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DATA EVALUATION RECORD

FENVALERATE

Metabolism Study in Rats

STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of [^{14}C]-phenoxyphenyl-SD 92459 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-023-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254118.

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Date: 8-14-85

1. CHEMICAL: SD 92459, Pydrin Y Rich, insecticide, benzeneacetic acid, (R*,R*-(±)-)-4-chloro-α-(1 methylethyl)-, (S*,S*-(±)-)-cyano-(3-phenoxyphenyl) methyl ester.

2. TEST MATERIAL: The test material was [^{14}C]-phenoxyphenyl]-SD 92459 with a specific activity of 24.5 μCi/mg and a radiochemical purity greater than 99.5 percent. The ratio of x/y isomers was 5/95. A stock solution of [^{14}C]-phenoxyphenyl]-SD 92459 in dichloromethane was stored at -4°C prior to use.

3. STUDY/ACTION TYPE: Metabolism Study in Rats.

4. STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of [^{14}C]-phenoxyphenyl-SD 92459 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-023-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254118.

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7. CONCLUSIONS:

When Pydrin Y rich (x:y isomer being 5:95) [^{14}C]-phenoxyphenyl-SD 92459 was administered to male and female rats in a single dose at 8.4 mg/kg, radioactive material was rapidly eliminated in the feces and urine; the majority of the radioactivity was eliminated in 24 hours. [^{14}C]O_2 was not detected in the expired air of the treated animals. There was no observable difference in the total elimination of SD 92459 between male and female test animals. The major route of elimination in both the male and female test animals was by fecal excretion. The quantities of the recovered SD 92459 metabolites varied among individual test animals. There was no apparent sex difference in the chemical nature of these metabolic products. The primary urinary metabolic pathway was the cleavage of the ester linkage of SD 92459 which subsequently underwent further oxidation and conjugation. The alcohol moiety of SD 92459 was rapidly excreted as free molecules or as glucuronide or sulfate conjugates. SD 44607 and SD 46114 were identified as the primary urinary degradation products. SD 48838, and SD 46114 were identified as the primary fecal degradation products. Undegraded SD 92459 (approximately 34 percent of the applied radioactivity) was recovered in the fecal excreta. Tissue residue distribution data indicated the lack of bioconcentration of the SD 92459 equivalent residues in the blood, lung, heart, kidney, gonad, muscle, and brain tissue of the test animals. Significant levels of the SD 92459 equivalent residues were detected in liver (approximately 0.13 ppm) and fat tissues (approximately 2.00 and 1.70 ppm in the male and female test animals, respectively). Further analysis indicated that approximately 9-16 percent of the total residues in the body fat were undegraded SD 92459, the remaining radiolabeled materials were primarily water-soluble materials and unextractable bound residues.

Under the conditions in which the study was conducted, it is acceptable.

Items 8 through 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test animals were male and female Sprague-Dawley rats (Simonsen's Laboratories, Gilroy, CA), 7 weeks of age, and weighing 175-200 g.

2. Control groups of 5 males and 5 females received a single dose of 5 ml/kg corn oil and were maintained to monitor basic physiological parameters under sham conditions of the experimental groups. Four/sex were maintained in Nalgene metabolism cages and one/sex in Stanford all glass metabolism cages. Daily food and water consumption and urine and feces elimination were measured.

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[1] Only items appropriate to this DER have been included.

3. Treatment group 1 consisted of 1 male and 1 female given single oral doses of 8.4 mg/kg ^{14}[C]-phenoxyphenyl-SD 92459. The animals were housed for two days in Stanford metabolism cages to monitor for radioactivity expired as $[^{14}C]O_2$ and/or excreted in urine and feces.

4. Treatment group 2 consisted of 5 rats of each sex given a single oral dose of 8.4 mg/kg test material and maintained individually for 5 days in Nalgene metabolism cages with slightly modified feeders that eliminated food contamination of collected urine and feces. Food and water intake were measured daily and urine and feces collected daily.

5. Urine volume was measured, the volume then brought to 25 ml with a 0.01 M phosphate buffer (pH 7.2) and aliquots radioassayed in triplicate by a liquid scintillation counter (LSC). For metabolite identification, 15 ml aliquots of urine samples for days 1 and 2 were combined, adjusted to pH 3 with 1 ml 6N HCl and extracted 3 times with equal volumes of ethyl acetate. The resulting aqueous phase was adjusted to pH 5.0, enzymatically hydrolyzed with sulfatase and β-glucuronidase, and then extracted 3 times with equal volumes of ethyl acetate. If more than 5 percent of the initial radioactivity remained in the aqueous phase, the sample was subjected to acid hydrolysis for 4 hours at 90°C at pH 1.0 and then extracted with solvent. The organic phases were concentrated and subjected to thin layer chromatography (TLC).

6. Feces were weighed wet then freeze dried and reweighed. Radioactivity was determined by LSC after combusting a 100 mg sample. For identification of metabolites, 3 g samples of feces collected on days 1 and 2 were combined, extracted 3 times with 30 ml MeOH:H_2O (9:1) and an aliquot of the extract radioassayed to determine total radioactivity. The extracts were concentrated, adjusted to pH 7.4 with 0.01 M phosphate buffer, extracted 3 times with equal volumes of ethyl acetate, and the organic phase brought to dryness. If the aqueous phases contained greater than 5 percent of the radioactivity, they were hydrolyzed at pH 1.0 and 90°C for 4 hours and extracted with ethyl acetate. The organic phases were subjected to two-dimensional TLC.

   Because of the possible low level of radioactivity (the residual level of SD 92459 equivalent) residues in the fecal excreta and other biological tissues associated with these samples (1.5 to 2 times above the background level), the normal background in each of the control urine, fecal and tissue samples were individually determined in order to establish a valid limit and sensitivity of detection. From these control data, a 99 percent upper confidence limit (UCL) was calculated. This 99 percent UCL was chosen as the concentration value that a treated tissue must exceed to represent a significant radioactive residue.

7. Animals were sacrificed at day 5, 2 ml blood collected in EDTA, and the following tissues trimmed and weighed: lung, liver, heart, kidneys, gonads, inguinal and back fat, muscle, and brain. Radioactivity was determined in triplicate 100 mg subsamples by combustion and LSC.

8. Radioactivity was determined for liquid samples in Aquasol-2, and for CO_2 from combusted samples in Carbosorb-Permafluor by liquid scintillation counting. Quench correction was determined monthly, and efficiency was determined by the external standard counting method. Combustion efficiency was determined on tissue samples of control animals to which an internal standard ^{14}[C]-test material was added.

9. Thin layer chromatography solvents were: hexane/acetone/HOAc (25/25/1) and toluene/ether/HOAc (75/25/1). Reference standards were visualized with UV light and ^{14}[C]-areas located by a Beta scanner and confirmed with autoradiography. Capillary GLC was performed with a Varian chromatograph equipped with an electron capture detector and radio-gas-liquid chromatography used a flame ionization detector and a Packard gas proportional counter. Mass spectrometry used the electron impact mode.

B. Protocol:

Materials and Methods are given in Appendix A in lieu of protocol.

12. REPORTED RESULTS:

A. Determination of the Limit and Sensitivity of Selection:

The combustion efficiency on all tissues ranged from 90-96 percent. The sensitivity level of detection for all control biological tissues ranged from 0.006 to 0.010 ppm. These findings indicate the uniformity of the test samples and the efficiency of the analytical procedures used in this study.

Based on the results obtained from the control treatment group, the specific activity of the SD 92459 treatment solution was adjusted to approximately 5 µCi/mg to allow the detection of a significant level of 0.5 to 1.0 percent of the applied radioactivity.

B. Preliminary All-Glass Metabolism Chamber Study: By monitoring an ethanolamine carbon dioxide trap at the 24 and 48 hour post treatment time intervals for the one male and one female dosed rats, it was determined that no [^{14}C]-carbon dioxide or other volatile radioactive material was generated in the respired air of the treated animals. Urine and feces from these two animals were not analyzed further.

C. Animal Physiological Parameters: Under the experimental conditions of the study, there were no observable behavioral or toxicological abnormalities among the control and treated animals. There were also no differences in body weight gain, water and food intake, or fecal and urine excretion.

D. Urine and Feces Excretion Rate Profile (Treatment Group II):

Most of the applied radioactivity in the urine was recovered from the test animals during the initial 24 hours post treatment (Table 1); males, approximately 34 percent (range 31-38 percent); females, 31 percent (range 12-51 percent). A large variation was observed in the urinary elimination rate of the administered radioactivity among individual females. Radioactivity recovered in the day-2 urine accounted for approximately 1-6 percent of the administrated dose in the males and females. Approximately 2-4 percent of the radioactivity was recovered in the combined day 3, 4, and 5 urine. The total radioactivities detected in the urine of dosed male and female animals were approximately 39 percent (range 36-42 percent) and 36 percent (range 14-60 percent) of the administered dose, respectively.

In the feces, approximately 64 percent (range 56-67 percent) and 59 percent (range 38-85 percent) of the total applied radioactivity was recovered in the males and females respectively, during the initial 24 hours after treatment. Radioactivity recovered in day 2 feces accounted for about 2-3 percent of the dose in males and females. About 1-2 percent of the radioactivity was recovered in the combined day 3, 4, and 5 fecal excreta. The total radioactivities detected in the entire feces in the male and female test animals was approximately 66 percent (range 60-74 percent) and 63 percent (range 43-87 percent), respectively.

The total recovered radioactivity from the urine and feces of dosed male and female animals was approximately 105 percent (range 99-112 percent) and 98 percent (range 89-106 percent), respectively at the end of the day-5 holding period.

E. Distribution of Urinary Metabolites: In males, approximately 36.9 ± 2.5 percent of the administered radioactivity was eliminated in the urine during the initial 48 hours (combined day 1 and day 2 urinary excreta). Approximately 4.5 ± 1.5 percent of the administered radioactivity (i.e., 12 percent of the recovered radioactivity in the urine) was extractable by the ethyl acetate. Of the remaining radioactivity, 29.4 ± 3.5 percent (80 percent) was water-soluble materials recovered by organic solvent extraction after enzyme hydrolysis with sulfatase and β-glucuronidase. About 2.9 percent of the administered radioactivity remained in the aqueous fraction after the initial organic solvent extraction and enzyme hydrolysis. This aqueous fraction was not further analyzed.

