Heath, D. F.; Vandekar, M. *Br. J. Ind. Med.* **1964**, *21*, 269.
Himbry, F. G.; Smart, L. I.; Binder, T. D.; Dixon, J. M. *J. Anim. Sci.* **1975**, *41*, 269.
Iverson, F. *J. Agric. Food Chem.* **1976**, *24*, 1238.
Ivie, G. W.; Casida, J. E. *J. Agric. Food Chem.* **1971**, *19*, 410.
Ivie, G. W.; Gibson, J. R.; Bryant, H. E.; Begin, J. J.; Barnett, J. R.; Dorough, H. W. *J. Agric. Food Chem.* **1974**, *22*, 646.
Koss, G.; Koransky, W. *Arch. Toxicol.* **1975**, *34*, 203.
Koss, G.; Koransky, W.; Steinbach, K. *Arch. Toxicol.* **1976**, *35*, 107.
Kunze, F. M.; Laug, E. P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1953**, *12*, 339.
Lin, T.; Dorough, H. W. *Arch. Environ. Contam. Toxicol.* **1974**, *2*, 364.
Ludwig, G.; Weiss, J.; Korte, F. *Life Sci.* **1964**, *3*, 123.
Mangold, H. In "Thin-Layer Chromatography"; Stahl, E., Ed.; Springer-Verlag: New York, 1969.
Mehendale, H. M.; Fields, M.; Matthews, H. B. *J. Agric. Food Chem.* **1975**, *23*, 261.
Mehendale, H. M.; Fishbein, L.; Fields, M.; Matthews, H. B. *Bull. Environ. Contam. Toxicol.* **1972**, *8*, 200.
Menzie, C. M. "Metabolism of Pesticides"; U.S. Department of the Interior: Washington, DC, 1969.
Menzie, C. M. "Metabolism of Pesticides: an Update"; U.S. Department of the Interior: Washington, DC, 1974.
Mollenhauer, H. H.; Johnson, J. H.; Younger, R. L.; Clark, D. E. *Am. J. Vet. Res.* **1975**, *36*, 1777.
Mumma, C. E.; Lawless, E. W. "Survey of Industrial Processing Data: Task I—Hexachlorobenzene and Hexachlorobutadiene Pollution from Chlorocarbon Processes"; Midwest Research Institute: Kansas City, MO, 1975; pp 1–175.
National Academy of Sciences "Assessing Potential Ocean Pollutants"; National Academy of Sciences: Washington, DC, 1975.
Negherbon, W. O. "Handbook of Toxicology, Vol. III: Insecticides"; W. B. Saunders: Philadelphia, PA, 1959.
Parke, D. V. "The Biochemistry of Foreign Compounds"; Pergamon Press: London, 1968.
Rajamanickam, C.; Padmanaban, G. *Indian J. Biochem. Biophys.* **1974**, *11*, 119.
Renner, G.; Schuster, K. P. *Toxicol. Appl. Pharmacol.* **1977**, *39*, 355.
Schmid, R. *N. Engl. J. Med.* **1960**, *263*, 397.
Stein, V. B.; Pittman, K. A.; Kennedy, M. W. *Bull. Environ. Contam. Toxicol.* **1976**, *15*, 140.
Stonard, M. D. *Biochem. Pharmacol.* **1975**, *24*, 1959.
Stonard, M. D.; Nenov, P. Z. *Biochem. Pharmacol.* **1974**, *23*, 2175.
Street, J. C.; Chadwick, R. W.; Wang, M.; Phillips, R. I. *J. Agric. Food Chem.* **1966a**, *14*, 545.
Street, J. C.; Wang, M.; Blau, A. D. *Bull. Environ. Contam. Toxicol.* **1966b**, *1*, 6.
Turner, J. C.; Green, R. S. *Biochem. Pharmacol.* **1974**, *23*, 2387.
U.S. Environmental Protection Agency (EPA) "Environmental Contamination from Hexachlorobenzene"; Office of Toxic Substances, EPA: Washington, DC, 1973.
Villeneuve, D. C.; Phillips, W. E.; Panopio, L. G.; Mendoza, C. E.; Hatina, G. V.; Grant D. L. *Arch. Environ. Contam. Toxicol.* **1974**, *2*, 243.
Wallcave, L.; Bronczyk, S.; Gingell, R. *J. Agric. Food Chem.* **1974**, *22*, 904.

Received for review March 31, 1980. Revised November 18, 1980. Accepted February 27, 1981. Mention of a trade name, proprietary product, vendor, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable. This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended.

Biokinetics and Metabolism of *N*-(2,3-Dichlorophenyl)-3,4,5,6-tetrachlorophthalamic Acid in Rats

David Kirkpatrick,* Christina M. Finn, Bethan Conway, David R. Hawkins, Takeo Honda, Mitsuo Ishida, and Graham P. Powell

The biokinetics and metabolism of the bactericide *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalamic acid, techlofthalam, have been studied in rats after repeated oral doses of the ^{14}C-labeled compound for 7 days. Up to 6 days after the last of seven daily doses, means of 92 and 66% of the total dose were excreted in the feces of male and female rats, respectively, and means of 5 and 25%, respectively, in the urine. Only 0.2% dose was retained in the carcasses at this time. There was no extensive accumulation of radioactivity in tissues. Techlofthalam was metabolized by hydroxylation in the dichloroaniline group, and this metabolite was eliminated as a conjugate in bile and also partly unconjugated in the urine of female rats. No metabolites resulting from amide hydrolysis were detected. An oral dose of techlofthalam imide was excreted almost entirely unchanged in feces, indicating a very low absorption of this compound.

