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2-Chlorotoluene Metabolism by Rats

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When rats were given a single oral dose of 2-chloro[U-*ring*-^{14}C]toluene at 1 mg/kg, 85–92 and 5–8% of the applied ^{14}C were excreted in urine and feces, respectively. The major urinary metabolites were a glycine conjugate of 2-chlorobenzoic acid, a β-glucuronide of 2-chlorobenzyl alcohol, and a mercapturic acid, representing 20–23, 35–42, and 21–28% of the urinary ^{14}C, respectively. Within 4 days after dosage virtually all of the administered 2-chlorotoluene and its ^{14}C residues had been eliminated from the rats (<1% of applied dose in carcass). No significant metabolic differences were found between males and females.

Monochlorotoluenes are prepared by the catalyzed ring chlorination of toluene. The 2-chloro isomer is used extensively as a solvent and as a chemical intermediate in the synthesis of pesticides, dyes, and pharmaceuticals (Gelfand, 1979). Since workers are exposed occupationally to 2-chlorotoluene and environmental contamination could result in even greater human exposure, knowledge of the metabolic fate of 2-chlorotoluene could be useful in assessing its toxicity and environmental impact. Hence, we used the rat as a model for metabolic fate analysis.

Methyl group oxidation is the major metabolic pathway for toluene and xylenes, resulting in benzyl alcohol, benzoic acid, hippuric acid, benzoyl glucuronide, and their methylated analogues (van Doorn et al., 1981). Although mercapturic acids are minor metabolites of toluene, *m*-, and *p*-xylene (<2%), these thioethers contribute 21% of the applied dose for *o*-xylene which is isosteric with 2-chlorotoluene (van Doorn et al., 1981).

There are only a few limited studies on the metabolic fate of 2-chlorotoluene. This compound appears to be environmentally labile since bacteria have been isolated from a landfill waste site that are able to use 2-chlorotoluene as a sole carbon source (Vandenbergh et al., 1981). As early as 1903 Hildebrandt (1903) reported that 2-chlorotoluene is converted to 2-chlorohippuric and 2-chlorobenzoic acids by dogs and rabbits, respectively. Callow and Hele (1926) studied the likelihood that 2-chlorotoluene was metabolized by the mercapturic acid pathway in dogs by monitoring neutral sulfur excretion; they concluded that it was not involved in this pathway. More recently Wold and Emmerson (1974) reported on the metabolic fate of 2-chlorotoluene in rats given a relatively high dose (320 mg/kg). We initiated this work as an extensive examination of the metabolic fate of 2-chlorotoluene in rats in order to supplement and amplify on the data of Wold and Emmerson (1974), which is published in abstract form only with few details.

EXPERIMENTAL SECTION

Analytical Methods. Thin-layer chromatography (TLC) was performed with precoated, silica gel GF plates (Analtech), and radioactive metabolites were detected with a radiochromatogram scanner (Packard Model 7201). A Spectra-Physics 8000 instrument was used for reversed-phase liquid chromatography (LC): 10-μm LiChrosorb RP-8 column, 0.46 × 25 cm; ultraviolet detection at 254

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nm; 35 °C; 1.6 mL/min. The following LC solvents contained either acetonitrile or methanol mixed with 0.1% acetic acid: SS 1 (25% methanol for 15 min, then linear gradient from 25 to 60% methanol over 10 min, isocratic at 60% methanol for 5 min, gradient 60–100% methanol over 5 min); SS 2 (15% methanol for 15 min, linear gradient 15–60% methanol over 5 min, isocratic 60% methanol for 5 min, gradient 60–100% methanol over 5 min); SS 3 (linear gradient 30–60% methanol over 20 min); SS 4 (40% acetonitrile); SS 5 (60% methanol). The following conditions were used for normal-phase LC: Haskel Model 28030 pump; Spectra-Physics Model 8200 UV detector; Zorbax SIL column, 25 × 0.46 cm, 10 μm; elution at 0.3–0.9 mL/min with pentane–ethyl acetate (40:60 for SS 6; 75:25 for SS 7).

^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra were determined by Dr. Robert L. Carney using a JEOL FX90Q Fourier transform spectrometer and are expressed in parts per million from Me_4Si as the standard in $CDCl_3$. Mass spectral analysis employed a Hewlett-Packard Model 5985A instrument in the electron impact (EI) mode at 70 eV or in the chemical ionization (CI) mode. Fast atom bombardment (FAB) ionization of the methylated glucuronide conjugate (**5**) used the same instrument fitted with a fast atom gun (Phrasor Scientific, Duarte, CA) with the sample dissolved in a glycerol matrix and then bombarded with argon atoms.