TABLE 1. Distribution of the Applied [^{14}C]-Phenoxyphenyl-SD 92459 Equivalent Radioactivity in the Urine and Feces of Male and Female Animals Following a Single Oral Administration of 8.4 mg/kg of the Test Compound

	Percent Recovery of the Administered Radioactivity on Days [a]					
	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Urine						
Male	34.4 ± 2.6	2.5 ± 0.3	0.8 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	38.6 ± 2.4
Female	31.3 ± 17.6	3.1 ± 2.0	0.9 ± 0.5	0.5 ± 0.2	0.5 ± 0.2	36.2 ± 20.1
Feces						
Male	63.6[b] ± 7.3	2.4[c] ± 0.9	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	66.3 ± 6.4
Female	58.8 ± 20.9	2.7 ± 1.7	0.3 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	62.2 ± 19.3

[a] Mean value ± standard deviation.

[b] Includes one value that is a combination of day 1 and day 2 excreta samples.

[c] Mean value based on 4 animals.

Undegraded SD 92459 was not detected in the ethyl acetate extract of urine. The following degradation products of [^{14}C]-phenoxy-phenyl SD 92459 were detected: SD 44607 (1-3 percent of the applied radioactivity), SD 46114 (approximately 1 percent) and several other unidentified minor urinary degradation products (Table 2; Figure 1).

Analysis of water-soluble conjugates after enzyme hydrolysis with sulfatase and β-glucuronidase indicated that SD 46114 (23-33 percent of the administered radioactivity) was the major SD 92459 metabolite in males. There were also several other unidentified minor urinary metabolites.

Radioactive material remaining in the aqueous phase after the initial ethyl acetate extraction and enzyme hydrolysis was not analyzed further.

In females, approximately 36.4 ± 18.8 percent of the administered radioactivity was eliminated in the urine during the initial 48 hours. Approximately 6.9 ± 2.9 percent of the administered radioactivity (or 19 percent of the recovered radioactivity in the urine) was recovered by ethyl acetate extraction. SD 44607 (1-3 percent of the applied radioactivity) and SD 46114 (1-4 percent) were identified as the primary degradation products of SD 92459. There were several additional unidentified minor metabolites observed (Table 2; Figure 1).

There was no qualitative difference in the chemical nature or distribution of SD 92459 urinary metabolites between the male and females. Analysis of water-soluble conjugates indicated that SD 46114 (8-44 percent of the administered radioactivity) was the major degradation product of SD 92459. There were also several unidentified minor metabolites.

F. Distribution of Fecal Metabolites: In males, approximately 48.8 ± 10.2 percent of the administered radioactivity (or 75 percent of the recovered radioactivity in the combined day 1 and day 2 feces) was extractable by the methanol-water (9:1) solvent system. The remaining 16.7 ± 6.2 percent of the administered radioactivity (or 25 percent of the recovered radioactivity) was unextractable residues, and no attempt was made to analyze them further.

The methanol-water extract was then partitioned with ethyl acetate. The majority of the extractable residues were recovered in the ethyl acetate phase with less than 6 percent of the administered radioactivity remaining in the aqueous fraction as water-soluble materials.

The SD 92459 metabolites in the ethyl acetate extract were identified by TLC and mass spectral analysis. In addition to the undegraded SD 92459, SD 44838 (2-3 percent of the administered

TABLE 2. Distribution of ^{14}[C]-Phenoxyphenyl-SD 92459 Metabolites in the Urine of Male and Female Animals

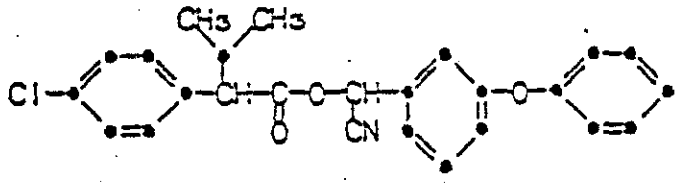
	Percent Recovery of Administered Radioactivity	
	Male	Female
Organic Extractable Fraction		
SD 44607	2.2 ± 0.9[b]	1.9 ± 0.9
SD 46114	1.1 ± 0.2	2.8 ± 1.1
Other	1.2 ± 0.5	2.2 ± 1.1
Total	4.5 ± 1.5	6.9 ± 2.9[c]
Water Soluble Fraction		
SD 46114	27.5±3.7	25.2±15.5
Other[a]	1.9±0.3	1.7± 0.5
Unextractable	2.9±0.5	2.6± 1.0
Total	32.4±3.0	29.6±16.6[c]

[a] Radioactivity associated with the origin of the TLC plate and other minor metabolites.

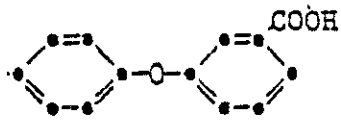
[b] Mean value ± standard deviation.

[c] The total radioactivity found in both the organic and aqueous phases in females is 36.5 percent of the administered dose, which is slightly higher than that reported earlier (34.4 percent), and may represent the total radioactivity found in the 5-day urine rather than the initial 2 days.

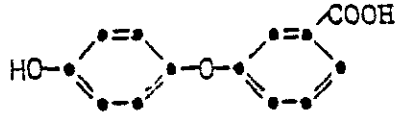
TABLE OF COMPOUNDS



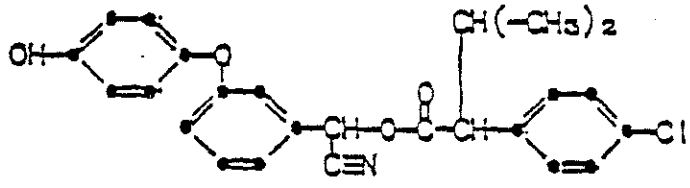
SD 43775: Benzeneacetic acid, 4-chloro--α-(1-methylethyl)-, cyano-(3-phenoxyphenyl)methyl ester.



SD 44607: 3-Phenoxy-benzoic acid



SD 46114: 3-(4-Hydroxyphenoxy)-benzoic acid



SD 48838: Benzeneacetic acid, 4--chloro-α-(1-methylethyl)-, cyano--(3-phenoxy-4-hydroxyphenyl)methyl ester.

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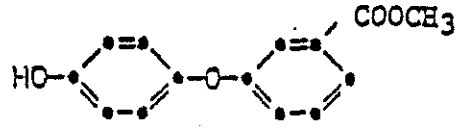
TABLE OF COMPOUNDS



SD 48389: 3-(4-Hydroxyphenoxy)-benzoic acid methyl ester

radioactivity), SD 44607 (1-2 percent) and SD 46114 (1-3 percent) were identified as the primary degradation products (Table 3; Figure 1). Undegraded SD 92459 accounted for approximately 31.9 ± 11.7 percent of the administered radioactivity (ranging from 20-51 percent). There were 4-5 other unidentified minor metabolites observed.

In females, approximately 54.0 ± 23.3 percent of the administered radioactivity (or 87 percent of the radioactivity recovered in the combined day 1 and day 2 feces) was extractable by the methanol-water (9:1) solvent system. The remaining 7.8 ± 5.3 percent of the administered radioactivity (13 percent of the recovered radioactivity) was unextractable residues, and no attempt was made to analyze them further.

The methanol-water extract was then partitioned with ethyl acetate. Most of the radioactivity was recovered in the ethyl acetate phase with less than 6 percent of the radioactivity remaining in the aqueous fraction. There were no observable qualitative differences in the chemical nature of the SD 92459 fecal metabolites between male and female animals. Undegraded SD 92459 accounted for 34.9 ± 27.1 percent of the administered radioactivity (range from 12-72 percent), and SD 48838 (2-4 percent), SD 44607 (1-2 percent), and SD 46114 (2-5 percent) were identified as the primary fecal metabolites. There were 4-5 other unidentified minor metabolites observed (Table 3; Figure 1).

G. Identification of Urinary Metabolites: The metabolic degradation pattern of the alcohol moiety of SD 43775 (the S,R-isomer, Figure 1) in the rat was examined using ^{14}[C]-phenoxyphenyl-SD 43775 and ^{14}[C]-phenoxyphenyl-SD 92459. TLC autoradiograms indicated that there was no qualitative difference in the urinary metabolic degradation pattern between SD 43775 and SD 92459. SD 44607, SD 46114, and 4-8 other unknown minor radiolabeled products were observed (Figure 1). The chemical identities of these degradation products had been confirmed by GC-mass spectral analysis and were reported in the ^{14}[C]-phenoxyphenyl-SD 43775 metabolism study (RIR-22-020-080).

H. Identification of Fecal Metabolites: The metabolic degradation pattern of the alcohol moiety of SD 43775 in the rat was examined using ^{14}[C]-phenoxyphenyl-SD 43775 and ^{14}[C]-phenoxyphenyl-SD 92459. TLC autoradiograms indicated that there was no qualitative difference in the fecal metabolic degradation pattern between SD 43775 and SD 92459. SD 48838, SD 44607, SD 46114, and 4-8 other unknown minor radiolabeled products were observed (Figure 1). Undegraded SD 43775 and SD 92459 were also recovered in the fecal excreta of the treated animals. The chemical identities of these degradation products had been confirmed by GC-mass spectral analysis and were reported in the ^{14}[C]-phenoxyphenyl-SD 43775 metabolism study.

TABLE 3. Distribution of ^{14}[C]-Phenoxyphenyl-SD 92459 Fecal Metabolites in Male and Female Rats

	Percent Recovery of Administered Radioactivity	
	Male	Female
$CH_3OH - H_2O$ Extractable	48.8±10.2	53.98±23.2
Organic Extractable	44.4±10.2	48.6 ±24.0
SD 92459	31.9±11.7	34.9 ±27.1
SD 48838	2.4± 0.6	2.5 ± 0.8
SD 44607	1.1± 0.4	0.9 ± 0.3
SD 46114	1.9± 0.7	3.6 ± 1.2
Others[a]	7.0± 2.4	6.6 ± 2.0
Water-soluble Conjugates	5.9± 1.2	5.4 ± 1.7
Unextractable Residues	16.7± 6.2	7.8 ± 5.3
Total	65.5± 6.4	61.8 ±19.2

[a] Radioactivity remaining in the origin of TLC plate and minor metabolites.