The compound *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalamic acid, techlofthalam, is a new systemic bactericide for the control of bacterial leaf blight (*Xanthomonas oryzae*) in rice (Nakagami et al., 1980). Techlofthalam possesses low acute mammalian toxicity, and the oral LD 50 for both sexes of rats and mice is in the region of 2000 mg/kg (M. Ishida, unpublished results). In rice straw, but not grain, two major residues have been identified as techlofthalam and the imide, *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalimide (M. Ishida, unpublished results). This paper describes the syntheses of [^{14}C]techlofthalam and [^{14}C]imide and studies on their fate in rats. Since hydrolysis of the amide group in techlofthalam could occur resulting in the formation of 2,3-dichloroaniline and tetrachlorophthalamic acid, these

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon, PE18 6ES England (D.K., C.M.F., B.C., D.R.H., and G.P.P.), Ube Industries, Ltd., Central Research Laboratory, 1978 Ogushi, Ube, Yamaguchi-ken, Japan (T.H.), and Sankyo Co. Ltd., Agricultural Chemicals Research Laboratories, 1041 Yasucho, Yasugun, Shiga-ken, Japan (M.I.).

0021-8561/81/1429-0608$01.25/0

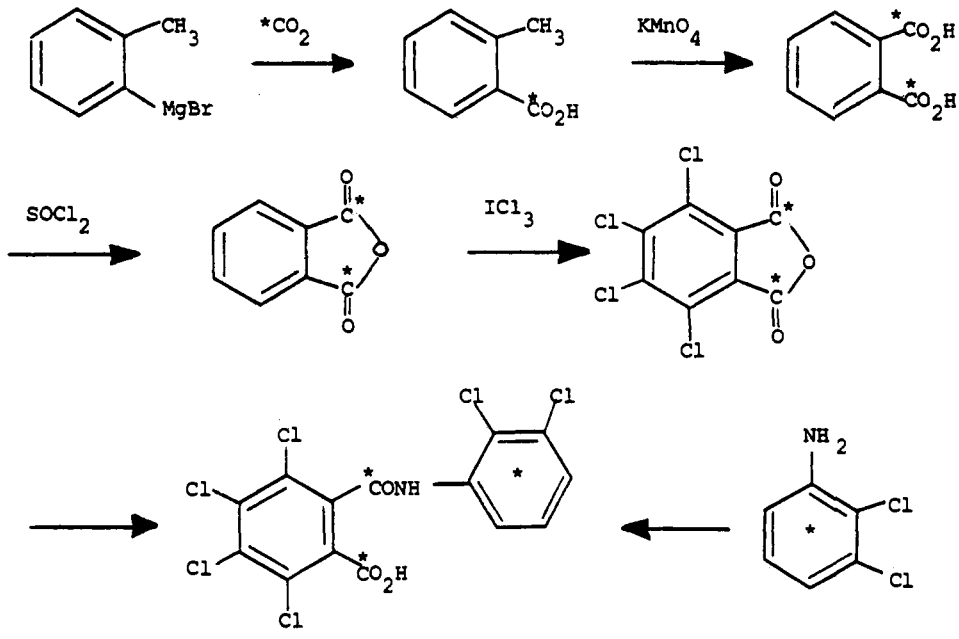

Figure 1. Scheme for the synthesis of [^{14}C]techlofthalam.

studies with techlofthalam were conducted using a mixture of two labeled compounds in order to obtain information on the fate of each moiety. For the animal studies, seven daily oral doses were administered in order to assess accumulation or changes in metabolism during repeated dosing. Doses of 30 mg kg^{-1} day^{-1} were used which corresponds to a low dose level used in rat toxicology studies.

EQUIPMENT AND METHODS

All radioactive liquid samples were measured by liquid scintillation counting (LSC) in a premixed toluene–Triton X-100 (2:1 v/v) based scintillator system, containing 0.39% (w/v) 2,5-diphenyloxazole and 0.008% (w/v) *p*-bis(*o*-methylstyryl)benzene (Patterson and Greene, 1965). A Philips liquid scintillation analyzer (Philips N.V., Holland) was used, and quench correction was by the external standard channel ratio method. The radioactivity in homogenized solid samples was determined by combustion analysis in a Model 306 Mk II sample oxidizer (Packard Instruments Co.), followed by LSC.

The radiochemical purity of ^{14}C-labeled compounds was determined by thin-layer chromatography (TLC) analysis on 250-μm Kieselgel F 254 plates (E. Merck AG., Germany) using the following solvent systems: (a) chloroform–methanol–98% formic acid (80:20:1 v/v); (b) ethyl acetate–2-propanol–35% ammonia (9:7:4 v/v); (c) dioxane–methanol–acetic acid (8:1:1 v/v); (d) toluene–ether–acetic acid–methanol (60:30:10:1 v/v). Radioactive bands were located with a Berthold Model LB 2722 radiochromatogram scanner and by autoradiography using Kodak Kodirex X-ray film. Areas of silica gel were removed from the plates, and adsorbed radioactivity was measured by suspending the finely ground powder in water (4 mL) and Triton X-100 based scintillator cocktail (10 mL).

The ^{14}C-labeled metabolites in urine and extracts of feces were separated by TLC in solvent systems a and b. Detection and quantitation were performed as described above.

Mass spectra were recorded on a VG Micromass 16F mass spectrometer (V.G. Organic Ltd., U.K.). Electron impact spectra were recorded at an ion source temperature of 200–220 °C, electron beam energy of 70 eV, and trap current of 100 μA. Samples were introduced on the direct insertion probe, and spectra were recorded while the probe was heated from 30 to 250 °C.