Radioactivity was quantified by liquid scintillation counting (LSC, Packard Model 2425 and 460 counters). Unextractable ^{14}C residues were quantified by combustion to $^{14}CO_2$ (Biological Oxidizer, Model OX-300, R. J. Harvey Instrument Co.) with collection in Carbon 14 Cocktail (Harvey Instrument Co.) followed by LSC.

Metabolic Standards. Authentic samples of 2-chlorotoluene (**1**), 2-chlorobenzoic acid (**2**), and 2-chlorobenzyl alcohol (**3**) were commercially available. The methylated, peracetylated glucuronide of **2** (i.e., **4**) was prepared in 81% yield from **2** and methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate by using previous methodology (Quistad et al., 1982). The methylated, peracetylated ether glucuronide (**5**) was synthesized in 15% yield from methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-D-glucopyranuronate (Quistad et al., 1982) and **3** with $CdCO_3$ in toluene (Goto et al., 1979).

Amino acid conjugates of 2-chlorobenzoic acid were prepared from the acid chloride of **2** and the respective methylated amino acid (Quistad et al., 1983). Thus, the glycine (**6**), glutamic acid (**7**), and alanine (**8**) conjugates of **2** were synthesized (as methyl esters) in 74, 22, and 36% yields, respectively. The free acids of these conjugates were produced by treatment with 1 M methanolic NaOH.

Radiosynthesis. 2-Chloro[U-*ring*-^{14}C]toluene was synthesized by Pathfinder Laboratories (St. Louis, MO) in 97.1% purity and 16.2 mCi/mmol specific activity. Since 2-chloro[^{14}C]toluene is quite volatile at low mass levels, further purification of the material presented technical difficulties in manipulation. A portion of the 2-chloro[^{14}C]toluene (8 mg, 1 mCi) in pentane (2 mL) was gently concentrated under a N_2 stream after addition of methanol (100 μL). When the sample reached about 100 μL, it was injected for reversed-phase LC purification (SS 5). Extraction of the appropriate eluate fraction with pentane gave 2-chloro[^{14}C]toluene in only 9% yield but 98.7% purity. Analysis by gas–liquid chromatography demonstrated the absence of meta and para isomers.

Treatment. Four female and four male albino rats (Sprague-Dawley, Simonsen Laboratories, Gilroy, CA) weighing 151–180 g were given a single oral dose of 2-chloro[U-*ring*-^{14}C]toluene at 1 mg/kg by gavage in corn oil (0.5 mL). Two additional female rats were treated similarly at 91 and 102 mg/kg. All animals had been fasted for 16 h before dosing and then were housed in all-glass metabolism chambers (Stanford Glassblowing Laboratories, Palo Alto, CA) for separate collection of urine, feces, expired $^{14}CO_2$, and expired organic ^{14}C. A 5% KOH solution was used to trap $^{14}CO_2$ and was monitored by LSC. Radiolabeled organic volatiles were trapped by passing an air stream from the metabolism chamber through Amberlite XAD-2 (ca. 16 g, Eastman). The Amberlite resin was extracted with methanol and dichloromethane, and then an aliquot was analyzed by LC (SS 1, $k' = 21.3$ for **1**).

Three additional male rats were dosed with 2-chloro-[^{14}C]toluene (1 mg/kg) as described. At various intervals blood was collected in heparinized tubes from the orbital sinus (Riley, 1960) and, after being cooled with ice, was centrifuged for 10 min. Aliquots (ca. 30 μL) of the resulting plasma were quantified by LSC.

Urine Analysis. The general method for urine analysis involved mild acidification (pH ~2) followed by reversed-phase LC (SS 2) to quantify the glycine conjugate (**6**, $k' = 3.9$), glucuronide (**5**, $k' = 9.8$), and mercapturic acid (**9**, $k' = 16.3$). The credibility of this procedure was verified by a preliminary separation of the three major components (i.e., **5**, **6**, and **9**) by TLC (ethyl acetate–methanol–acetic acid, 10:1:0.2) into two radioactive zones ($R_f = 0.26$ for **5** and $R_f = 0.50$ for **6** plus **9**). LC (SS 2) of the two TLC zones confirmed that the three major metabolites were separated effectively by LC of acidified urine and that no new metabolites were evident (<3% urinary ^{14}C).