I. Tissue Distribution of SD 92459 Equivalent Residues:

A summary of the distribution of SD 92459 equivalent residues in various organ tissues of male and female test animals is presented in Table 4 of the report. The concentrations of SD 92459 equivalent residues detected in blood, lung, heart, gonads, and muscle were slightly above the level of detection. In the liver, where the SD 92459 equivalent residues were approximately three times the upper limit of detection, their distributions in whole tissue, the soluble fraction or the proteinaceous fraction were determined. The data showed that 30 percent of the residues were present in the soluble fraction and that the remaining 70 percent of the equivalent residues were present in the proteinaceous fraction. The SD 92459 equivalent residues detected in the kidney were approximately two times the upper limit of detection.

The SD 92459-equivalent residues in the fat were 40-50 times the upper limit of detection. The distribution of radiolabel residues was determined in whole tissue, in the hexane extract following homogenization, and in the non-extractable residues. In males, approximately 60-85 percent of the radioactivity associated with fat tissues was extracted by hexane, and approximately 15-20 percent of the extractable residues (or about 9-16 percent of the total fat residues) (0.2-0.4 ppm) was identified as unchanged SD 92459. In females, approximately 37-85 percent of the radioactivity associated with fat tissues was extracted and approximately 15-17 percent of the extractable residues or about (6-13 percent of the total fat residues) (0.15-0.23 ppm) was unchanged SD 92459. The level of SD 92459-equivalent residues in the brain were below the limit of detection.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. After a single oral dose of 8.4 mg/kg [^{14}C]-phenoxyphenyl-SD 92459 was administered to male and female rats, there was rapid elimination of radioactivity in the urine and feces. Most of the radioactivity was eliminated in the first 24 hours, and no exhaled radioactivity was detected. Excretion was similar in males and females. The major route of elimination was the feces.

The examination of urinary metabolites by chromatography indicated metabolic cleavage of the ester linkage in the parent compound followed by further oxidation and conjugation. The alcohol moiety of SD 92459 was rapidly excreted as free molecules or as glucuronide or sulfate conjugates. SD 44607 and SD 46114 were the primary urinary degradation products; SD 48838 and SD 46114 were the primary fecal products. Approximately 34 percent of the administered radioactivity was recovered as fecal SD 92459.

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TABLE 4. A Summary of the Residue Level of ^{14}[C]-Phenoxyphenyl-SD 92459 Equivalent in the Various Organ Tissues of Male and Female Test Animals[a]

	µg SD 92459 Equivalent/ gram wet tissue (ppm)	
	Male	Female
Blood	**0.067±0.004**	**0.075±0.022**
Lung	0.061±0.111	0.061±0.009[b]
Heart	0.046±0.003	0.051±0.009
Liver	0.127±0.011	0.129±0.046
Kidney	0.078±0.009	0.097±0.035
Fat	2.002±0.319	1.698±0.825
Gonad	0.074±0.026[b]	0.331±0.078
Muscle	0.071±0.036	0.047±0.007[c]
Brain	BLD[e]	0.041±0.0[d]

[a] 5 male and 5 female test animals.

[b] Data obtained from 4 test animals (1 animal was below the limit of detection).

[c] Data obtained from 3 test animals (2 animals were below the limit of detection.

[d] Data obtained from 2 test animals (3 animals were below the limit of detection).

[e] Below the limit of detection.

Tissue residue distribution data showed a lack of bioconcentration of radioactive residues in blood, lung, heart, kidney, gonad, muscle, and brain. Detectable levels of radioactive residues were found in the liver (approximately 0.13 ppm equivalents) and fat (2.00 and 1.70 ppm equivalents/g in males and females, respectively). Approximately 9-16 percent of the total residues in body fat were undegraded SD 92459. The remaining labeled materials in the fat were water soluble materials and unextractable bound residues.

B. A quality assurance statement was not included in the report. However, this study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guidelines.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors' conclusions were supported by individual animal data. The protocol was adequate to determine the metabolism, tissue distribution, and excretion of the test material and its metabolites, and the study was well conducted and reported; we assess that the study was acceptable for showing the metabolism of SD 92459. Although there was variability among animals in percent of radioactivity in the various fractions or metabolites, this is not unusual in metabolism studies, and sufficient animals were used to calculate mean values for all parameters. The data indicate rapid elimination of radiolabeled material in urine and feces with most of the radioactivity being eliminated during the first 24 hours. The compound is hydrolyzed at the ester linkage followed by oxidation and/or conjugation and elimination in the urine; the alcohol moiety is also excreted as free molecules. Several metabolites were also found in the feces, but the undegraded parent compound was the major component. Tissue residue were very low except for the liver (0.13 ppm) and fat tissues (1.9 ppm) which were relatively higher. About 9-16 percent of the total residues in fat were the parent compound. There were no major differences in the metabolic pattern among females and males.

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 4-19.

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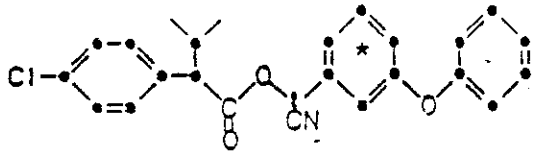
APPENDIX A

Materials and Methods

III. Description of the Conduct of Experiment

A. Test Compound

SD 43775 labeled with carbon-14 at the phenoxyphenyl-postion was used for the separation of SD 92459.



SD 43775

*Denotes carbon-14.

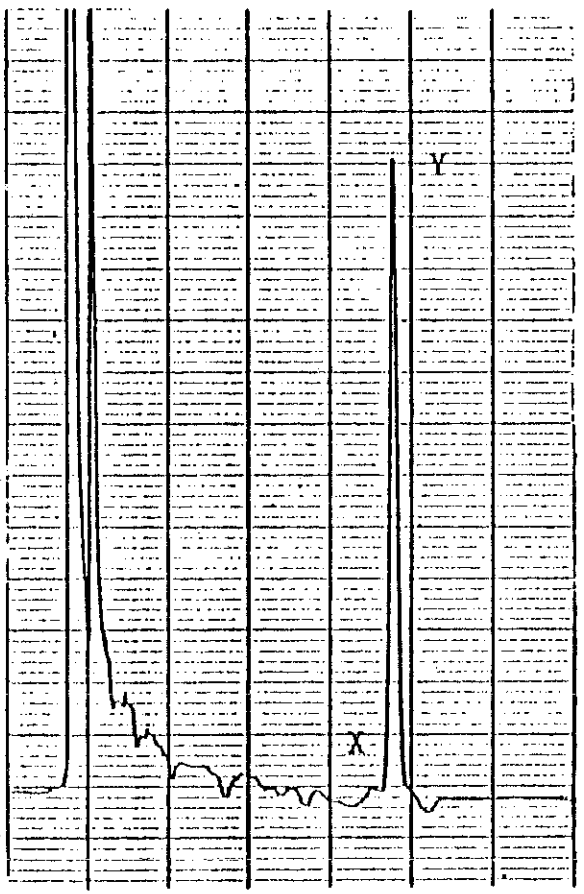
The specific activity of the ^{14}C-phenoxyphenyl-SD 43775 was not determined prior to the chromatographic separation of ^{14}C-phenoxyphenyl-SD 92459. Two different preparations of ^{14}C-phenoxyphenyl-SD 43775 were used in the rat metabolism study (RIR-22-020-80, specific activity 52.7 microCuries/mg) and for the preparation of ^{14}C-phenoxyphenyl-SD 92459.

^{14}C-phenoxyphenyl-SD 92459 was prepared by the TLC preparative separation of ^{14}C-phenoxyphenyl SD 43775. ^{14}C-phenoxyphenyl-SD 43775 (82 mg, 2887 microCuries) was applied onto a 1.0mm preparative silica gel TLC plate and developed six consecutive times in the hexane-tetrahydrofuran (97:3) solvent system. SD 43775 was separated into two close bands and the bottom band corresponded to the SD 92459. The yield of ^{14}C-phenoxyphenyl-SD 92459 was 824 microCuries. The specific activity of ^{14}C-phenoxyphenyl-SD 92459 was 24.5 microCuries/mg as determined by capillary gas-liquid chromatography (GLC) and liquid scintillation counting (LSC). A detailed calculation of the specific activity of ^{14}C-phenoxyphenyl-SD 92459 is presented in Appendix II and III. The X/Y isomeric ratio of ^{14}C-phenoxyphenyl-SD 92459 was 5/95 as determined by capillary GLC (Figure 1). The radiochemical purity of ^{14}C-phenoxyphenyl-SD 92459 is greater than 99.5% as determined by autoradiography of two-dimensional TLC (Figure 2) and LSC. Stock solution of ^{14}C-phenoxyphenyl-SD 92459 in dichloromethane is stored at -4°C prior use.

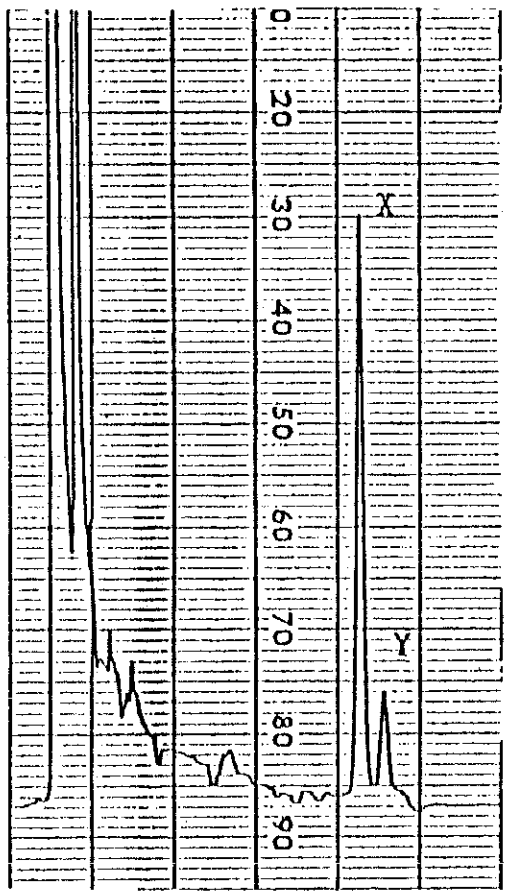
B. Test Animals

Male and female Sprague-Dawley albino rats (SIM:SD f1 strain, 7 weeks old, weighing from 175-200 grams each) were obtained from Simonsen's Laboratories, Gilroy, California. The number of total test animals, their experimental identification number, sexes, arrival date, initial body weight, experimental treatment date and their corresponding body weights are summarized in the Animal Resource Record (Appendix IV).

