Metabolism of 4-Chlorobenzotrichloride in Rats

Gary B. Quistad,* Kathleen M. Mulholland, Gary Skiles, and Gene C. Jamieson

Special analytical methodology was developed for purification of 4-chloro[^{14}C]benzotrichloride, which is both volatile and hydrolytically unstable at milligram mass levels. When rats were given a single oral dose of 4-chloro[^{14}C]benzotrichloride at 1.5 mg/kg, within 4–6 days 87 and 9% of the applied ^{14}C were excreted in urine and feces, respectively. The major urinary metabolite was identified as 4-chlorohippuric acid, representing 78% of the applied dose. While about two-thirds of the fecal ^{14}C residues were unextractable with organic solvents, free 4-chlorobenzoic acid and $\alpha,\alpha',4,4'$-tetrachlorostilbene contributed 10 and 8% of the fecal ^{14}C. The metabolic production of $\alpha,\alpha',4,4'$-tetrachlorostilbene appears to occur by a novel metabolic pathway.

Since there is a paucity of data concerning the metabolic fate of 4-chlorobenzotrichloride (4-chloroBTC), a high-volume intermediate used in the synthesis of certain herbicides [e.g., trifluralin (Boudakian, 1980)], we studied the degradation of 4-chloroBTC in rats. Interest in the disposition of 4-chloroBTC in mammals is enhanced by reports of bacterial mutagenicity (Yasuo et al., 1978) and carcinoma in mice (Matsushita et al., 1975) for unsubstituted benzotrichloride.

Since 4-chloroBTC is rapidly hydrolyzed to 4-chlorobenzoic acid in water, we anticipated that these two compounds would follow essentially the same metabolic pathways. The conjugation of 4-chlorobenzoic acid with glycine is one of the oldest known metabolic conversions (Schultzen and Gräbe, 1867). Mammals efficiently excrete free 4-chlorobenzoic acid, as well as its glycine and ester glucuronide conjugates (Bray et al., 1952, 1955). Tissue residues in rats treated with 4-chlorobenzoic acid are insignificant (Lang and Lang, 1956).

EXPERIMENTAL SECTION

Radiosynthesis. 4-Chloro[U-*ring*-^{14}C]benzotrichloride (4-chloroBTC) was prepared by Pathfinder Laboratories, Inc. (St. Louis, MO) according to the following sequence of reactions: barium carbonate to acetylene to benzene to nitrobenzene to aniline to chlorobenzene to 4-chloroacetophenone to 4-chlorobenzoic acid to 4-chloroBTC. A portion of the [^{14}C]-4-chloroBTC (ca. 25 mg) was distilled by using a sublimation apparatus (1–5 mmHg; 22 °C; dry ice cooled condenser; 1 h) to give a 63% yield of ^{14}C products. A 5-mg sample of the distillate was purified by reversed-phase liquid chromatography (LC) with the following conditions: Waters M-6000 pump; 10-μm LiChrosorb RP-8 column, 0.46 × 25 cm; acetonitrile–water, 60:40; ultraviolet (UV) detection at 254 nm; 1.6 mL/min. The 4-chloroBTC was introduced onto the column via a 0.5-mL loop by using acetonitrile (200 μL) before rapid addition of water (100 μL) and injection. The effluent containing 4-chloroBTC ($k' = 3.6$) was partitioned into pentane immediately upon its appearance from the column. This methodology gave a 52% recovery of 4-chloroBTC (98.1% radiochemical purity by analytical LC in SS 4).

The specific activity of the 4-chloroBTC was determined spectrophotometrically by LC and liquid scintillation counting (LSC). Variable masses of 4-chloroBTC were used for LC in order to prepare a standard curve for UV response at 254 nm. [^{14}C]-4-ChloroBTC was then injected onto the LC column. The mass of 4-chloroBTC was determined from the standard curve and the amount of ^{14}C by LSC. Hence, the specific activity of 4-chloroBTC was 4 mCi/mmol.