EXPERIMENTAL SECTION

Synthesis of [^{14}C]Techlofthalam. Techlofthalam was prepared, labeled with carbon-14 at the carbonyl carbons and in the aromatic ring of the 2,3-dichlorophenyl moiety. The syntheses are outlined in Figure 1 and consisted of the following steps.

(*a*) *[carbonyl-^{14}C]-3,4,5,6-Tetrachlorophthalic Anhydride.* [*carbonyl-*^{14}C]Phthalic anhydride was prepared from $^{14}CO_2$ and the Grignard reagent of 2-bromotoluene by the usual route (Murray and Williams, 1958). For chlorination, according to the method of Baessler et al. (1971), the ^{14}C-labeled anhydride (645 mg, 4.4 mmol, 65 mCi) was dissolved in chlorosulfonic acid (2 mL). Iodine trichloride (1.4 g) was added and the mixture was heated at 130 °C for 24 h. During the time, four further amounts of iodine trichloride (0.7 g) in chlorosulfonic acid (1 mL) were added at intervals. The reaction mixture was cooled and poured onto ice–water. The resulting precipitate was filtered, washed with water, and dried to give the product as a white powder (yield 738 mg, 59%). The identity of the product was confirmed by mass spectrometry and TLC.

(*b*) *[carbonyl-^{14}C]Techlofthalam.* To a solution of [^{14}C]-3,4,5,6-tetrachlorophthalic anhydride (285 mg, 1.0 mmol, 15 mCi) in benzene (15 mL) was added 2,3-dichloroaniline (161 mg, 1.0 mmol). The solution was kept at 10 °C for 4 days. The resulting precipitate was filtered and dried to give the product as a white powder (yield 290 mg, 65%; mp 198–199 °C; sp act. 15 mCi/mmol; radiochemical purity >98%).

(*c*) *[2,3-dichlorophenyl-^{14}C]Techlofthalam.* A solution of [U-^{14}C]-2,3-dichloroaniline (24 mg, 0.15 mmol, 3 mCi; C.E.A., Gif-sur-Yvette, France) and 3,4,5,6-tetrachlorophthalic anhydride (60 mg, 0.21 mmol) in benzene (3 mL) was kept at 10 °C for 4 days. The product was isolated as described above (yield 50 mg, 75%; mp 200–201 °C; sp

act. 20 mCi/mmol; radiochemical purity >98%).

Synthesis of [*carbonyl*-^{14}C]-*N*-(2,3-Dichlorophenyl)-3,4,5,6-tetrachlorophthalimide ([^{14}C]Techlofthalam Imide). A suspension of [*carbonyl*-^{14}C]techlofthalam (250 mg, 0.56 mmol, 0.3 mCi) in dichloromethane (50 mL) was added dropwise over 40 min to a stirred, cooled (0–5 °C) solution of dicyclohexylcarbodiimide (160 mg, 0.78 mmol) in ether (10 mL). The reaction was stirred at 0–5 °C for 100 min, after which the precipitated dicyclohexylurea was filtered off. The filtrate was evaporated to a small volume and methanol added. The resulting precipitate was filtered, washed with methanol, and redissolved in the minimum quantity of dichloromethane. The product was precipitated by the dropwise addition of methanol and filtered to give [^{14}C]techlofthalam imide as a white solid (yield 160 mg, 67%; sp act. 0.49 mCi/mmol; radiochemical purity >98%).

Animal Experiments with [^{14}C]Techlofthalam. Adult CFHB rats (bodyweight ~200 g), a Wistar-derived strain, were obtained from Anglia Laboratory Animals, Huntingdon, U.K. The animals were provided with food (Laboratory Diet No. 1, Spratts Limited, Barking, U.K.) and water ad libitum.

Techlofthalam dose solutions were prepared by dissolving equal amounts, in terms of radioactivity, of the two labeled forms of techlofthalam together with nonradioactive techlofthalam in an aqueous solution containing an equimolar amount of sodium hydroxide (0.06% w/v). Dose solutions were prepared separately for each part of the study and the specific activity of the administered techlofthalam therefore varied. Specific activities for sections a–d of the study listed below were 0.77, 0.69, 0.66, and 0.80 mCi/mmol, respectively.

For all experiments the animals were dosed by gastric intubation with [^{14}C]techlofthalam at a nominal dose level of 30 mg (kg of bodyweight)$^{-1}$ day^{-1} in aqueous solution (1 mL).

(*a*) *Excretion and Retention of Radioactivity.* For the excretion studies, six rats (three males and three females) were housed individually in glass metabolism cages which facilitated separation of urine and feces. Animals were dosed once daily at 24-h intervals for 7 days. Urine was collected at 0–8, 8–24, and every 24 h thereafter for 12 days in receivers cooled with solid CO_2.

Feces were collected at 0–24 h, 24–48 h, 48–72 h, and 3-day intervals thereafter. Expired air was passed through an ethanolamine–2-ethoxyethanol mixture (1:4 v/v) for 2 days after the last dose. After 12 days, the animals were sacrificed and the interior of the cages washed with water. The gastrointestinal tracts were removed from the carcasses, and these samples together with the samples of urine and feces were stored at −20 °C until taken for analysis.

(*b*) *Plasma Concentrations of Radioactivity.* For the measurement of plasma concentrations of radioactivity, six rats (three males and three females) were dosed once daily at 24-h intervals for 7 days. After administration of the first dose (day 1), blood samples were withdrawn from the tail veins at 30 and 60 min and 3 and 6 h. Blood samples were taken immediately before the administration of the next six doses, and after the seventh dose (day 7) blood samples were taken at intervals up to 144 h. Cells were removed by centrifugation and separated plasma was taken immediately for analysis.