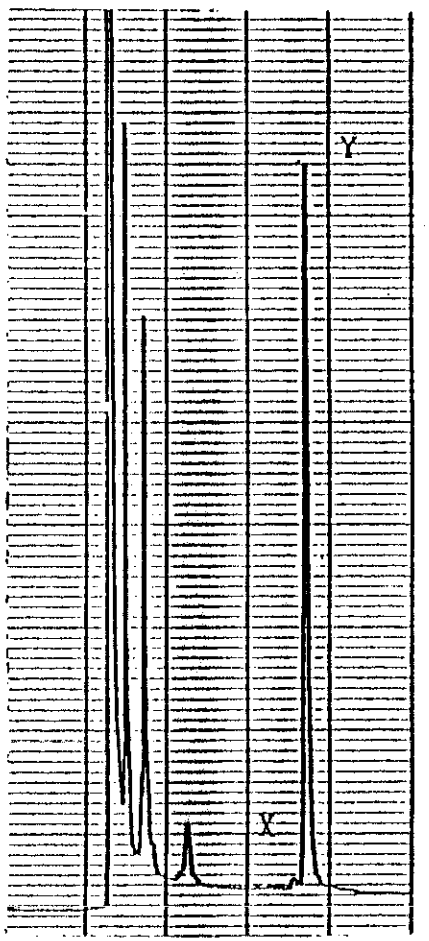
Figure 1 Isomeric composition of SD 51142 (PYDRIN-Y), SD 51143 (PYDRIN-X) and ^{14}C-phenoxyphenyl-SD 92459 (PYDRIN-Y rich)



SD 51142 X/Y=1/99



SD 51143 X/Y=85:15



^{14}C-phenoxyphenyl-
SD 92459 X/Y=5:95

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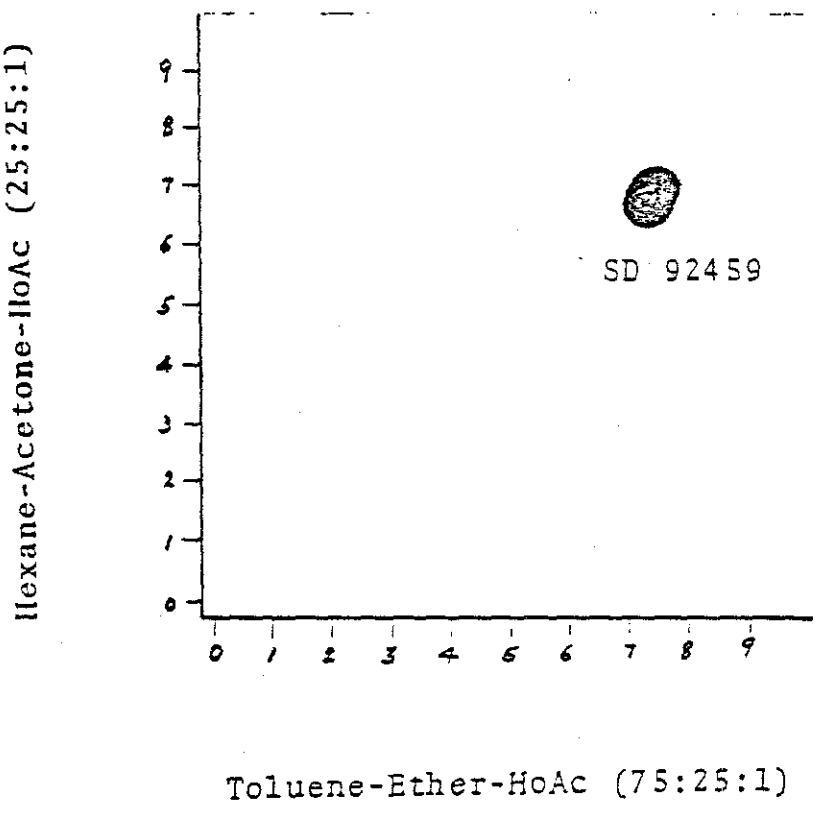
Figure 2 Autoradiogram of ^{14}C-phenoxyphenyl-SD 92459 used in this study



C. Test Animals Housing and Caring

All newly received animals were identified by ear code, weighed an housed individually in a suspended cage system (Lab Products, Rochelle Park, NJ equipped with an automatic water feeding system (System Engineering, Napa, CA and were given free access to food (Purina Rodent Chow) and water. The chemica analysis of the lab chows and the water used in this study are presented i Appendix V A and B. The physiological conditions and the behavioral patterns o the test animals were observed and recorded twice daily. Daily observation were recorded in the raw data file submitted for storage at BSRC Chemica Archives under Protocol No. PPL-22-009-80. The daily food intake, wate consumption, urine volume and feces weight for each experimental animal wer recorded and presented in the Animal Physiological Record (Appendix VI).

D. Route of Administration

^{14}C-phenoxyphenyl-SD 92459 was formulated in corn oil immediatel prior to treatment and the appropriate dose (8.4 mg/kg) was administrated to th test animals by stomach intubation at a constant volume dose of 5 ml/kg ^{14}C-phenoxyphenyl-SD 92459 treatment solution (30 mg) was prepared by dilutin ^{14}C-phenoxyphenyl-SD 92459 stock solution (6.4 mg, X/Y ratio 5/95, specifi activity 24.5 microCuries/mg) with unlabeled SD 51142 (PYDRIN® Y, 22.0 mg, X/ ratio 1/99, Figure 1) and SD 51143 (PYDRIN® X, 1.6 mg, X/Y ratio 85/15, Figur 1) in the final volume of 17.8 ml of corn oil. The final concentration o SD 92459 in this treatment solution was 1.68 mg/ml and had the final specific activity of 5.0 microCuries/mg. The X/Y ratio of the ^{14}C-phenoxyphenyl-SD 9245 treatment compound was approximately 10/90 as determined by capillary GL (Figure 3). The level of specific activity of ^{14}C-phenoxyphenyl-SD 9245 allowed the the detection of SD 92459 equivalent residues in the animal tissue at the level of 0.5 to 1% of the applied radioactivity. Animals were fasted fo 16 hours prior to dosing with free access to water. Control animals were treated with corn oil only. The actual dose of ^{14}C-phenoxyphenyl-SD 92459 for each individual animal received is summarized and presented in the Animal Physiological Record (Appendix VI). Food was returned to all control and treated animals one hour after dosing.

E. Animal Treatment Groups

Experimental animals were organized into the following treatment groups.

1. Control Treatment Groups

Five male and 5 female (80R001-80R010) (single oral, corn oil, 5 ml/kg). Animals were treated on June 18, 1980 and were maintained individually in a Nalgene Metabolism Cage unit (4 males and 4 females) and an all glass Stanford metabolism chamber (1 male and 1 female) for 7 days. The purpose of this contol treatment group was to provide the basic animal physiological parameters (such as daily food and water intake, urine and feces excretion profile,

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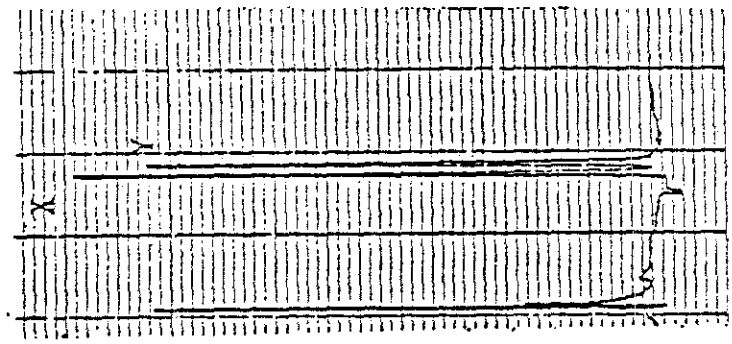
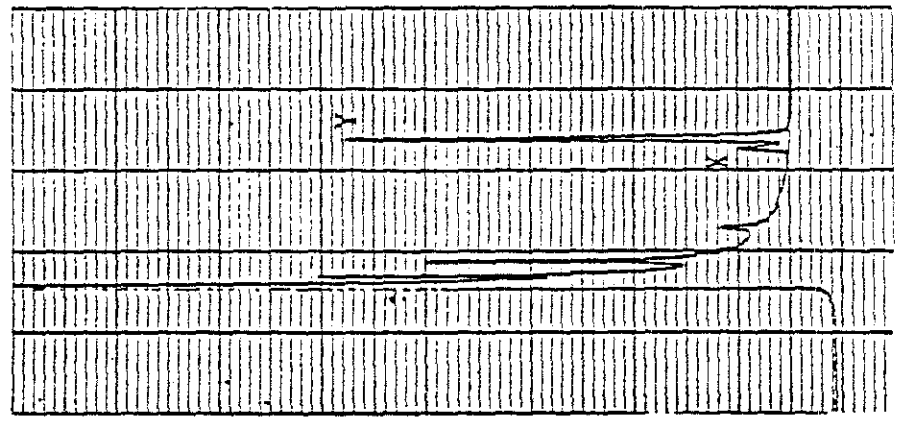
Figure 3 Isomeric composition of the dosing solution of ^{14}C-phenoxyphenyl-SD 92459 and ^{14}C-phenoxyphenyl-SD 43775



SD 43775
X/Y=55/45



SD 92459
X/Y=10/90

experimental conditions. Individual body organs and tissues o the animal in the control treatment group were also used t establish the normal background level, sensitivity of th radioanalysis and the limit of determination o ^{14}C-phenoxyphenyl-SD 92459 equivalent residues by analytica procedures used in this study.

