Retention, Excretion and Metabolism of Phthalic Acid Administered Orally to the Rat

by D. T. WILLIAMS and B. J. BLANCHFIELD

Food Research Laboratories
Health Protection Branch
Tunney's Pasture
Ottawa K1A OL2

Phthalic acid was recovered from the urine in almost quantitative yield after subcutaneous injection in the dog (POHL, 1909) and in man (SHEMIAKIN and SCHUKINA, 1944) but was almost completely metabolised when orally administered to the dog (PORCHER, 1908). McBain et al. (1968) reported that rats orally dosed with phthalic acid-^{14}C excreted this compound in the urine but gave no quantitative data. ALBRO et al. (1973) have shown that phthalic acid was one of the metabolic products excreted in the urine when di-(2-ethylhexyl) phthalate was administered orally to the rat. To facilitate our work on the retention, excretion and metabolism of phthalate esters in the rat we have quantitatively investigated the retention, excretion and metabolism of phthalic acid orally administered 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. 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.

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 1 © 1974 by Springer-Verlag New York Inc.

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.

Phthalic anhydride-7-^{14}C (New England Nuclear) was hydrolysed and the phthalic acid-7-^{14}C purified by preparative thin layer chromatography using chloroform-ethanol-ammonium hydroxide 21/65/14 as solvent. The specific activity was 10.5 mCi/mmole and portions were appropriately diluted with phthalic acid and administered in aqueous solution (0.5 ml) by oral intubation.

RESULTS

Two rats were intubated with 40 mg/kg (0.40 μCi) and 3.3 mg/kg (0.28 μCi) phthalic acid-7-^{14}C respectively and individually maintained in a controlled ventilation cage for four days. Expired carbon dioxide was collected in ethanolamine-monomethyl ethylene glycol at 24 hr intervals. $^{14}CO_2$ was only detected in the first 24 hr after dosing and represented 0.15 and 0.11% respectively of the administered dose.

The excretion of phthalic acid in the urine and feces after a single oral dose is shown in Table 1 for three different dose levels. The urine, after concentration to a small volume was examined by thin layer chromatography-autoradiography, chloroform-ethanol-ammonium hydroxide 21/65/14 as solvent, and was found to contain only one radioactive compound and this had the same R_f as phthalic acid. Reverse isotope dilution with unlabelled phthalic acid accounted for 97% of the radioactivity. The sodium hydroxide extract of the feces, which contained all of the feces radioactivity, was acidified to pH1 and extracted four times with an equal volume of ether. The combined ether extracts contained 98% of the radioactivity and showed only a single radioactive compound when analysed by thin layer chromatography-autoradiography. Methylation with diazomethane of a portion of the ether extract followed by thin layer chromatography-autoradiography, methylene chloride as solvent, showed one radioactive spot with the same R_f as dimethyl phthalate.

Twelve rats were intubated with 4 mg/kg (0.4 μCi) of phthalic acid-7-^{14}C and were killed in groups of four at 4, 8 and 24 hr after dosing. The tissues and organs were analysed for radioactivity and the results, expressed as ppm of phthalic acid, are given in Table 2.

TABLE I

% Excretion of Phthalic Acid in Urine and Feces at Different Dose Levels

	0.4 mg/kg		4 mg/kg		40 mg/kg	
	U	F	U	F	U	F
0-4 hrs	14.2±3.0	2.2± 1.7	14.8±2.4	8.1±3.5	-	-
0-8 hrs	19.3±3.0	31.0±10.3	19.9±2.6	46.7±6.8	-	-
0-24 hrs	23.1±3.0	68.2± 4.5	22.7±4.0	70.9±3.7	23.3±3.5	76.7±6.4
0-48 hrs	24.4±3.3	71.4± 3.5	20.6±1.9	74.3±3.0	23.8±3.5	78.4±6.4

Values are means ± standard deviation; N = 4 for each dose level.

TABLE 2 Distribution and Retention of Phthalic Acid in Organs and Tissues

	Phthalic Acid (ppm)		
	4 hr	8 hr	24 hr
Spleen (0.2 g)	1.72±1.53	0.69±0.42	0
Kidney (0.8 g)	0.95±0.95	0.87±0.67	0
Liver (3.0 g)	0.68±0.55	0.54±0.33	0
Adipose	1.26±0.67	0.66±0.21	0
Skeletal Muscle	0.18±0.18	0.14±0.15	0
Lungs	0.05±0.07	0	0
Testes (0.38g)	1.13±0.56	0.38±0.26	0
Heart (0.35g)	0.03±0.02	0.01±0.01	0
Brain	0	0	0
Skin	0.04±0.02	+	0

Values are means ± standard deviation; N = 4 for each group; + indicates trace amounts.

DISCUSSION

Following a single oral dose of carbonyl labelled phthalic acid to rats 95% of the radioactivity was recovered as phthalic acid in the feces and urine. The distribution between the feces and the urine was relatively independent of the dose level (Table I) with 70-80% of the radioactivity in the feces and 20-25% in the urine. No metabolites of phthalic acid could be detected in the feces or the urine but a small amount of the phthalic acid (0.15%) was converted to carbon dioxide. Four hours after dosing approximately 2% of the radioactivity was distributed throughout the organs and tissues with most of this radioactivity detected in the liver, kidney, spleen and testes (Table 2). No radioactivity could be detected in these organs twenty-four hours after dosing.

These results indicate that phthalic acid administered orally to the rat is not appreciably metabolised and is not retained in the organs or tissues.

REFERENCES

ALBRO, P.W., R. THOMAS and L. FISHBEIN: J. Chromatogr. 76, 321 (1973).
MCBAIN, J.B., J.J. MENN and J.E. CASIDA: J. Agr. Food Chem. 16, 813 (1968).
POHL, J.: Biochem Z. 16, 68 (1909).
PORCHER, M.Ch.: Biochem Z. 14, 351 (1908).
SHEMIAKIN, M.M. and L.A. SCHUKINA: Nature 154, 513 (1944).