(*c*) *Tissue Distribution of Radioactivity.* For the quantitative tissue distribution study, ten rats (five males and five females) were administered seven daily oral doses at 24-h intervals. Pairs of rats (one male and one female) were sacrificed at various times after administration of the final dose. The animals were lightly anaesthetized with halothane and blood was removed from the heart by cardiac puncture. The animals were then sacrificed by cervical dislocation, and all organs and tissues were surgically removed from each carcass.

For the qualitative tissue distribution study, six male rats were administered seven daily oral doses at 24-h intervals, and single animals were killed by asphyxiation with CO_2 at various times after the final dose.

The animals were processed for whole-body autoradiography as previously reported (Hawkins et al., 1977). The freeze-dried whole-body sections were placed in contact with Kodak Industrex C X-ray film in light-tight cassettes, and the autoradiographs developed after an exposure time of 120 days.

(*d*) *Biliary Excretion.* For studies of the biliary excretion of radioactivity, the bile ducts of three male rats (under halothane–oxygen anaesthesia) were cannulated by using 0–0 gauge nylon catheter. After recovery from the operation, the animals were administered single oral doses of [^{14}C]techlofthalam and placed in restraining cages where they were allowed access to food and an isotonic saline solution containing 5% (w/v) glucose. Room temperature was maintained at 24 °C. Bile was collected from each animal at 3-h intervals. Urine was collected during 0–24 and 24–48 h and feces were collected as a 48-h sample. After 2 days, the rats were sacrificed, and the livers and gastrointestinal tracts were removed from the carcasses.

Animal Experiments with [^{14}C]Techlofthalam Imide. Six adult CFHB rats (three males and three females), maintained as described above, were administered a single oral dose (by gastric intubation) of [^{14}C]techlofthalam imide, suspended in gum tragacanth (1 mL) at a nominal dose level of 30 mg/kg of bodyweight. Each animal was housed separately in a glass metabolism cage. Urine was collected (in receivers cooled with solid CO_2) at 0–8 and 8–24 h and at 24-h intervals for a further 4 days. Feces were collected at 24-h intervals for 5 days. Expired air from two animals (one of each sex) was monitored as described above up to 48 h after dosing. After 5 days, the animals were sacrificed and the interiors of the cages washed with water.

Metabolite Investigation. Samples of urine from male and female rats were pooled separately and evaporated to dryness under reduced pressure at 35 °C. The residues were triturated with methanol, and these methanol extracts which contained more than 95% of the urinary radioactivity were applied directly to TLC plates. Feces were extracted with methanol and additionally with dichloromethane in the case of rats dosed with the ^{14}C-labeled imide. Extracts (which contained 50–70% fecal radioactivity) from male and female animals were pooled separately, concentrated to small volumes under reduced pressure, and applied directly to TLC plates. For examination of radioactive components in bile, one male rat was administered an oral dose of [^{14}C]techlofthalam, and bile was collected deep frozen at 0–24 and 24–48 h after dosing. Samples of bile were treated by the procedure described above for urine.

Portions of urine and bile, from animals dosed with [^{14}C]techlofthalam, were mixed with an equal volume of sodium acetate buffer (pH 5.0) and incubated with β-glucuronidase (Type H-1, from Helix Pomatia, Sigma Chemical Co., Ltd., London) for 18 h at 37 °C. Portions of urine and bile were also incubated with buffer alone as controls. Both enzyme-treated and control samples were evaporated to dryness, the residues were triturated with methanol, and

Table I. Mean Cumulative Excretion of Radioactivity by Rats during and after Seven Daily Oral Doses of [^{14}C]Techlofthalam

	excretion, % of dose[a]			
	males		females	
day	urine	feces	urine	feces
1	3.6	58.3	19.1	36.9
2	4.4	74.7	21.4	37.4
3	4.9	79.8	23.3	54.2
4	4.8		23.5	
5	5.0		24.0	
6	4.9	87.5	24.0	62.0
7	4.9		24.5	
8	4.9		25.0	
9	4.9	91.4	25.2	65.5
10	5.0		25.2	
11	5.0		25.3	
12	5.0	92.1	25.3	66.4

[a] Results are expressed as percent of cumulative dose administered up to and including the specified day.

the extracts were applied to TLC plates.

For the isolation and identification of metabolites, methanol extracts of feces were concentrated and applied to preparative TLC plates of layer thickness 2 mm. The plates were developed in solvent system a (chloroform–methanol–98% formic acid, 80:20:1 v/v). After autoradiography, bands of silica gel corresponding to zones of radioactivity were removed from the plate and extracted with ethanol. The procedure was repeated with the ethanol eluates using solvent system b (ethyl acetate–2-propanol–35% ammonia, 9:7:4 v/v) to obtain purer samples of the metabolites, which were then subjected to mass spectrometry.

RESULTS

Excretion of Radioactivity. After daily oral administration of [^{14}C]techlofthalam to rats, radioactivity was excreted fairly rapidly and mainly in the feces, but there was a considerable difference between the excretion patterns in males and females (Table I). At 6 days after the final dose (12 days after the first dose), male rats had excreted means of 92 and 5% of the total (seven) doses in feces and urine, respectively. During the same period, female rats excreted means of 66 and 25% of the total dose in feces and urine, respectively. Virtually all this radioactivity had been excreted within 48 h of the final dose. No radioactivity was detected in expired air during 48 h after the final dose, indicating that the radioactivity was situated in metabolically stable positions. Small amounts of radioactivity (mean values in parentheses) were measured in the gastrointestinal tracts (0.03% of the total dose) and carcasses (0.1% of the total dose) of the animals at 6 days after the final dose.