Analytical Methods. Metabolites were analyzed initially by thin-layer chromatography (TLC, silica gel GF, Analtech) with radiolabeled zones located with a Packard Model 7201 radiochromatogram scanner. In order to enhance resolution of metabolites, TLC was followed usually by reversed-phase LC (Spectra-Physics 8000 instrument; UV detection at 254 nm; 10-μm LiChrosorb RP-8 column; 35 °C; 1.6 mL/min; elution with various mixtures of acetonitrile–0.1% acetic acid). The following solvent combinations were used for LC: SS 1 (20% acetonitrile), SS 2 (25% acetonitrile), SS 3 (15% acetonitrile), SS 4 (isocratic at 60% acetonitrile for 17 min, linear gradient 60–100% over 8 min), SS 5 (65% acetonitrile), SS 6 (isocratic at 60% acetonitrile for 10 min, 60–100% over 15 min), SS 7 (60% acetonitrile). In general, metabolite identification and quantification were based on collection of the total column effluent in timed fractions for radioassay by LSC (Packard Model 2425).

Certain tissues and residual solids were quantified for radiolabel by combusion to $^{14}CO_2$ [Harvey biological material oxidizer (OX 300) with collection in ^{14}C-cocktail for biological oxidizers (Harvey)] followed by LSC.

Mass spectra were obtained by coupled gas–liquid chromatography–mass spectrometry (GLC–MS) using a Hewlett-Packard Model 5985 instrument in the electron impact (EI) or chemical ionization modes. Nuclear magnetic resonance (^{1}H NMR) spectra were determined by using a Varian T-60 spectrometer.

Metabolite Standards. An authentic sample of 4-chlorobenzoic acid (**2**) was purchased from Aldrich Chemical Co., whereas 4-chloroBTC was supplied by Occidental Chemical Co.

The glycine conjugate of **2** (i.e., **3**) was synthesized from **2** and the methyl ester of glycine. 4-Chlorobenzoic acid (500 mg, 3.2 mmol) was reacted with oxalyl chloride (0.3 mL, 3.5 mmol) in ether (10 mL) containing dimethylformamide (1 drop) to give the acid chloride of **2**. The ether phase containing this acid chloride was decanted into a separate flask and the ether was evaporated. The acid chloride of **2** was dissolved in tetrahydrofuran–toluene (1:1), and to this solution was added the hydrochloride salt of the methyl ester of glycine (440 mg, 3.5 mmol) dissolved in pyridine (10 mL). After being stirred for 16 h, the mixture was acidified and extracted with ethyl acetate to give the methyl ester of **3** (81% yield after purification by a silica gel column eluted with hexane–ethyl acetate, 2:1). The methyl ester of **3** was characterized by its mass and NMR spectra: ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3, OCH_3), 4.19 (d, 2, CH_2, J = 5 Hz), 6.64 (br s, 1, NH), 7.35 (d, 2, ar, J

Biochemistry Department, Zoecon Corporation, Palo Alto, California 94304.

0021-8561/85/1433-0095$01.50/0

= 8 Hz), 7.73 (d, 2, ar, J = 8 Hz). Saponification of the methyl ester of **3** with 1 M methanolic NaOH (1 h, 22 °C) gave **3** in 44% yield. The crude **3** was purified by preparative TLC (ethyl acetate–hexane–acetic acid, 6:1:0.3; R_f = 0.35).

A methylated, peracetylated glucuronide of **2** (i.e., **4**) was synthesized by reacting **2** (100 mg, 0.65 mmol) with methyl 2,3,4-tri-*o*-acetyl-D-glucopyranuronate (217 mg, 0.65 mmol) in the presence of dicyclohexylcarbodiimide (132 mg, 0.65 mmol) and 4-(dimethylamino)pyridine (8 mg, 0.07 mmol) in methylene chloride (3 mL). Purification of the crude product by TLC (ether–hexane, 4:1) gave **4** (R_f = 0.49) in 35% yield. The standard was separable by GLC (1-m 3% OV-17) into α- and β-glucuronides, which gave essentially identical mass spectra: m/z (rel intensity) 415 (0.2, M – CO_2CH_3 for Cl = 37), 413 (0.2, M – CO_2CH_3 for Cl = 35), 141 (32), 139 (90), 111 (15), 43 (100).