2. ^{14}C-Phenoxyphenyl-SD 92459 Treatment Group I

1 male (80R096) and 1 female (80R099) (single oral ^{14}C-phenoxyphenyl-SD 92459, 8.4 mg/kg). Animals were treated o October 29, 1980 and were maintained individually in an all-glas Stanford metabolism chamber for 2 days. The purpose of thi treatment group was to demonstrate that no $^{14}CO_2$ or other volatil radioactive materials were exhaled in the respired air of th treated animals during the initial 48 hours immediately afte dosing. Data from this study justified that the requirement fo the monitoring of the exhaled air from the treatment group II i waived.

3. ^{14}C-Phenoxyphenyl-SD 92459 Treatment Group II

5 male (80R086,88,90,92 and 94) and 5 female (80R087, 91,93,95 an 97) (single oral, ^{14}C-phenoxyphenyl-SD 92459 8.4 mg/kg); 1 mal (80R084) and 1 female (80R085) (control, corn oil, single oral, ml/kg). Animals were treated on October 29, 1980 and wer maintained individually in Nalgene metabolism cage units for days. Animal physiological parameters (daily food and wate intake, urine and feces excretion profile) were recorded daily The rates of excretion of ^{14}C-phenoxyphenyl-SD 92459 equivalen residues in the urine and feces were also monitored daily. At th end of the Day-5 holding period, animals were sacrificed an individual organ tissues were collected and radioassayed fo ^{14}C-phenoxyphenyl-SD 92459 equivalent residues.

The actual dose of ^{14}C-phenoxyphenyl-SD 92459 and control corn oi carrier administered to each individual test animals ar summarized in Table 1.

TABLE 1 SUMMARY OF TEST ANIMALS AND THEIR TREATMENT SCHEDULE

	ANIMAL ID	SEX	DOSE	HOLDING CONDITION
Control Treatment Group (corn oil)				
	80R001	F	0.94 ml[a)]	PC[b)]
	80R002	M	1.07 ml	PC
	80R003	F	0.91 ml	PC
	80R004	M	1.14 ml	PC
	80R005	F	0.93 ml	PC
	80R006	M	0.93 ml	PC
	80R007	F	0.92 ml	PC
	80R008	M	1.10 ml	PC
	80R009	F	1.02 ml	GC[c)]
	80R010	M	1.12 ml	GC
^{14}C-Phenoxyphenyl-SD 92459 Treatment Group I				
	80R096	M	1.60 mg[d)]	GC
	80R099	F	1.55 mg	GC
^{14}C-Phenoxyphenyl-SD 92459 Treatment Group II				
	80R086	M	1.70 mg[d)]	PC
	80R087	F	1.48 mg	PC
	80R088	M	1.68 mg	PC
	80R090	M	1.66 mg	PC
	80R091	F	1.55 mg	PC
	80R092	M	1.72 mg	PC
	80R093	F	1.54 mg	PC
	80R094	M	1.70 mg	PC
	80R095	F	1.48 mg	PC
	80R097	F	1.61 mg	PC
	80R084[e)]	M	1.31 ml[a)]	PC
	80R085	F	1.14 ml	PC

a) Dosage of corn oil (5 ml/kg)
b) Nalgene Metabolism Cage Unit
c) Stanford All-Glass Metabolism Chamber
d) Dosage of ^{14}C-phenoxyphenyl-SD 92459 (8.4 mg/kg)
e) Control animal

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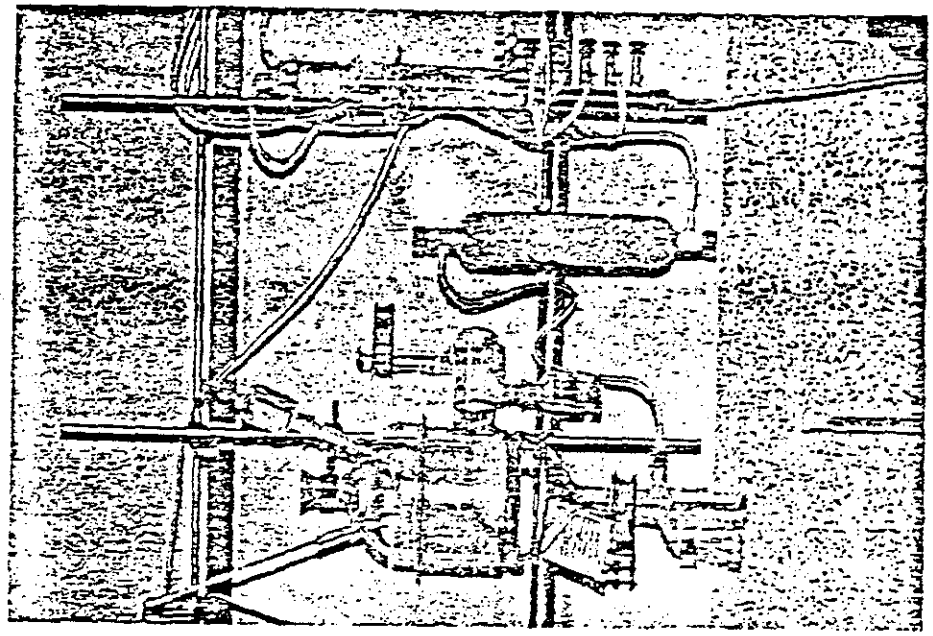
F. Preliminary All-Glass Metabolism Chamber Holding Study

A preliminary range-finding experiment was carried out using 1 female (80R099) and 1 male (80R080) rat treated with a single oral dosage of 8.4 mg/kg of ^{14}C-phenoxyphenyl-SD 92459. Animals were maintained in individual Stanford all-glass metabolism chamber (Model MC 3000, Stanford Glassblowing Laboratories, Palo Alto, CA) and allowed free access to food and water one hour after dosing. A schematic diagram of this all-glass metabolism chamber unit is presented in Appendix VII. This is an air-tight structure that allows a continuous monitoring of $^{14}CO_2$ generation and the separate collection of urine and feces excreta. $^{14}CO_2$ was collected over a CO_2 absorption tower which contained 250 ml of ethanolamine. Several minor modifications were added to this existing unit. A manifold system was used which allowed an independent fine control of the amount of air passing through each individual metabolism chamber. Gas washing bottles (Laboratory Glass Apparatus, Berkeley, CA) were modified to provide a continuous monitoring of the generation of $^{14}CO_2$ during the entire holding periods. A detailed description of this experimental setup is presented in Figure 4.

G. Nalgene Metabolism Cages Holding Study

A control treatment group (5 male and 5 female) and the ^{14}C-phenoxyphenyl-SD 92459 treatment group II (5 male and 5 female) plus 2 control animals (1 male and 1 female) were maintained individually in Nalgene metabolism cage units (Sybron Nalge Co., Rochester, NY). A description of the assembly of the Nalgene metabolism cage unit is presented in Appendix VIII. Although these metabolism units are not designed for the collection of $^{14}CO_2$ from the exhaled air of the treated animals, they were excellent and efficient in the separate collection of urine and feces excreta. A group of 10-12 metabolism cages were used in each experiment. Animals were allowed free access to food and water. Because of the poor design of the food container, animals in control treatment group were able to carry extra food pellets back into the main metabolism chamber, which resulted in the contamination of both the urine and feces excreta. Modification of the food container on these Nalgene metabolism cages was carried out prior to the initiation of the ^{14}C-phenoxyphenyl-SD 92459 treatment group II study and resulted in the elimination of the cross contamination of the urine and feces excreta by the food diet (Figures 5 and 6). A Detailed description of this modification is presented in a separate report (RIR-22-018-80).[a)]

a) RIR-22-018-80 Modification of the Diet Feeder in the Nalgene Metabolic Cages for Rats to Minimize Contamination of Excreta.