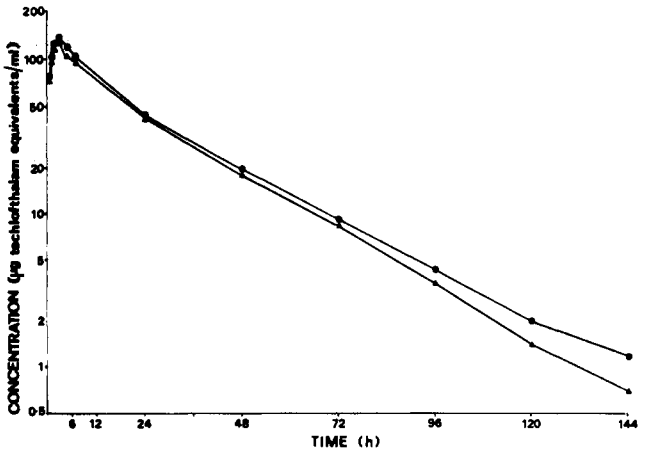

Figure 2. Mean plasma concentrations of radioactivity in male (▲) and female (●) rats after the last of seven daily oral doses of [^{14}C]techlofthalam.

After single oral doses of [^{14}C]techlofthalam to male rats with cannulated bile ducts, individual amounts of 21, 41, and 48% of the dose were excreted in the bile by the three animals during 48 h. Biliary excretion of radioactivity was most rapid between 3 and 18 h after dosing. During 48 h, means of 10 and 35% of the dose were excreted in urine and feces, respectively.

Following single oral doses of [^{14}C]techlofthalam imide to rats, almost all of the administered radioactivity (94.4 ± 8.1% SD) was excreted in the feces in 48 h. A mean of 97.0 ± 4.9% SD of the dose was excreted in feces during 5 days. Urinary excretion accounted for only 0.3% of the dose during 5 days, and no radioactivity was detected in expired air during 48 h after dosing or in the carcasses of the animals sacrificed at 5 days.

Plasma Concentrations of Radioactivity (Figure 2). After the first oral dose of [^{14}C]techlofthalam to rats, peak mean concentrations of radioactivity in plasma were measured at 3 h in both males (115 μg equiv/mL) and females (140 μg equiv/mL). During the dosing period, predose plasma concentrations of radioactivity were similar in both sexes and reached a maximum (~40 μg equiv/mL) within 3 days.

After the final dose, peak mean plasma concentrations of radioactivity again occurred at 3 h in both males (129 μg equiv/mL) and females (137 μg equiv/mL). Between 7 and 144 h after the final dose, concentrations declined with mean half-lives of about 19.7 h in males and 21.3 h in females.

Table II. Concentrations of Radioactivity in Rat Tissues after the Last of Seven Daily Oral Doses of [^{14}C]Techlofthalam

	concn of radioact, μg/g,[a] at time of sacrifice after last dose									
tissue	3 h		7 h		24 h		48 h		120 h	
brain	1.2	1.5	1.8	1.6	0.3	0.6	0.6	0.4	0.1	<0.1
kidneys	26.6	53.4	28.0	43.5	7.4	9.6	4.6	9.3	1.4	2.7
spleen	6.7	6.9	5.7	6.4	1.3	3.5	0.7	1.5	<0.1	<0.1
heart	18.8	17.7	22.2	28.6	3.9	7.6	2.5	4.1	1.3	0.3
lungs	20.8	28.1	24.0	27.9	4.9	11.2	2.6	4.6	0.3	0.4
muscle	4.7	4.6	6.0	6.4	1.7	3.2	0.8	1.3	0.3	0.1
fat	4.7	6.3	5.4	3.3	1.7	2.1	0.8	0.9	<0.2	<0.2
eyes	3.0	4.7	5.5	5.1	1.8	3.2	0.8	1.1	<0.2	<0.2
gonads	8.9	20.9	12.3	19.8	2.5	11.4	1.3	4.4	0.1	0.3
adrenals	15.2	12.4	17.6	17.4	3.8	7.1	3.1	2.9	<1.1	<0.7
pancreas	15.0	16.2	12.1	13.9	3.1	7.0	1.6	2.6	0.1	0.2
liver	38.0	29.0	34.6	23.3	5.2	7.3	3.2	3.1	0.7	0.5
GIT[b]	354	244	295	232	17.9	48.3	9.9	12.2	2.3	0.8
plasma	97.7	105	91.8	92.4	19.0	42.5	10.3	19.6	0.8	1.2

[a] Results are expressed as microgram equivalents per gram of tissue (wet weight). [b] Gastrointestinal tract and contents.