The reaction of 4-chloroBTC (1.0 g, 4.4 mmol), red phosphorus (135 mg, 4.4 mmol), and iodine (3 mg, 0.01 mmol) at 210 °C for 4 h gave $\alpha,\alpha',4,4'$-tetrachlorostilbene (**5**) in 88% yield [cf. LeSuer and Stuebe (1961)]. The product was purified by TLC (hexane, R_f = 0.42); reversed-phase LC analysis (SS 7) gave a single UV-active peak (k' = 14.4) whereas the standard eluted at the solvent front upon normal-phase LC analysis (Zorbax SIL column, 100% pentane). The standard was separable by GLC into cis and trans isomers, which gave essentially identical mass spectra: m/z (rel intensity) 320 (19), 318 (29), 316 (28, M^+ for Cl = 35), 248 (64), 246 (100), 176 (49), 123 (32). This synthetic sample consisted of a 62:38 ratio of trans:cis isomers. After two recrystallizations of this mixture using acetone, the cis isomer was isolated [mp 167–168.5 °C; lit. mp 166–167 °C (Kenner and Witham, 1910; Fleck, 1948)].

Treatment. Female albino rats (Sprague-Dawley, Simonsen Laboratories, Gilroy, CA) weighing 166–186 g were given a single oral dose of [U-*ring*-^{14}C]-4-chloroBTC (ca. 5 μCi for 1.5 mg/kg and 38 μCi for 102 mg/kg) by gavage in corn oil (0.5 mL). All animals had been fasted 16 h before dosing.

Immediately after dosing, the rats were housed in all-glass metabolism chambers (Stanford Glassblowing Laboratories, Palo Alto, CA) for separate collection of urine, feces, and expired CO_2. The rats were fed a diet of rat chow (Ralston Purina). The 5% KOH solution (25 mL) used to trap $^{14}CO_2$ was quantified daily by LSC (1.0-mL aliquots). Four to six days after dosing, the animals were sacrificed with ether. Tissues were dissected, weighed, and frozen for subsequent analysis.

Urine. The urine was removed at daily intervals and the collection flask rinsed with acetonitrile (~5 mL). Aliquots of urine were quantified by LSC. For each day the urine from the two rats dosed at 1.5 mg/kg was analyzed by TLC (hexane–ethyl acetate–acetic acid, 2:1:0.1). The coapplication of authentic standards of **1**, **2**, and **3** allowed quantification of these components by scraping discrete UV-active zones of silica gel (R_f = 0.74, 0.43, and 0.11, respectively) followed by LSC. The glycine conjugate (**3**) was converted to methyl 4-chlorohippurate with CH_2N_2 and was coincident with a synthetic standard by TLC (hexane–ethyl acetate–acetic acid, 1:1:0.1; R_f = 0.48) and reversed-phase LC (k' = 9.1, SS 1). The presence of 4-chlorohippurate was verified by EI mass spectral analysis of 8 μg isolated from the 1-day urine of a high-dose (102 mg/kg) rat: m/z (rel intensity) 229 (4, M^+ for Cl = 37), 227 (11, M^+ for Cl = 35), 195 (6), 168 (18), 141 (33), 139 (100), 111 (33).

Feces. The feces were extracted with acetonitrile (3×), and extracts were quantified by LSC, and then analyzed by TLC (hexane–acetic acid, 1:0.1). Synthetic standards of **1**, **2**, and **3** were spotted with the extract prior to TLC development in order to permit scraping of TLC zones coincident with these compounds (R_f = 0.65, 0.21, and 0.0, respectively) for further analysis. The metabolites **2** and **3** were quantified by LC (for **2**, k' = 9.1 in SS 2; for **3**, k' = 7.6 in SS 3). When the TLC zone containing **1** was analyzed by LC (SS 4), **1** was only a minor component (k' = 5.9) and most of the ^{14}C residue was identified as $\alpha,\alpha',4,4'$-tetrachlorostilbene (**5**, k' = 14.8).