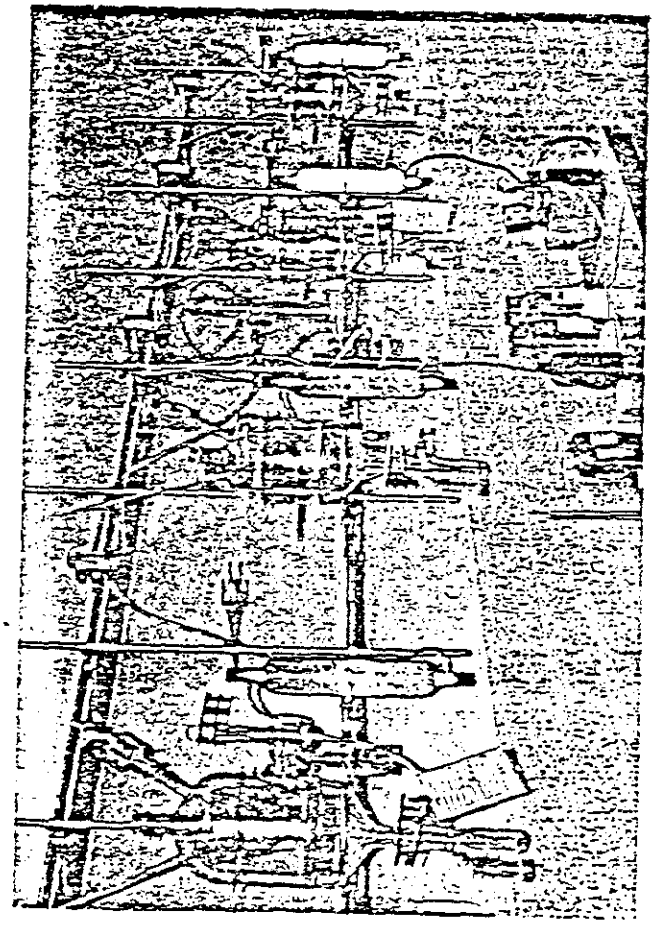


Figure 4 Experimental setup for the all glass metabolism chamber holding study

Figure 5 Experimental setup for the Nalgene plastic metabolism chamber unit

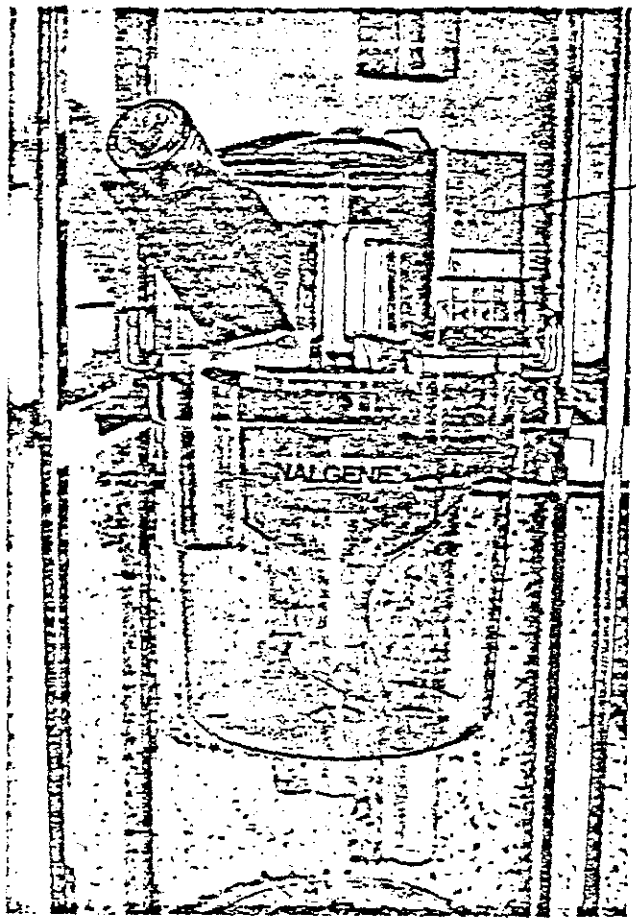
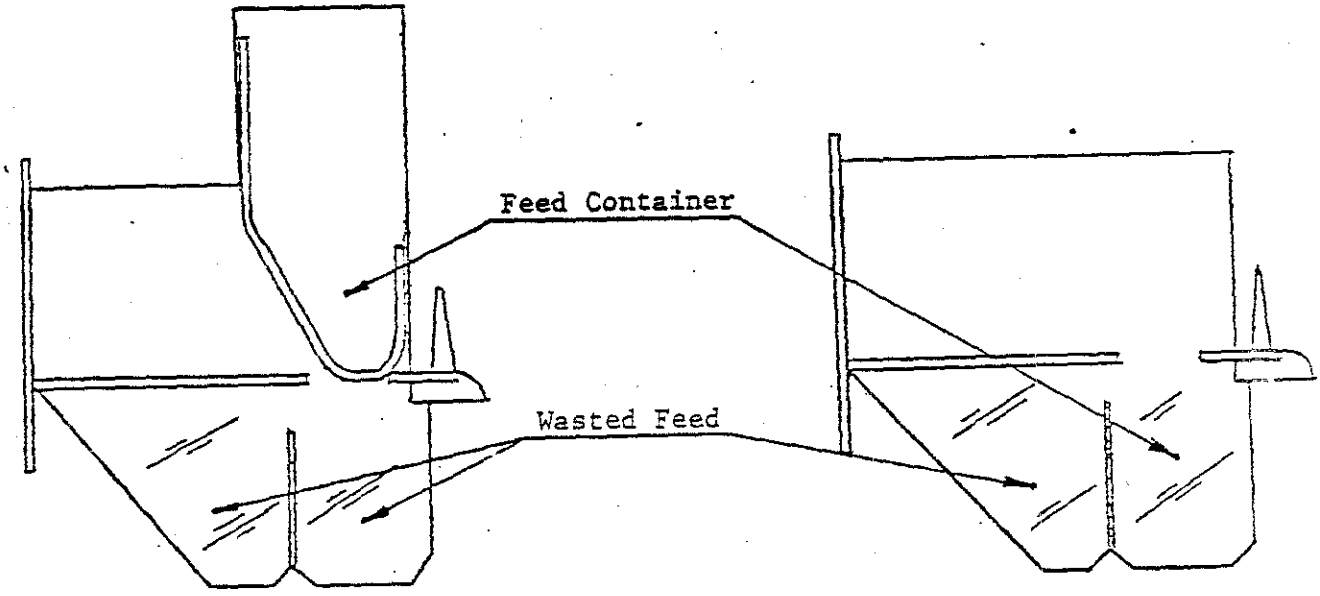


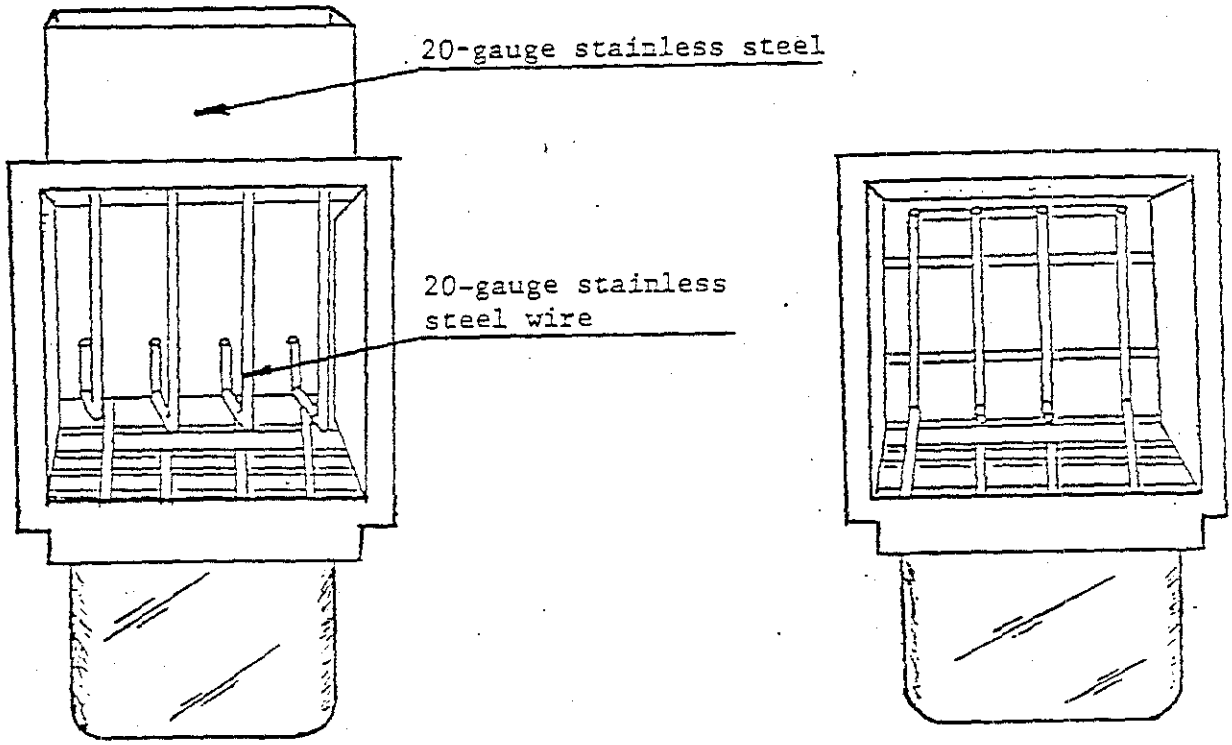
Figure 6 . MODIFICATION OF METABOLISM FEED CHAMBER FOR RATS

Modified Form

Original Form



Cross-Sectional Side View



Front View

H. Urine Sampling and Analysis

Urine excreta from all the control and ^{14}C-phenoxyphenyl-SD 92459 treated animals were collected daily and the total urine volume from each individual animal was recorded into the Animal Physiology Record (Appendix VI). The final volume of each individual daily urine excreta was then adjusted to a final volume of 25 ml with 0.01 M phosphate buffer (pH 7.4). Triplicate 100-µl aliquots were sampled and the total daily ^{14}C-phenoxyphenyl-SD 92459 equivalent residues present in the urine excreta was quantitatively analyzed by LSC. All samples were analyzed immediately after collection. All urine samples were stored at 4°C prior to further qualitative analysis.

For the quantitative and qualitative examination of the excreted urinary metabolites, 15 ml aliquots of the Day-1 and Day-2 urine samples from each treated animal were combined for analysis. Analysis was carried out immediately after the termination of the animal holding period. Triplicate 100-µl aliquots were subjected to LSC quantitation. The pH of the combined urine sample was adjusted to pH 3 with 1 ml of 6N hydrochloric acid, and extracted three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate extraction were subjected to enzyme hydrolysis. Enzyme hydrolysis was carried out at 35 ± 1°C for 12 hours using approximately 1×10^4 units of the sulfatase/β-glucuronidase enzyme (No. S-9126, Sigma Chemical Co., one enzyme unit will hydrolyze 1 mole of nitrocatechol sulfate per hour at pH 5 at 37°C). Water-soluble conjugates released during enzyme hydrolysis were recovered by extracting the aqueous phase three times with equal volumes of ethyl acetate. Organic solvents were dried over anhydrous magnesium sulfate, concentrated and further analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after enzyme hydrolysis was quantitated by LSC. If the amount of the radioactivity remaining in this aqueous fraction represented greater than 5% of the initial applied radioactivity, this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by the acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the urine sample after the initial organic solvent extraction, enzyme hydrolysis and acid hydrolysis were considered as unextractable materials and were not further qualitatively analyzed.

I. Sampling and Analysis of Fecal Material

Fecal excreta from all the control and ^{14}C-phenoxyphenyl-SD 92459 treated animals were collected daily and the total fecal wet weight from each animal was recorded into the Animal Physiological Record (Appendix VI). Daily fecal excretion was freeze dried in a freeze dryer (Thermovac Industries Corp.) for 24 hours and the final resultant dry weight was also recorded. Dried fecal materials were pulverized by using a microanalytical mill (Tekmar Company). The percent of the applied radioactivity recovered in the daily fecal excreta was

quantitatively analyzed by oxygen combustion of triplicate fecal subsamples (approximately 100 mg each) by using a Packard Model 306B sample oxidizer and subsequent LSC quantitation. All samples were analyzed after collection. All fecal samples were stored at 4°C prior to further qualitative analysis.

