7-^{14}C is excreted in the urine or feces within 48 hr. No breakdown of DEHP-7-^{14}C to $^{14}CO_2$ could be detected. The extent of metabolism and pathway of excretion are dependent on the magnitude of the phthalate ingestion. With a large single dose (Experiment 2) 30 - 50% of the activity was excreted in the feces, most as unchanged DEHP-7-^{14}C plus some 5 - 10% of an unknown metabolic product. The urine contained 4% of monoethylhexylphthalate-7-^{14}C and at least seven other radioactive metabolic products in roughly equal concentration. Hydrolysis of the urine gave only phthalic acid-7-^{14}C indicating that metabolism of DEHP-7-^{14}C did not involve modification or substitution of the aromatic ring. This is in agreement with ALBRO et al. (1973) who identified four metabolites in the urine of rats orally dosed with di-(2-ethylhexyl) phthalate as w and (w-1) oxidation products of mono-(2-ethylhexyl)phthalate and the fifth metabolite as phthalic acid.

When low levels of DEHP-^{14}C are fed in the diet (Experiment 4, 5) 90 - 97% of the activity is excreted in the urine with 2 - 5% of unchanged DEHP-7-^{14}C excreted in the feces. Metabolic products are excreted in the feces with a diet containing 0.2% DEHP-7-^{14}C but with a diet containing only 10 ppm of DEHP-7-^{14}C all metabolic products are excreted in the urine.

Analysis of the urine of rats fed on a diet containing 0.1% DEHP-7-^{14}C showed no monoethylhexylphthalate but did show other metabolic products similar to the single dose experiment, with some changes in the ratios of these products. Hydrolysis of the urine gave only phthalic acid 7-^{14}C.

These results are similar to those found by SCHULZ and RUBIN (1973) for intravenous administration of DEHP-7-^{14}C to the rat. They similarly found a greater percentage of metabolic products at lower dose than higher dose levels and that most of the activity was excreted in the urine and feces within 24 hr. In a single experiment with an oral dose of 200mg/Kg DEHP-7-^{14}C they found after 24hr 12.8% activity excreted as DEHP, 61.7% activity excreted as metabolic products and negligible activity in the organs and tissues.

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