Compound **5** was isolated from the 1-day acetonitrile extract of feces from a rat dosed at 102 mg/kg. The fecal extract was evaporated to dryness, and the residue was partitioned between hexane and acetonitrile–H_2O (70:30). Evaporation of the hexane phase gave **5** as the major ^{14}C metabolite, which was purified by TLC (hexane–ether, 5:1, R_f = 0.64) and LC (k' = 9.5, SS 5). Analysis of this metabolite by GLC/MS revealed two isomeric peaks (11:89 ratio) with essentially identical mass spectra: m/z (rel intensity) electron impact 320 (53), 318 (73), 316 (68, M^+ for Cl = 35), 281 (28), 248 (93), 246 (100), 210 (39), 176 (89); m/z (rel intensity) chemical ionization (CH_4), 321 (39), 319 (24), 317 (22, M + H for Cl = 35), 283 (70), 281 (76), 249 (86), 247 (100).

Tissues. Selected organs and tissues were combusted to $^{14}CO_2$ for quantification of ^{14}C residues by LSC. The stomach and intestines, as well as the carcass remains, were extracted with acetonitrile (3×), and aliquots of the extract were quantified by LSC. The residual solids after acetonitrile extraction were extracted further with methanol in order to monitor the efficiency of the extraction process.

A sample of abdominal fat was extracted with chloroform (2×) and acetonitrile (1×). The combined extract was analyzed by TLC (100% hexane and hexane–ethyl acetate–acetic acid, 2:1:0.1). TLC zones coincident with added standards of **1** and **5** were analyzed further by LC (SS 2 and SS 6, respectively).

RESULTS AND DISCUSSION

The purification of 4-chlorobenzotrichloride (**1**, 4-chloroBTC) offered several technical challenges. First of all, it is extremely reactive with water (total hydrolysis within minutes). Since 4-chloroBTC is unretained upon normal-phase LC (LiChrosorb SI 100 column, elution with pentane), it was necessary to find reversed-phase LC conditions with aqueous solvents for purification. The hydrolysis of 4-chloroBTC was minimized by introducing it into the LC injection loop with acetonitrile. Sufficient water was added rapidly to reach a 2:1 ratio of acetonitrile:water, and the sample was injected immediately onto the LC column. After elution from the LC column, the eluate with 4-chloroBTC was collected directly in pentane. The usage of pentane allowed gentle removal of solvent (4-chloroBTC volatile at milligram mass levels) and prevented hydrolysis. With these conditions we were able to recover 4-chloroBTC in good yield and 98% purity.

Radiolabel Balance. When female rats were given a single oral dose of [U-*ring*^{14}C]-4-chloroBTC, most of the radiolabel was excreted readily in the urine and feces (Table I; Figure 1). Exhaled organic ^{14}C residues (including 4-chloroBTC itself) and $^{14}CO_2$ were essentially absent. When the rats were sacrificed 4–6 days after dosage, only about 4% of the applied dose remained in the carcass. The recovery of radiolabel from the rats was virtually quantitative.

Urine. 4-chloroBTC is substantially hydrolyzed in the rat to 4-chlorobenzoic acid (**2**). As expected from previous work (Bray et al., 1952, 1955), **2** is excreted largely as 4-chlorohippuric acid (**3**) in the urine. Indeed, for the 1.5

Table I. Radiolabel Balance for Rats Given a Single Oral Dose of 4-Chloro[^{14}C]benzotrichloride

	% applied dose	
	1.5 mg/kg[a]	102 mg/kg[b]
urine	87	77
feces	9	14
carcass	4	4
$^{14}CO_2$	≤0.02	
volatile organics	≤0.002	
total recovery	100	95

[a] Average for two females, 6-day sacrifice. [b] One female, 4-day sacrifice.