For the qualitative and quantitative examination of the excreted fecal metabolites, a 3 gram subsample of the Day-1 and Day-2 combined fecal excreta from each treated animal was analyzed. Analysis was carried out immediately after the termination of the animal holding period. Fecal excreta was first extracted three times (15 minutes each) using 30 ml of methanol-water (9:1) solvent mixture. The solvent extract and solid fecal materials were separated by centrifugation (2000 rpm for 10 minutes). The combined methanol-water solvent extract was quantitatively analyzed by LSC for the total amount of solvent extractable metabolites. The volume of the methanol-water solvent extract was then concentrated to approximately 15 ml by rotor evaporation. The final volume of this concentrated fecal solvent extract was adjusted to 30 ml using 0.01M phosphate buffer (pH 7.4) and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate solvent extract was dried over anhydrous magnesium sulfate, concentrated and the organo-soluble fecal metabolites of ^{14}C-phenoxyphenyl-SD 92459 were analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after organic solvent extraction was considered as fecal water-soluble conjugates and was quantitated by LSC. If the amount of radioactivity remaining in the aqueous fraction represented greater than 5% of the initial applied radioactivity, then this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the solid fecal residues after the initial methanol-water solvent extraction was considered as unextractable materials and was not further qualitatively analyzed.

J. Animal Sacrifice and Tissue Sampling Procedures

Control and treated animals were sacrificed 5 days after the administration of ^{14}C-phenoxyphenyl-SD 92459. Each animal was removed from its respective metabolism cage and their final body weight was recorded. Sacrifice was carried out by decapitation. Control animals were sacrificed prior to the ^{14}C-phenoxyphenyl-SD 92459 treated animals to minimize cross contamination.

The required tissues were excised from each experimental animal in the order that follows. Special care was taken to prevent contamination between individual tissues.

Whole blood, approximately 2 ml anticoagulated with ethylenediamine tetraacetate (EDTA, 10 mg), was collected from the severed carotid arteries in a 15-ml centrifuge tube immediately after sacrifice. Lung, heart, liver, kidney, gonads, inguinal fat, back fat, muscle (from the hind leg), and brain were obtained. All organs and tissue samples, except blood, were individually weighed, placed in prelabeled vials and stored frozen prior to analysis. All organ and tissue weights were recorded in the Animal Tissue Record (Appendix IX). The remaining carcasses were labeled accordingly and sealed in polyester bags and stored frozen (-10°C).

Tissues were subsampled (triplicate subsamples approximately 100 mg) and quantitatively analyzed for total ^{14}C-phenoxyphenyl-SD 92459 equivalent residues by oxygen combustion techniques and LSC.

K. Radioassay

Radioactivity was quantitated by using a Packard Model 2660 Liquid Scintillation System. Counting efficiency determination was carried out by using the external standard ratio (ESR) technique. The actual quench curve was determined at a monthly interval to insure its validity. An example of the instrument print-out for the counting efficiency correlation with ESR is presented in Appendix X. Radioactivity was analyzed in 15 ml of Aquasol-2 scintillation solution. Radioactive areas on the TLC plate after solvent development were removed by scraping and analyzed for radioactivity in an Aquasol-2/water (11:4 ml) gel system.

Radioactive residues associated with animal tissues and fecal excreta were analyzed by weighing approximately 100 mg each of the subsample into a Combusto-Cone sample holder (Packard Instrument Co.) and combusted in a Packard Model 306B TriCarb sample oxidizer. Combustion efficiency of the individual tissue was determined using control animals and calibrated ^{14}C-chlorophenyl-SD 43775 solution as internal recovery standard. Counting solution included Carbo-Sorb and Permafluor V (10:12 ml) mixture.

L. Chromatographic Procedures

The chemical nature of the radioactivity recovered in the urine, feces and other selected organ tissues was qualitatively and quantitatively analyzed by TLC. Two-dimensional TLC (silica gel F-254, 0.25mm thickness, E. Merck) was performed in the following solvent system combinations.

(A) Hexane-acetone-HoAc (25:25:1)

(B) Toluene-ether-HoAc (75:25:1)

The R_f values of SD 43775 and other model metabolites are presented in Table 2. Reference standards were visualized under UV light. TLC plates were scanned for radioactivity by either using a Berthold LB 2760 TLC scanner or a Berthold LB 292 Beta Camera (Beta Analytical Inc.). Final visual confirmation of the distribution of ^{14}C-phenoxyphenyl-SD 92459 and its metabolites were carried out by autoradiography on Kodak SB-54 single-coated blue sensitive x-ray film (Eastman Kodak Co.).

Capillary gas-liquid chromatography was carried out using a 25m x 0.37mm I.D. SE-30 WCOT glass column in a Varian 3700 gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector. Isothermal analysis was carried out at injector, column and detector temperatures of 280, 245 and 320°C, respectively. The helium carrier gas and nitrogen make up gas flow rate through the detector were 3 and 36 ml/minute, respectively. On-column split ratio was controlled at 10:1 ratio.

Radio-gas-liquid chromatography (RGLC) was carried out using a Varian 1440 gas-liquid chromatograph equipped with a flame ionization detector and a Packard Model 894 gas-proportional counter. The column used was a 1m x 2mm I.D.

TABLE 2 R_f values of SD 43775 and its metabolites on Silica gel F 254 plates using two solvent systems

	R_f	
	System 1	System 2
SD 43775	0.78	0.72
SD 48838	0.60	0.40
SD 44607	0.49	0.38
SD 46114	0.41	0.16

Solvent system 1 Hexane-acetone-HoAc (25:25:1)

Solvent system 2 Toluene-ether-HoAc (75:25:1)

Mass spectroscopy in election impact mode (EIMS) was carried out on the Finnigan 3200 mass spectrometer with the instrument settings as follows:

GC setting

Stationary phase	SE-30
Solid support	WCOT column
Column size and material	1.2m x 0.3 mm ID, glass
Carrier	He approximately 3 ml/min
Column temp	100°C for 2 min;program @ 15°C/min. to 280°C
Transfer line	Approximately 250°C
Solvent Vent	at 2 min

MS setting

Electron energy	70 eV
Electron multiplier voltage	1800 V
Emission current	1.0 ma
Preamplifier sensitivity	10^{-7} a/v

glass column packed with 3% OV-101 on 80/100 mesh Supelcoport (Supelco Co.). The air, hydrogen and helium flow rates for RGLC were 210, 30 and 30 ml/minute, respectively. The helium and propane quench gas flow rates for the gas-proportional counter were 120 and 15 ml/minute, respectively.

M. Data Handling and Presentation

All raw data obtained from this metabolism study were recorded directly and promptly into laboratory notebooks designated for this study. Raw data are records of any original experimental observations which are necessary for the reconstruction of this experiment and the evaluation of this report. The following experimental records were also established as a permanent file on the HP-3000 computer system. Such files include the Animal Resource Record (Appendix IV), and the Animal Physiology Record (Appendix VI).

All experimental observations (urine volume, feces weight, tissue weight, subsample volume and weight and the radioactivity associated with these samples) were also presented as the Animal Tissue Record (Appendix IX) and the Excretion and Tissue Distribution Profile (Appendix XI). The percent of applied ^{14}C-phenoxyphenyl-SD 92459 equivalent residues recovered in the urine and fecal excreta of the treated animals were calculated by using Computer Program-2 (Appendix XII). Standard student's t-test was used to calculate the significant level between experimental and control animals.

All raw data from this study were submitted to the BSRC Archives under designated Protocol PPL-22-009-80 code number for permanent storage.

N. Biological Samples Storage and Retention

All biological samples generated from this study (animal excreta, organ tissues, animal carcasses, etc.) were packaged and stored in the Building 16 walk-in freezer at BSRC at -10°C under the designated Protocol PPL-22-009-80 code number for a minimum of five years.

O. Good Laboratory Practice Compliance Program

This metabolism study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guideline (CFR, vol. 43, no. 247, page 59986-60020, December 22, 1978). A Standard Operation Procedures (SOP) package designated for this metabolism study under the code number of Protocol PPL-22-009-80 was prepared and submitted as raw data to the BSRC Archives for permanent storage. The table of contents of this SOP package is presented in Appendix XIII.

EPA: 68-01-6561
TASK: 112
July 3, 1985

00 4681
PC 109301

DATA EVALUATION RECORD

FENVALERATE

Metabolism Study in Rats

STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of ^{14}[C]-chlorophenyl-SD 43775 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-021-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254117.

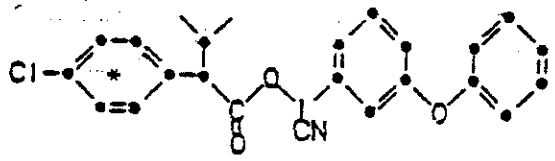
APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-3-85

1. CHEMICAL: SD 43775, Pydrin insecticide, benzeneacetic acid, 4-chloro-α-(1 methylethyl)-cyano-(3-phenoxyphenyl) methyl ester:



2. TEST MATERIAL: The test material was ^{14}[C]-chlorophenyl-SD 43775 labeled at the chlorophenyl position. The specific activity was 43.7 **μCi/mg and the radiochemical purity greater than 99.5%. A concentrated stock solution, 6.57 mg/ml in dichloromethane, was stored at** -4°C. Unlabeled SD 43775 with a chemical purity of greater than 99.4% was also used. The ratio of x/y isomers was 45:55.

3. STUDY/ACTION TYPE: Metabolism study in rats.

4. STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of ^{14}[C]-chlorophenyl-SD 43775 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-021-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254117.