A sufficient mass of each metabolite was isolated from the 0–24-h urine of a rat dosed at 102 mg/kg in order to permit verification of structural assignments by spectral methods. TLC separation (hexane–ethyl acetate, 1:1) of methylated (CH_2N_2) urine produced two radioactive zones ($R_f = 0$ and $R_f = 0.29$). The more mobile TLC zone at $R_f = 0.29$ was separated into methylated glycine conjugate (**6**, $k' = 5.3$) and mercapturic acid (**9**, $k' = 11.6$) by LC (SS 1). The methylated glycine conjugate (**6**) was identical with a synthetic standard by mass spectral analysis: m/z (rel intensity), EI, 229 (2.5, M^+ for ^{37}Cl), 227 (7.4, M^+ for ^{35}Cl), 195 (7), 168 (24), 141 (40), 139 (100), 111 (19); m/z (rel intensity), CI (CH_4), 268 (16, M + 41), 256 (40, M + 29), 230 (63, M + H, ^{37}Cl), 228 (100, M + H, ^{35}Cl), 196 (39), 194 (41). The methylated mercapturic acid (**9**) was purified further by normal-phase LC (SS 6, $k' = 3.3$) and gave the following spectral data: mass spectrum m/z (rel intensity), EI, 303 (1.0, M^+ for ^{37}Cl), 301 (3.5, M^+ for ^{35}Cl), 242 (21), 176 (49), 144 (14), 134 (23), 127 (29), 125 (100), 117 (46); mass spectrum m/z (rel intensity) CI (CH_4), 342 (8.5, M + 41), 330 (26, M + 29), 304 (48, M + H, ^{37}Cl), 302 (100, M + H, ^{35}Cl), 270 (18), 260 (33), 242 (24); ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3, CH_3CO), 2.97 (d, 2, CH_2, $J = 5$ Hz), 3.75 (s, 3, OCH_3), 3.83 (s, 2, CH_2), 4.84 (m, 1, CH_2CH), 6.49 (br s, 1, NH), 7.3 (m, 4, ar); ^{13}C NMR ($CDCl_3$) δ 171.1 and 170.4 (C=O), 135.4, 134.0, 130.8, 129.9, 128.8, 127.0 (ar), 52.8 and 51.9 (CH_3O and ar – CH_2S), 34.4 and 33.9 (SCH_2CH and CH_3CO), and 23.0 (CH_2CH).

The methylated glucuronide (**5**) was recovered from the origin zone after TLC (hexane–ethyl acetate, 1:1). Methylated **5** was acetylated (acetic anhydride–pyridine, 1:1) and then purified by TLC (ether–hexane, 4:1, $R_f = 0.5$), reversed-phase LC (SS 4, $k' = 11$), and normal-phase LC (SS 7, $k' = 5.3$). The methylated, peracetylated glucuronide (**5**) was identified from the following spectral data: mass spectrum m/z (rel intensity), CI (CH_4), 499 (0.4, M

+ 41), 487 (2.2, M + 29), 459 (0.2, M + H), 399 (9), 317 (76), 257 (100); mass spectrum m/z (rel intensity), EI, 279 (1.1), 245 (5), 231 (11), 198 (11), 155 (16), 143 (25), 139 (57), 127 (44), 125 (100); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 9, $COCH_3$), 3.77 (s, 3, OCH_3), 4.06 (d, 1, H at C-5 of pyranose, J = 7 Hz), 4.65 (d, 1, H at C-1 of pyranose, J = 7 Hz), 4.73 (d, 1, OCH_2, J = 13 Hz), 5.00 (d, 1, OCH_2, J = 13 Hz), 5.2 (m, 3, H at C-2, C-3, and C-4 of pyranose), 7.3 (m, 4, ar). Underivatized glucuronide **5** was isolated by TLC (ethyl acetate–methanol–acetic acid, 10:1:0.2; R_f = 0.26). Treatment of **5** with β-glucuronidase (*Helix pomatia*, Sigma) in citrate–phosphate buffer at pH 4.5 gave **3** in 51% yield as determined by reversed-phase LC (SS 1, k' = 10.5). When the methylated, peracetylated glucuronide of **5** was treated with β-glucuronidase under the same conditions as underivatized **5**, methylated **5** was recovered in 36% yield and purified by LC (SS 1) for spectral analysis: mass spectrum m/z (rel intensity), CI (isooctane), 333 (6, M + H), 315 (8, M + H – H_2O), 191 (100); FAB, 333 (4, M + H), 315 (6, M + H – H_2O); ^{1}H NMR ($CDCl_3$) δ ca. 3.7 (m, 3, C*H*OH at C-2, C-3, and C-4), 3.85 (s, 3, OCH_3), 4.13 (d, 1, C*H*OH at C-5, J = 7 Hz), 4.48 (d, 1, H at C-1 of pyranose, J = 7 Hz), 4.81 (d, 1, OCH_2, J = 12 Hz), 5.02 (d, 1, OCH_2, J = 12 Hz), 7.3 (m, 4, ar).