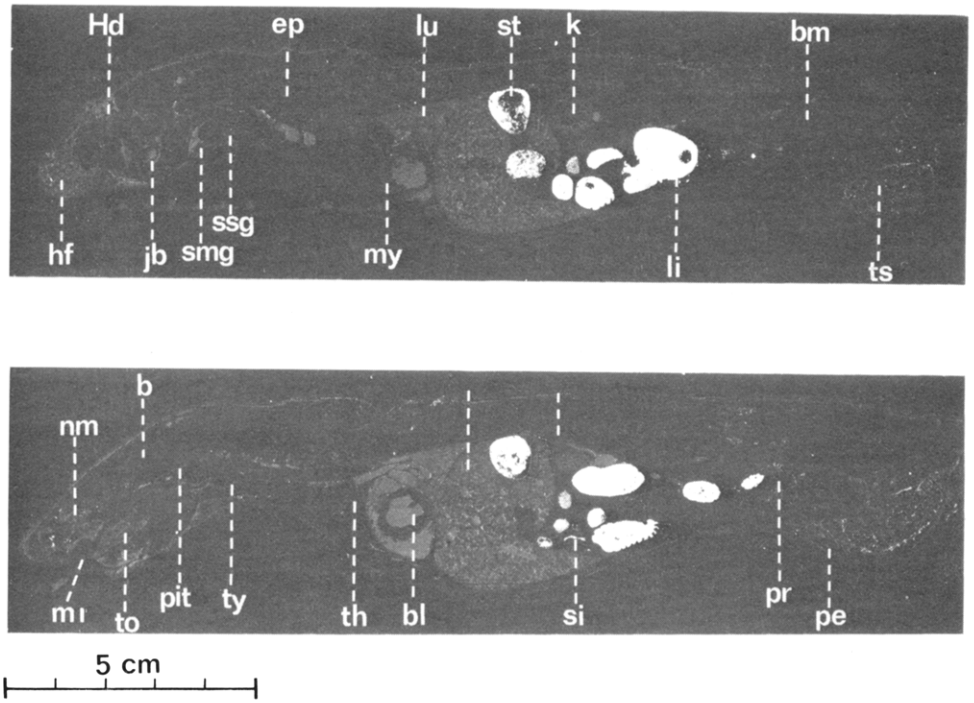

Figure 3. Whole-body autoradiographs of a rat sacrificed 7 h after the last of seven daily oral doses of [^{14}C]techlofthalam; ad, adrenal; b, brain; bl, blood; bm, bone marrow; ep, epimysium; Hd, harderian gland; hf, hair follicle; jb, jaw bone; k, kidney; l, liver; li, large intestine; lu, lung; m, mouth; my, myocardium; nm, nasal mucosa; pe, penis; pit, pituitary; pr, prostate; si, small intestine; smg, submaxillary salivary gland; ssg, sublingual salivary gland; st, stomach; th, thymus; to, tongue; ts, testes; ty, thyroid gland.

Concentrations of Radioactivity in Tissues (Table II). After daily oral administration of [^{14}C]techlofthalam to rats for 7 days, the highest tissue concentrations of radioactivity were found in those animals killed at 3 and 7 h after the final dose. In these rats, concentrations of radioactivity in tissues were considerably lower than those in plasma, in all tissues examined. Liver, kidneys, lungs, and heart were the tissues with the greatest concentrations. At 24, 48, and 120 h after the final dose, the distribution of radioactivity was similar with almost all tissue concentrations lower than the corresponding plasma concentration. Radioactivity concentrations in kidneys were higher in females than in males at all times, indicating the greater importance of renal excretion in this sex.

Whole-body autoradiography of male rats, sacrificed at various times after daily oral administration of [^{14}C]techlofthalam, confirmed the results of the quantitative tissue study (Figure 3). Radioactivity was mainly confined to the gastrointestinal tract, blood, liver, kidneys, and lungs, and levels in other tissues appeared to depend on the blood supply. Moderate levels of radioactivity were detected in the testes and adrenal glands and the epimysium. Radioactivity was not detected in the central nervous system. The relative distribution of radioactivity did not change over the time course of the experiment, with highest concentrations occurring in the animals killed at 3 and 7 h. At 5 days, radioactivity was only detected in kidneys and gastrointestinal tract, and, at 10 days, it could not be detected in any tissue.

Metabolite Investigation. TLC analysis of fecal extracts from rats which had received repeated oral doses of [^{14}C]techlofthalam showed two major radioactive components which together accounted for 94–99% of the extracted radioactivity (Table III). One of these components was identified as unchanged techlofthalam by cochromatography in solvent systems a and b and by mass spectrometry. The mass spectrum which was very similar to that of authentic techlofthalam did not contain a molecular ion but showed small peaks corresponding to the loss of CO_2 or H_2O from the molecular ion (m/e 401 and 427). The most prominent fragments, m/e 392 and 241, correspond to loss of water and chlorine from the molecular ion and to the ion $C_6HCl_4C{\equiv}O^+$, respectively. Relative intensities of ions in the spectrum were very dependent on the probe temperature at which the spectrum was recorded. The mass spectrum of the second radioactive component (metabolite A) in fecal extracts which was more polar than techlofthalam was very similar to that of techlofthalam, with the exception that all the ions containing the dichloroaniline part of the molecule occurred 16 mass units higher, indicating the substitution of a hydroxyl group in the aromatic ring. As with techlofthalam, there was no molecular ion but there were peaks corresponding to the loss of CO_2 (m/e 417) and H_2O (m/e 443). The most prominent ions in the spectrum were at m/e 408, corresponding to loss of water and chlorine, and at m/e 241. The radioscans in Figure 4 represent the TLC behavior of techlofthalam and metabolite A in solvent system a. Relative proportions of techlofthalam and metabolite A in fecal extracts from male and female rats at different times are shown in Table III. Techlofthalam accounted for the larger proportion of the extracted radioactivity at

Table III. Proportions of Radioactive Components in Rat Fecal Extracts and Urine

	% of sample radioact			
	males		females	
sample	techlofthalam	metabolite A	techlofthalam	metabolite A
fecal extract, day 1	62	36	74	25
fecal extract, days 4–6	51	44	59	35
urine, day 1	ND[a]	ND	16	54
urine, day 3	ND	3	14	54
urine, day 6	ND	5	16	56

[a] ND = not detected.