Table II. Analysis of Urinary Metabolites from Female Rats Dosed with 4-Chloro[U-*ring*-^{14}C]benzotrichloride

	% ^{14}C in urine				
	1.5 mg/kg[a]				
	1 day	2 day	3 day	4–6 day	102 mg/kg,[b] 1 day
4-chlorobenzotrichloride (1)	≤0.3	≤0.9			≤0.4
4-chlorobenzoic acid (2)	0.7	4	6	9	5
4-chlorohippuric acid (3)	96	37	27	8	88
TLC origin zone[c] (would include glucuronides)	4	59	64	73	3

[a] Average for two rats. [b] One rat. [c] Silica gel GF, hexane–ethyl acetate–acetic acid, 2:1:0.1.

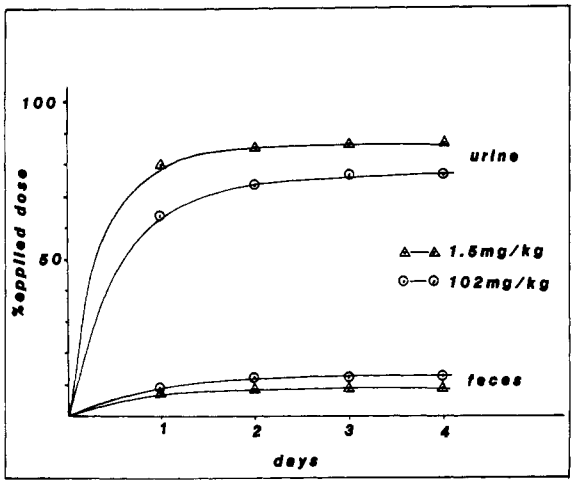

Figure 1. Excretion of radiolabel from rats given a single oral dose of 4-chloro[^{14}C]benzotrichloride.

mg/kg dose rate, **3** represented 78% of the applied dose and was the uncontested major metabolite of 4-chloroBTC in rats. Free 4-chlorobenzoic acid was found in trace amounts in 1-day urine (0.7% of urine ^{14}C) and **2** became relatively more abundant with increasing time (Table II). Parent 4-chloroBTC was undetectable in rat urine.

Somewhat surprisingly, glucoronides of **2** were not found in urine [cf. Bray et al. (1952)]. The attempted cleavage of polar ^{14}C residues with β-glucuronidase and also derivatization of such polar residues to methylated, peracetylated glucuronides (**4**) both failed. Hence, we conclude that glucuronides of **2** are minor urinary metabolites of 4-chloroBTC in rats.

Feces. The feces contained considerably less radiolabel from [^{14}C]-4-chloroBTC than the urine (Figure 1). Interestingly, two-thirds of the ^{14}C residues in feces were not extractable with acetonitrile, which is generally a very effective solvent for such extractions. Hence, 6–9% of the applied 4-chloroBTC may have reacted chemically with the contents of the alimentary canal to form polar ^{14}C residues.

Table III. Analysis of Fecal Metabolites from Rats Given a Single Oral Dose of 4-Chloro[^{14}C]benzotrichloride

	% ^{14}C in feces		
	1.5 mg/kg, 1 day[a]	102 mg/kg	
		1 day[b]	2 day[c]
CH_3CN extract	28	38	64
4-chlorobenzotrichloride (1)	≤0.3	1	1
4-chlorobenzoic acid (2)	10	7	6
4-chlorohippuric acid (3)	0.8	13	27
α,α′,4,4′-tetrachlorostilbene (5)	8	13	13
residual solids	72	62	36

[a] 8.3% applied dose. [b] 7.8% applied dose. [c] 3.9% applied dose.

For the organoextractable radiolabel in feces (3–5% of the applied dose), the major metabolites were free 4-chlorobenzoic acid and α,α′,4,4′-tetrachlorostilbene (**5**), representing 10 and 8% of the ^{14}C in the 1-day feces of rats dosed at 1.5 mg/kg (Table III). Although a trace of 4-chlorohippuric acid was found in feces (~1% of fecal ^{14}C), 4-chloroBTC represented only 1% of the fecal ^{14}C from a rat dosed at 102 mg/kg. Hence, very little 4-chloroBTC survives transit through the rat.