The glutamic acid and alanine conjugates (**7** and **8**) were isolated also from 0–24-h urine. Treatment of urine with CH_2N_2 and subsequent TLC (hexane–ethyl acetate, 1:1) gave a zone of ^{14}C metabolites at R_f = 0.3. LC analysis (SS 2) showed that the methylated glycine conjugate (**6**) was the major ^{14}C metabolite, but methylated **7** and **8** were recovered as trace components (k' = 16). Normal-phase LC (SS 6) separated methylated **7** and **8** from each other (k' = 2.0 and 1.0, respectively) and allowed spectral identification: for **7** (as dimethyl ester), mass spectrum m/z (rel intensity), EI, 313 (0.9, M^+ for ^{35}Cl), 281 (2), 254 (18), 174 (13), 139 (100), 111 (12); for **7** (as dimethyl ester), mass spectrum m/z (rel intensity), CI (CH_4), 354 (8, M + 41), 342 (23, M + 29), 314 (100, M + H), 139 (67); for **8** (as methyl ester), mass spectrum m/z (rel intensity), EI, 243 (2, M^+ for ^{37}Cl), 241 (4, M^+ for ^{35}Cl), 182 (46), 139 (100), 111 (15). Subsequent analysis of authentic standards confirmed these structural assignments.

Feces, Plasma, and Tissue Analysis. The feces were extracted with methanol. A portion of the extract was acidified (pH ~2) for analysis by reversed-phase LC (SS 2) as described for the urine. Unextractable ^{14}C was combusted to $^{14}CO_2$ for quantification.

Aliquots (30–60 μL) of the 1-h plasma were mixed with authentic standards (**1**, **2**, **3**, and **6**) for reversed-phase LC analysis in SS 1 (k' = 21.8, 16.5, 10.8, 6.3, and 2.3 for **1**, **9**, **3**, **2**, and **6**, respectively).

Selected organs and tissues were combusted for quantification of ^{14}C residues by LSC. The stomach and intestines, as well as the carcass remains, were extracted with methanol (2×) and chloroform (1×) prior to LSC.

RESULTS AND DISCUSSION

When rats were given a single oral dose of 2-chloro[U-*ring*-^{14}C]toluene at 1 mg/kg by gavage in corn oil, 85–92, 5–8, and 1–4% of the applied dose were eliminated in urine, in feces, and as volatile ^{14}C (Table I). At least 84% of the volatile ^{14}C was identified by LC analysis as unmetabolized 2-chloro[^{14}C]toluene. $^{14}CO_2$ was an insignificant metabolite (<1% applied dose), indicating relative stability of the aromatic ring to exhaustive metabolic degradation.

Excrement. Most of the administered 2-chloro[^{14}C]-toluene was extracted as three urinary metabolites [2-chlorohippurate (**6**), glucuronide (**5**), and mercapturic acid (**9**)]. Mild acidification of urine (pH ~2) allowed direct LC analysis of each of these metabolites in numerous urine samples (Table II). The β-glucuronide of 2-chlorobenzyl alcohol (i.e., **5**) was the single most abundant metabolite, representing about one-third of the applied ^{14}C. The β-anomeric structure for glucuronide **5** was assigned from the ^{1}H NMR spectra of methylated, peracetylated **5** and methylated **5** (δ 4.65 and 4.48, respectively, for the chemical shift of the hydrogen at C-1 of the pyranose). For both of these derivatized glucuronides the coupling constant for the C-1 hydrogen was 7 Hz, which suggests the presence of the β-anomer (vs. J = 4 Hz for an α-glucuronide). When underivatized **5** was treated with β-glucuronidase, a 51% yield of 2-chlorobenzyl alcohol (**3**) was obtained. Interestingly, when methylated, peracetylated **5** was treated with β-glucuronidase under the same conditions, the ether linkage was relatively stable, but the acetate moieties were hydrolyzed to produce methylated **5** in 36% yield. This result suggests (1) methylated, peracetylated **5** is a poor substrate for β-glucuronidase release of the aglycon and (2) β-glucuronidase from *H. pomatia* (Sigma) contains esterolytic enzymes. The structure of methylated **5** was verified by ^{1}H NMR and mass spectrometry (fast atom bombardment with argon and chemical ionization with isobutane).