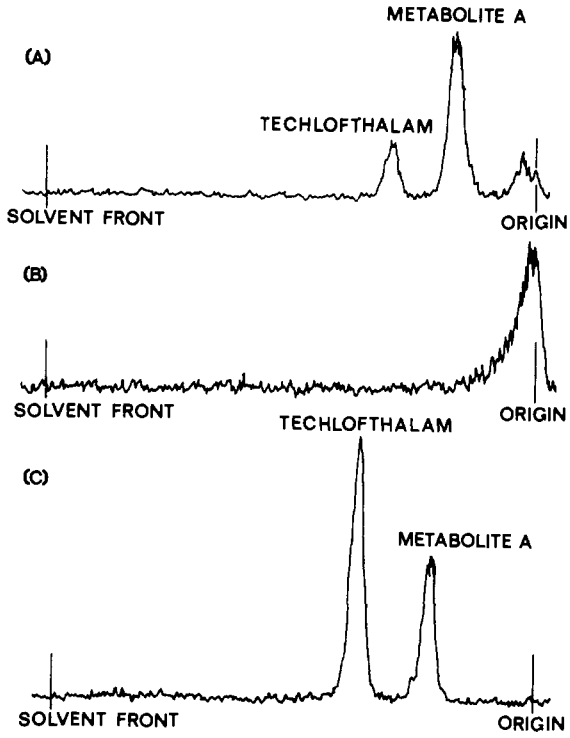

Figure 4. Radiochromatograms of (A) extract of day 1 female rat urine, (B) day 1 male urine, and (C) day 1 male fecal extracts after TLC using the solvent system chloroform–methanol–98% formic acid (80:20:1 v/v).

all times. The proportion of radioactivity associated with metabolite A was greater in males than in females and greater in the day 4–6 sample than in the day 1 sample. Several minor radioactive components were detected in fecal extracts, but none accounted for more than 2% of the sample radioactivity. One of these was found to cochromatograph with the imide of techlofthalam but was not present in sufficient quantity for isolation.

TLC analysis of rat urine showed considerable differences between the patterns of radioactive components in samples from male and female animals (Figure 4). Metabolite A and techlofthalam were the major radioactive components in female urine, and the relative proportions were similar throughout the dosing period. These two components accounted for a maximum of 5% of the radioactivity in male urine (which, of course, contained only one-fifth as much radioactivity as female urine). Two unidentified components accounted for most of the radioactivity in male urine and were also present in similar amounts in the female urine but represented lower proportions of the total radioactivity. The more important of the two was more polar than metabolite A and may have been a conjugate as its TLC behavior was similar to that of the conjugate identified in bile (see below).

TLC analysis (solvent system b) of bile from male rats which had received a single oral dose of [^{14}C]techlofthalam showed a major radioactive component (metabolite B, Table IV) which was hydrolyzed to metabolite A on incubation with β-glucuronidase containing some sulphatase activity. It was concluded, therefore, that this major biliary metabolite was probably a glucuronide conjugate of metabolite A. Unconjugated metabolite A was also present in rat bile, and the remaining radioactivity was divided between several minor components. The presence or absence of unchanged techlofthalam in bile was not confirmed, but it would have been a very minor component if present at all. Thin-layer cochromatographic analysis showed that radioactivity in extracts of feces from rats

Table IV. Proportions of Radioactive Components in Male Rat Bile

sample	% of sample radioact		
	metab-olite A	metab-olite B	others
0–24 h	26	56	18
24–48 h	20	69	11
0–24 h (enzyme treated)	76	2	22

dosed with [^{14}C]techlofthalam imide was mostly (~90%) associated with the unchanged imide. A small proportion (6%) of the extracted radioactivity was associated with techlofthalam. There was no significant difference in the patterns of radioactive components in samples from male and female animals.

DISCUSSION

There was an interesting difference in the disposition of [^{14}C]techlofthalam in male and female rats, which was well illustrated by the urinary excretion data. This could not be entirely attributed to a difference in absorption since studies in bile duct cannulated male rats showed that up to 48% of a single oral dose was eliminated in the bile. Plasma concentrations of radioactivity were also similar in both sexes, indicating similar absorption. If it is assumed that patterns observed in rat fecal extracts were representative of all the radioactivity in feces, then it can be calculated that approximately 46 and 38%, respectively, of the administered radioactivity was excreted in the feces as unchanged techlofthalam by males and females and this proportion could represent the unabsorbed portion of the oral doses since no significant amounts of unchanged techlofthalam (free or conjugated) were detected in the bile of male rats. Amounts of unchanged techlofthalam in urine corresponded to ~4% of the administered radioactivity in female rats and to less than 1% in males. Metabolite A accounted for about 40 and 23% of the administered radioactivity in the feces of males and females, respectively. The value for males is in good agreement with the results from the analysis of bile metabolites since most of the radioactivity in male rat bile was associated with metabolite A or its conjugate. Urinary excretion of metabolite A accounted for ~14% of the administered radioactivity in females and for less than 1% in males. Total excretion of this metabolite was, therefore, similar in both sexes (~40% of the administered radioactivity), only the route of excretion being different; this in turn resulted in the observed sex difference in urinary excretion of radioactivity. A possible explanation of this is that the conjugation of metabolite A with glucuronic acid is a prerequisite for biliary excretion and it is known that this conjugation reaction occurs more rapidly in male rats (Inscoe and Axelrod, 1960). This would imply that unconjugated metabolite A, detected in bile, was an artifact resulting from hydrolysis during the extraction procedure.

The major metabolite has been shown to be formed by hydroxylation in the dichloroaniline ring, although no information has been obtained on the position of hydroxylation. This metabolite was excreted as either an ester or ether glucuronide conjugate involving the carboxylic acid or phenolic groups, respectively. Since no significant amounts of conjugated techlofthalam were detected, it is perhaps more likely that the conjugate was an ether glucuronide. With salicyclic acid, which contains adjacent aromatic hydroxy and carboxyl groups, only the ether glucuronide is formed by rats (Quilley and Smith, 1952).

The imide of techlofthalam, a significant metabolite and residue in rice straw, was excreted almost entirely unchanged in the feces. Since the imide does not contain the

properties necessary for biliary excretion and is not able to form a conjugate directly, the results indicate that it is very poorly absorbed after oral administration and is, thus, toxicologically less important than techlofthalam.