The identification of α,α′,4,4′-tetrachlorostilbene (**5**) in rat feces is particularly intriguing. This unique metabolite obviously results from the condensation of two 4-chloroBTC molecules. Hence, one might expect a higher yield of **5** at the 102 mg/kg dose level than at the 1.5 mg/kg dose, but **5** represented 1.5% and 0.7% of the applied dose, respectively, which is a relatively small (if significant) dose effect. An explanation of the biochemical mechanism for formation of **5** is somewhat perplexing. If one considers 4-chloroBTC as an analogue of carbon tetrachloride, it is tempting to speculate that **5** may form by carbene intermediates (Pohl and George, 1983). By comparison, the chemical synthesis of **5** requires condensation of two molecules of **1** and a recipient for chlorine (elemental phosphorus) with iodine catalysis at 210 °C. Although the mechanism for the formation of **5** is unclear, we are confident that **5** was *not* an impurity in the 4-chloroBTC used for rat dosage. The initial radiochemical purity of **1** was 98.1%, but none of the radiochemical impurities were contributed by **5** (<0.2%). The largest single ^{14}C impurity was 4-chlorobenzoic acid (ca. 1% of total ^{14}C) while no other single ^{14}C impurity could be identified. The purification and purity assessment of [^{14}C]-4-chloroBTC was obtained by reversed-phase LC, which affords a good separation of **1** from **5**. In addition, we determined that **5** is not formed from **1** in corn oil prior to dosage, and after several months of storage of neat nonradiolabeled **1** at room temperature, **5** is undetectable. The α,α′,4,4′-tetrachlorostilbene was also present in feces as a 11:89 ratio for the trans:cis isomers based on GLC analysis. Hence, although the trans isomer of **5** is the favored synthetic product, the cis isomer is the predominant biosynthetic metabolite. α,α′,4,4′-Tetrachlorostilbene is alleged to be a DDT degradation product, and the crystal structures for both cis and trans isomers have been reported (Norrestam et al., 1977; De Kok and Romers, 1978).

Tissues. When rats were sacrificed 4–6 days after dosage, only about 4% of the applied dose remained in the carcass. Except for the abdominal fat from a rat given an artificially high dose of 4-chloroBTC (102 mg/kg), selected tissues showed no evidence of any selective concentration of ^{14}C residues (Table IV). At this high dose rate, compared to other tissues, the abdominal fat contained elevated levels of ^{14}C residues (16 ppm equiv as 4-chloroBTC). Further analysis by TLC and LC revealed that about a third of the ^{14}C residue in this fat actually was 4-

Table IV. Residues in Tissues of Rats Given a Single Oral Dose of 4-Chloro[^{14}C]benzotrichloride

	1.5 mg/kg[a]		102 mg/kg[b]	
	% applied dose	ppb equiv as 1	% applied dose	ppb equiv as 1
spleen	0.009	57	0.008	4.5
ovaries and fallopian tubes	0.006	53	0.013	6.4
pancreas	0.02	69	0.019	6.0
lung	0.02	77	0.044	7.4
kidney	0.04	78	0.060	7.2
brain	0.10	141	0.047	5.4
heart	0.01	42	0.013	4.3
muscle—leg[c]	0.07	114	0.030	4.1
muscle—pectoral[c]	0.06	134	0.049	5.3
fat—abdominal[c]	0.04	99	0.094	16.0
fat—pericardial[c]	0.004	36	0.005	4.5
liver	0.14	56	0.18	4.9
hide[c]	0.02	42	0.063	7.3
stomach and intestines	0.18	41	0.24	5.0
carcass remains	3.25	72	3.12	4.6

[a] Average for two rats sacrificed at 6 days posttreatment. [b] One rat only sacrificed 4 days posttreatment. [c] Aliquot only.