Free 2-chlorobenzoic acid (**2**) was a minor metabolite (<1% of urinary ^{14}C), but **2** was conjugated with amino acids (Table II). As expected, glycine is the favored amino acid for conjugation. While 2-chlorohippurate (**6**) con-

Table I. Distribution of Radioactivity after Four Days for Rats Given Single Oral Doses of 2-Chloro[U-*ring*-^{14}C]-toluene by Gavage in Corn Oil

	% of applied dose		
	1 mg/kg		97 mg/kg,
	female[a]	male[a]	female[b]
urine	92 ± 7	85 ± 11	92.7
feces	5.3 ± 6	7.6 ± 5	7.2
methanol extract	4.6 ± 5	6.3 ± 5	6.5
residual solids	0.7 ± 0.6	1.3 ± 0.8	0.7
carcass	0.04 ± 0.06	0.8 ± 0.7	0.21
volatiles	1.4 ± 0.8	4.4	3.5
total recovery	98.7 ± 2	97.8 ± 8	103

[a] Mean ± standard deviation for four rats. [b] Mean for two rats that received 91 and 102 mg/kg, respectively.

Table II. Metabolites of 2-Chlorotoluene in One-Day Urine

	% of ^{14}C in urine[a]		
	1 mg/kg		102 mg/kg,
	females	males	female
2-chlorobenzoic acid			
free (**2**)	<1		
conjugates:			
glucuronide	<2		<1
glycine (**6**)	23 ± 6[b]	20 ± 4[b]	25
glutamic acid (**7**)	1.2		<1
alanine (**8**)	0.3		<1
2-chlorobenzyl alcohol			
free (**3**)	<1		<1
glucuronide (**5**)	42 ± 11[b]	35 ± 6[b]	22
mercapturic acid (**9**)	21 ± 12[b]	28 ± 12[b]	33
2-chlorotoluene (**1**)	<1		<1

[a] 90 ± 8, 81 ± 15, and 94% of applied ^{14}C excreted in 0–24-h urine for females at 1 mg/kg, males at 1 mg/kg, and female at 102 mg/kg, respectively. [b] Mean ± standard deviation for four rats. All other data for urine from a single rat.

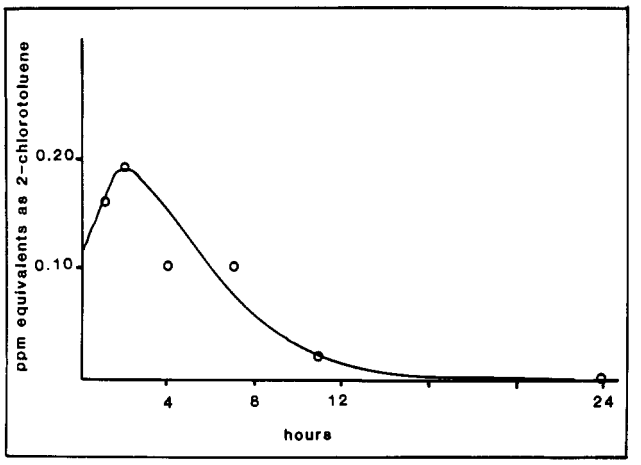

Figure 1. Profile of ^{14}C-labeled residues in blood plasma of rats given a single oral dose of 2-chloro[U-*ring*-^{14}C]toluene at 1 mg/kg.

tributed 20–23% of the urinary ^{14}C, radiolabeled hippuric acid was absent, which implies that the chlorine is not released from the aromatic ring by metabolic degradation. Lesser amounts of glutamic acid and alanine conjugates of **2** (i.e., **7** and **8**) were identified initially by mass spectrometry and then confirmed by synthesis of authentic standards. In contrast to the metabolism of toluene (van Doorn et al., 1981), the appropriately substituted benzoyl glucuronide was a minor metabolite (<2% of urinary ^{14}C). The absence of 2-chlorobenzoyl glucuronide also contradicts the generality of Caldwell (1978), who stated that ortho-substituted benzoic acids conjugate more with glucuronic acid than glycine because glucuronidation has broader substrate specificity than amino acid conjugation.