LITERATURE CITED

Baessler, K.; Scheidl, F.; Mayer, K. German Patent 1934174, 1971.
Hawkins, D. R.; Weston, K. T.; Chasseaud, L. F.; Franklin, E. R. *J. Agric. Food. Chem.* **1977**, *25*, 398.
Inscoe, J. K.; Axelrod, J. *J. Pharmacol. Exp. Ther.* **1960**, *129*, 128.
Murray, A.; Williams, D. L. "Organic Syntheses with Isotopes"; Interscience: New York, 1958; pp 439, 848.
Nakagami, K.; Tanaka, H.; Ishida, M.; Koremura, M.; Honda, T.; Yamazaki, T. *Nippon Noyaku Gakkaishi* **1980**, *5*, 237.
Patterson, M. S.; Greene, R. C. *Anal. Chem.* **1965**, *37*, 854.
Quilley, E.; Smith, M. J. H. *J. Pharm. Pharmacol.* **1952**, *4*, 624.

Received for review April 9, 1980. Accepted October 15, 1980.

A Simple Colorimetric Method for the Determination of Endrin in Emulsifiable Concentrates

Ramesh T. Sane* and Sudhir S. Kamat

A simple colorimetric method for the determination of endrin from emulsifiable concentrates is described. The method is based on the reaction of formaldehyde–sulfuric acid with endrin in the presence of sodium perborate. The method is sensitive and obeys Beer's law for the concentration range 4–40 $\mu g\ mL^{-1}$ with 99 ± 2% recovery.

Quantitative analysis of endrin contents by determining total organic chlorine and infrared spectrophotometric methods are recommended by AOAC (1975) in the case of endrin formulations. Bann et al. (1958) have reported a colorimetric method based on determination by dechlorination of endrin and subsequent coupling with diazotized sulfanilic acid. There are some other colorimetric methods reported in the literature (O'Donnell, 1954, 1955; Beckman, 1954). On thin-layer chromatograms many reagents such as zinc chloride in hydrochloric acid (Wienke and Burke, 1969), sulfuric acid (Chou and Cochrane, 1969), ethanolic silver nitrate followed by UV irradiation (Abbott et al., 1964), silver nitrate–formaldehyde–potassium hydroxide–nitric acid–hydrogen peroxide in succession (Salo et al., 1963), *O*-toluidine (Salo et al., 1963), dianisidine (Kawashiro and Hosogai, 1964), etc. are used for the quantitative estimation. Bioassays using vinegar flies (*Drosophila melanogaster*) (Phillips et al., 1962), water fleas (*Daphnia magna*), and goldfish (*Carassius auratus*) (Davidow and Schwartzman, 1955) are also reported for endrin quantitation. Excellent reviews of instrumental methods like GLC, mass spectrometry, etc. are also reported (Beynon and Elgar, 1966; Williams and Cook, 1967; Zweig, 1978).

There is a shift in the peak at the higher concentration of endrin by the infrared method (Indian Standards Institute, 1959), while in the sulfanilic acid method the excess sulfanilic acid and sodium nitrite give high background values. We observed that endrin reacts with formaldehyde–sulfuric acid in the presence of sodium perborate, producing a yellow-colored compound. This yellow-colored compound has an absorption maximum at 405 nm, and it can be used in the quantitative determination of endrin (Figure 1).

EXPERIMENTAL SECTION

Materials. The following chemicals were used: Carbon disulfide, methyl alcohol, acetone, sodium perborate ($NaBO_3 \cdot 4H_2O$), formaldehyde (40% v/v), sulfuric acid (concentrated), acetic acid (glacial), sodium hydroxide (10% m/v, aqueous), and endrin standard (Shell Chemical Corp). All the chemicals were analytical grade unless specified otherwise.

Preparation of Sodium Perborate Solution. To a mixture of 150 mL of methyl alcohol and 150 mL of glacial acetic acid 100 g of sodium perborate is added. The mixture is allowed to stand overnight to ensure saturation.

Preparation of Endrin Standard Solution. Accurately weighed pure endrin (500 mg) is dissolved in ~20 mL of the solvent acetone and diluted to 50 mL in a volumetric flask by using methyl alcohol. The concentration of endrin in this solution is 10 mg mL^{-1}. One milliliter of this solution is transferred to a 10-mL volumetric flask and diluted to the 10-mL mark by using methyl alcohol. The concentration of endrin in this diluted solution is 1 mg mL^{-1}.

Analytical Procedure. One milliliter of the sample under test was pipetted in a 25-mL volumetric flask. One millilter of the sodium perborate solution and 1 mL of formaldehyde were then added. The contents were properly mixed by gently shaking the flask. Two milliliters of sulfuric acid was added very slowly with constant swirling. The flask was allowed to stand for 30 min at room temperature after which the volume was made up to the 25-mL mark by using methyl alcohol. Absorbance of the color developed was measured at 405 nm, against a reagent blank. The reagent blank was prepared by treating 1 mL of methanol in place of the sample.

Standard Curve. A series of endrin standard solutions having concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μg were taken in 10 different 25-mL volumetric flasks and treated as per the analytical procedure described earlier. The graph of endrin concentration against optical density was a straight line passing through the origin and was found to obey Beer's law for the concentration range of 4–10 $\mu g\ mL^{-1}$.

Application of the Method to Emulsifiable Concentrates. The method was tried for five emulsifiable concentrates from the market. The amount of endrin determined and the percent of declared are shown in Table I.

Department of Chemistry, Ramnarain Ruia College, Matunga, Bombay 400 019, India.

0021-8561/81/1429-0614$01.25/0