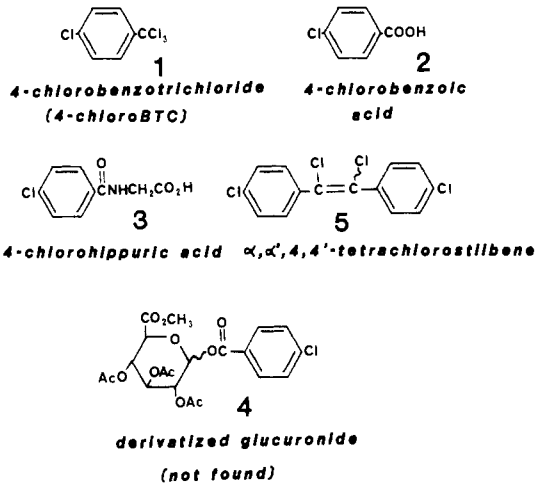

Figure 2. Structures of 4-chlorobenzotrichloride and metabolites.

chloroBTC. Hence, some (but very little) of the administered 4-chloroBTC survived hydrolysis and was deposited in fat. Another 15% of the radiolabel in abdominal fat was identified as α,α',4,4'-tetrachlorostilbene (**5**).

Conclusions. 4-Chlorobenzotrichloride (**1**) is rapidly degraded by rats with 96% excretion of the applied dose within 4–6 days. Since 4-chloroBTC is quickly and quantitatively hydrolyzed in aqueous solution to 4-chlorobenzoic acid, it is not surprising that its metabolic fate mirrors the latter (Figure 2). By artificially protecting 4-chloroBTC with a lipid matrix (corn oil), it is possible to preserve its chemical integrity as evidenced by detection of traces of **1** and a novel metabolite, α,α',4,4'-tetrachlorostilbene (**5**), in feces and fat. Residues of intact **1** and **5** would vanish if 4-chloroBTC were administered in aqueous medium since microgram masses of **1** are hydrolyzed to 4-chlorobenzoic acid within minutes [cf. Lang and Lang (1956)] and presumably 4-chlorobenzoic acid would not be metabolized to **5**.

ACKNOWLEDGMENT

We thank S. Gelfand (Occidental Chemical Corp.) for coordinating this research and D. A. Schooley for useful comments.

Registry No. **1**, 5216-25-1; **2**, 74-11-3; **3**, 13450-77-6; **5**, 93040-25-6.

LITERATURE CITED

Boudakian, M. M. In "Kirk-Othmer Encyclopedia of Chemical Technology", 3rd ed.; Wiley: New York, 1980; Vol. 10, p 921.
Bray, H. G.; Clowes, R. C.; Thorpe, W. V.; White, K.; Wood, P. B. *Biochem. J.* **1952**, *50*, 583.
Bray, H. G.; Humphris, G.; Thorpe, W. V.; White, K.; Wood, P. B. *Biochem. J.* **1955**, *59*, 162.
De Kok, A. J.; Romers, C. *Acta Crystallogr., Sect. B* **1978**, *B34*, 2477.
Fleck, E. E. *J. Am. Chem. Soc.* **1948**, *70*, 2173.
Kenner, J.; Witham, E. *J. Chem. Soc.* **1910**, *97*, 1965.
Lang, H.; Lang, K. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **1956**, *229*, 505.
LeSuer, W. M.; Stuebe, C. W. U.S. Pat. 2960542, Nov 15, 1960; *Chem. Abstr.* **1961**, *55*, 9352i.
Matsushita, H.; Fukuda, K.; Sakabe, H.; Takemoto, K., 49th Annual Meeting of the Japanese Industrial Hygiene Society, Sapporo, Japan, 1975, p 252.
Norrestam, R.; Hovmoller, S.; Palm, T. B.; Gothe, R.; Wachtmeister, C. A. *Acta Crystallogr., Sect. B* **1977**, *B33*, 370.
Pohl, L. R.; George, J. W. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 367.
Schultzen, O.; Gräbe, C. *Arch. Anat. Physiol.* **1867**, 166.
Yasuo, K.; Fujimoto, S.; Katoh, M.; Kikuchi, Y.; Kada, T. *Mutat. Res.* **1978**, *58*, 143.

Received for review June 28, 1984. Accepted September 24, 1984. This study was sponsored by Occidental Chemical Corp., Hooker Industrial and Specialty Chemicals.