The third major metabolic pathway in rats involved mercapturic acid formation from 2-chlorotoluene. Since 2-chlorobenzyl alcohol is a major primary metabolite of 2-chlorotoluene, the production of a mercapturic acid is predictable from the work of Seutter-Berlage et al. (1982), who showed a 13% yield of mercapturic acid from 2-chlorobenzyl alcohol by rats. Ortho substitution of toluene seems to decrease the conversion of CH_3 to CO_2H with a concomitant increase in glutathione-mediated metabolites (Seutter-Berlage et al., 1982; van Doorn et al., 1980). The intermediacy of a sulfate ester in the formation of mercapturic acid, **9**, from **3** has been suggested (Seutter-Berlage et al., 1982; Clapp and Young, 1970; van Doorn et al., 1981) since the direct alkylation of the glutathione SH moiety by an alcohol (i.e., **3**) is less likely. The sulfate ester of **3** also gives the same mercapturic acid as **3** itself (Clapp and Young, 1970), which reinforces the notion of an activated intermediate for reactivity with glutathione. Such a sulfate ester would be a good alkylating agent, but van Doorn et al. (1981) have shown that the related sulfate of 2-methylbenzyl alcohol (isosteric with **3**) is not mutagenic in the Ames test. Comparison of the ^1H NMR spectra revealed that 2-chlorotoluene and 2-chlorobenzyl alcohol produce the same mercapturic acids [cf. Seutter-Berlage et al. (1982)]. The structure of this mercapturic acid was affirmed by its mass and ^{13}C NMR spectra. Hence, as with *o*-xylene (van Doorn et al., 1980), benzylic attack by glutathione appears to be favored over addition to the aromatic ring.

Less than 10% of the applied dose was excreted in feces. In general, the same three metabolites (**5**, **6**, and **9**) as were found in urine were also major fecal products (Table III). Although some contact between urine and feces occurs during collection, our experience indicates a negligible quantitative contribution from contamination of feces by urine. A small fraction of the applied 2-chlorotoluene (<2%) survived metabolic degradation and was excreted in feces.

Table III. Metabolites of 2-Chloro[^{14}C]toluene in Fecal Extracts (0–24 h) from Rats Given a Single Oral Dose at 1 mg/kg

	% of ^{14}C in feces extract	
	female[a]	male[b]
2-chlorohippurate (6)	18	9
2-chlorobenzyl alcohol		
free (3)	3	2
glucuronide (5)	24	5
mercapturic acid (9)	22	33
2-chlorotoluene (1)		10
volatile ^{14}C		20

[a] 9.7% of applied ^{14}C. [b] 3.7% of applied ^{14}C.

Table IV. Distribution of Radioactivity in Tissues of Rats Four Days after a Single Oral Dose of 2-Chloro[^{14}C]toluene by Gavage in Corn Oil

	ppb (equiv as 2-chlorotoluene)		
	1 mg/kg		97 mg/kg,
tissue	female[a]	male[b]	female[c]
spleen	0.1 ± 0.2	0.7	32
ovaries and fallopian tubes	<0.1		26
testes		0.2	
pancreas	0.2 ± 0.2	<0.1	16
lungs	0.5 ± 0.6	5	84
kidneys	0.7 ± 0.4	6	168
brain	0.5 ± 1	<0.1	1
heart	0.1 ± 0.1	<0.1	15
muscle—leg	0.1 ± 0.2	<0.1	13
muscle—pectoral	0.2 ± 0.3	<0.1	40
fat—abdominal	0.5 ± 1	<0.1	45
fat—pericardial	0.2 ± 0.3	6	22
liver	0.2 ± 0.3	0.4	82
hide	1 ± 2	11	258
stomach, intestines	0.3 ± 0.4	1 ± 1[a]	32
carcass remains	0.6 ± 0.9	11 ± 9[a]	235

[a] Mean ± standard deviation for four rats. [b] Single rat. [c] Mean for two rats that received 91 and 102 mg/kg, respectively.

Pharmacokinetics and Tissues. 2-Chlorotoluene is quickly absorbed from the gastrointestinal tract into blood as evidenced by exhalation of 2-chloro[^{14}C]toluene and the rapid peak in ^{14}C residues at ca. 2 h in blood plasma (Figure 1). Analysis of the ^{14}C residues in plasma near the peak for radiocarbon levels (1 h) showed that the two major radioactive components were mercapturic acid, **9**, and glucuronide, **5** (38 and 25% of plasma ^{14}C, respectively), while trace levels of **1**, **2**, **3**, and **6** were detectable also (4, 10, 2, and 3% of plasma ^{14}C, respectively).

Virtually all of the administered 2-chloro[^{14}C]toluene was eliminated within 4 days with <1% remaining in the carcass (Table I). Only traces of ^{14}C residues were found in tissues, and in most cases the radioactivity levels were close to the limit of detection (Table IV). Relatively higher ^{14}C residues were found in the hide, but even there the residue was <1 ppm at a high dose rate of 97 mg/kg.

Conclusions. 2-Chlorotoluene is rapidly absorbed and quantitatively eliminated by rats within 4 days after a single oral dose (Figure 2). No significant sex-related metabolic differences were noticed between males and females. The same qualitative and quantitative distribution of metabolites was found for doses of 1–100 mg/kg.

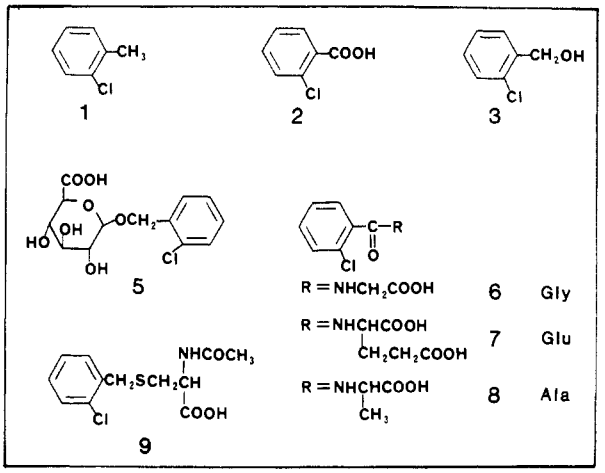

Figure 2. 2-Chlorotoluene and its metabolites from rats.

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Isolation and Identification of Novel Lactones from Male Mexican Fruit Flies

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Two isomeric γ-lactones were isolated from the male Mexican fruit fly, *Anastrepha ludens* (Loew), Diptera, Tephritidae, and characterized by ^1H and ^{13}C NMR, IR, MS, and X-ray analyses. The structures were [3a*R**-(3aα,4α,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3*H*)-benzofuranone [$C_{12}H_{18}O_2$, orthorhombic, $P2_12_12_1$, a = 7.448 (6) Å, b = 8.582 (4) Å, and c = 17.860 (9) Å] and [3a*R**-(3aα,4β,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3*H*)-benzofuranone [$C_{12}H_{18}O_2$, orthorhombic, $P2_12_12_1$, a = 6.389 (9) Å, b = 11.461 (15) Å, and c = 15.410 (21) Å]. X-ray data include final atomic coordinates, bond lengths, bond angles, and molecular numbering schemes. Two alcohols, (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol, were also isolated from the male Mexican fruit fly. These compounds are currently being evaluated for possible pheromonal activity.

Various fruit fly species can cause heavy production losses of cultivated varieties of fruit crops. The Mexican fruit fly, *Anastrepha ludens* (Loew), is responsible for a 10% loss in the annual citrus crop and a 5% loss to other food crops in Mexico. Although this pest is located predominantly in Mexico, trapping along the U.S.–Mexican border has shown the seasonal migration of this pest into citrus groves located in the southwestern United States (Baker et al., 1944).

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Currently, a protein lure is used as an attractant for assessing Mexican fruit fly infestation. However, a more potent attractant such as a sex pheromone would be advantageous for assessing or controlling infestations. Aguirre (1974) and Steer (1975) suggested that the male Mexican fruit fly attracts the female of this species by a sexual lure. With their work in mind, Gaxiola (1977) isolated four compounds from the male Mexican fruit fly. He identified two nine-carbon unsaturated alcohols, (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol, and proposed structures for two fused cyclohexyl lactones.

Our study was carried out to determine the chemical structures of these lactones from the male Mexican fruit fly.

EXPERIMENTAL SECTION

Materials. Mexican fruit fly male abdomens (317 000) in 95% ethanol were supplied by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service