5. REVIEWED BY:

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Date: July 3, 1985

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Date: 7-3-85

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Date: 7/9/85

Albin Kocialski, Ph.D.
EPA Section Head

Signature: Albin B. Kocialski
Date: 9/24/85

7. CONCLUSIONS:

When Pydrin, ^{14}[C]-chlorophenyl-SD 43775, was administered to male and female rats in a single oral dose at 8.4 mg/kg, radioactive material was rapidly eliminated in the feces and urine; the majority of the radioactivity was eliminated in 24 hours. There was a lack of accumulation of radioactive residues in most tissues; detectable levels were found after 5 days in the liver (approximately 0.16 ppm equivalents/g) and fat (1.67 and 0.81 ppm equivalents/g in males and females, respectively). Over 50% of the residues in fat were undegraded SD 43775. Examination of urinary metabolites indicated a metabolic cleavage of the ester linkage followed by further oxidation to an acid moiety which was excreted free or as a glucuronide or sulfate conjugate. Unmetabolized SD 43775 was found in feces, approximately 30% and 46% of the administered radioactivity in males and females, respectively.

Under the conditions of the assay, the study is acceptable.

Items 8 through 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test animals were male and female Sprague-Dawley rats (Simonsen's Laboratories, Gilroy, CA), 7 weeks of age, and weighing 175-200 g.

2. Control groups of 5 males and 5 females received a single dose of 5 ml/kg corn oil and were maintained to monitor basic physiological parameters under sham conditions of the experimental groups. Four/sex were maintained in Nalgene metabolism cages and one/sex in Stanford all glass metabolism cages. Daily food and water consumption and urine and feces elimination were measured.

3. Treatment group 1 consisted of 1 male and 1 female given single oral doses of 8.4 mg/kg ^{14}[C]-chlorophenyl-SD 43775. The animals were housed for seven days in Stanford metabolism cages to monitor for radioactivity expired in $^{14}[CO_2]$ and excreted in urine and feces. If no $^{14}[CO_2]$ was detected in 48 hours, collection of expired air was discontinued.

4. Treatment group 2 consisted of 5 rats of each sex given a single oral dose of 8.4 mg/kg test material and maintained individually for 5 days in nalgene metabolism cages with slightly modified feeders that eliminated food contaminating

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[1] Only items appropriate to this DER have been included.

urine and feces. Food and water intake were measured daily and urine and feces collected daily.

5. Urine volume was measured, the volume then brought to 25 ml and an aliquot radioassayed by a liquid scintillation counter (LSC). For metabolite identification, 15 ml aliquots of urine samples for days 1 and 2 were combined, adjusted to pH 3 with 1 ml 6N HCl and extracted 3 times with equal volumes of ethyl acetate. The resulting aqueous phase was adjusted to pH 5.0, enzymatically hydrolyzed with sulfatase and β-glucuronidase, and then extracted 3 times with equal volumes of ethyl acetate. If more than 5% of the initial radioactivity remained in the aqueous phase, the sample was subjected to acid hydrolysis for 4 hours at 90°C at pH 1.0 and then extracted with solvent. The organic phases were concentrated and subjected to thin layer chromatography (TLC).

6. Feces were weighed wet then freeze dried and reweighed. Radioactivity was determined by liquid scintillation counting after combusting an aliquot (100 mg). For identification of metabolites, 3 g samples of feces collected on days 1 and 2 were combined, extracted three times with 30 ml MeOH:H_2O (9:1) and radioactivity quantitated on an aliquot. The extracts were concentrated, adjusted to pH 7.4, extracted with equal volumes of ethyl acetate, and the organic phase brought to dryness. If the aqueous phases contained greater than 5% of the radioactivity, they were hydrolyzed at pH 1.0 at 90°C for 4 hours and extracted with ethyl acetate. The organic phases were subjected to two-dimensional TLC.

7. Animals were sacrificed at 5 days, 2 ml blood collected in EDTA, and the following tissues trimmed and weighed: lung, liver, heart, kidneys, gonads, inguinal and back fat, muscle, and brain. Radioactivity was determined in triplicate 100 mg subsamples by combustion and LSC.

8. Radioactivity was determined by liquid scintillation counting (LSC) in Aquasol (liquid samples) or Carbosorb-Permafluor (CO_2 from combusted samples). Quench correction was determined monthly, and efficiency was determined by the external standard counting method. Combustion efficiency was determined on tissue samples of control animals to which an internal standard ^{14}[C]-test material was added.

9. Thin layer chromatography solvents were: hexane/acetone/HOAc (25/25/1) and toluene/ether/HOAc (75/25/1). Reference standards were visualized with UV light and ^{14}[C]-areas located by autoradiography. GLC was performed with a Varian chromatograph with electron capture and radio-gas-liquid chromatography used a flame ionization detector and a Packard gas proportional counter. Mass spectrometry used the electron impact mode.

B. Protocol: Detailed methods are given in Appendix A.

12. REPORTED RESULTS:

There were no observable behavioral effects or toxic signs in dosed animals. Food consumption and water intake were similar in dosed and control rats. In the preliminary study, there were no $^{14}[CO_2]$ or ^{14}[C]-volatiles in the exhaled air of the one male and one female administered 8.4 mg/kg ^{14}[C]-test material and maintained in an all glass metabolism chamber for 2 days. Of the administered radioactivity in the male, 38.9% and 56.7% was excreted in urine and feces (7 days), respectively. In the female, 40.1% and 46.2% of the administered radioactivity was excreted in urine and feces, respectively. Further studies with treatment group 2 were carried out in Nalgene metabolism chambers without the collection of expired air.

Urinary and Fecal Excretion:

There was a rapid elimination of the administered radioactivity in the urine and feces. Approximately 28% and 21% of the radioactivity was recovered in the urine of males and females, respectively, 24 hours after dosing. The second-day urine contained 7.7% and 5.3% of the administered label in males and females. The total recovery in 5 days was about 39% and 28% of the administered label in males and females, respectively. Likewise, elimination of radioactivity in the feces at 24 hours was 43% and 54% of the administered dose in males and females, and the total recovered in the 5 days was 52% in males and 60% in females. Recovery of administered radioactivity ranged from 86.9%-93.4% for the 5 males and 78.5-96.6% for the 5 females.

Urine samples of day 1 and day 2 were pooled for each animal and extracted with ethyl acetate. The water soluble conjugates were then enzymatically hydrolyzed and extracted with ethyl acetate. The aqueous phase was acid hydrolyzed and reextracted with ethyl acetate. Table 1 shows the distribution of radioactivity in the various fractions. The organic fractions were then concentrated, and qualitatively and quantitatively analyzed by two-dimensional TLC and liquid scintillation counting. Urinary metabolites were tentatively identified by comparing R_f values on TLC to those of authentic standards. Structures were further confirmed by mass spectroscopy analysis. The nonconjugated and conjugated metabolites were analyzed for each animal. There was no qualitative difference in the distribution of metabolites between males or females (Table 2). However, there was some variability in the amounts of metabolites between individual animals. Undegraded SD 43775 was not detected. The major urinary metabolites were: 4-chloro-α-(1-methyl-ethyl)benzeneacetic acid (SD 44064; 10.0-17.7% in males and 10.1-20.1% in females) and 4-chloro-α(2-hydroxy-1-methyl-ethyl)benzeneacetic acid (SD 53919; 4.1 to 5.6% in males and 1.1 to 4.0% in females). Seven to eight minor metabolites were not identified.

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TABLE 1. Distribution of the Eliminated ^{14}[C]-Chlorophenyl-SD 43775 Residues in the Urine of Male and Female Rats

	Percent of Administered Radioactivity Recovered	
	Male[a]	Female[a]
Organic Extractable	16.5±7.2[b]	18.8±7.9
Water Soluble Conjugates	19.2±4.2	7.2±4.7
Released after enzyme hydrolysis	11.0±2.2	3.3±1.7
Released by acid hydrolysis	7.7±4.6	2.9±4.0
Unextractable	0.6±0.1	1.0±0.4
Total urinary radioactivity	35.7±4.6	26.0±5.5

[a] N = 5.

[b] Mean ± standard deviation.

TABLE 2. Radiolabeled Metabolites in Urine of Rats after Administration of ^{14}[C]-Chlorophenyl-SD 43775

Metabolite[b]	Sex	Percent of Administered Dose[a]			
		Noncon-jugated	Conjugated[c]	Acid Released	Total
SD 52667	M	0.6	1.0	0.6	2.3
	F	1.0	0.2	0.3	1.8
SD 44064	M	10.8	2.5	0.5	13.8
	F	10.8	1.3	1.4	13.5
SD 53065	M	0.3	0.4	0.6	1.4
	F	0.1	ND[e]	0.5	0.6
SD 53919	M	2.8	1.9	--	4.7
	F	2.1	ND	--	2.4
SD 52666	M	0.7	1.0	--	1.7
	F	1.6	ND	--	1.6
SD 90930	M	0.4	0.2	--	0.6
	F	0.2	ND	--	0.2
Other[d]	M	0.8	3.8	5.9	11.2
	F	2.7	1.7	0.7	5.9

[a] Mean of 5 males and 5 females combined.

[b] The structural formulas of the metabolites are given in Appendix B.
SD 52667 (α isomer), 3-(4-chlorophenyl) dihydro-4-methyl, 2(3H)-furanone
SD 44064, 4-chloro-α-(1-methylethyl)benzeneacetic acid
SD 53065, 4-chloro-α-hydroxy (1-methylethyl)benzeneacetic acid
SD 53919 (b isomer), 4-chloro-α-(2-hydroxy-1-methyl-ethyl)benzene-acetic acid
SD 52666, α isomer of SD53919
SD 90930, 4-chloro-α-hydroxy-α-(2-hydroxy-1-methylethyl)benzene-acetic acid

[c] Released by enzyme hydrolysis.

[d] Includes radioactivity remaining in the aqueous phase after initial organic extraction or after organic extraction following enzyme hydrolysis.

[e] ND, not detected; (-), not tabulated.

Most of the radioactivity in feces collected on days 1 and 2 was extracted into ethyl acetate, 93.7% in males and 90.7% in females (Table 3). Approximately 3.1±1.7% and 3.5±4.3% of the administered radioactivity in males and females, respectively, remained bound. Undegraded test material (SD 43775) accounted for 30.5±10.2% (19-43%) and 46.2±8.3% (37-55%) of the radioactive dose in males and females, respectively. Major metabolites were the hydroxylated test compound (3 phenoxy-4-hydroxy derivative, SD 48838), and 4-chloro-α-(1-methylethyl)benzeneacetic acid (SD 44064).

Tissue Distribution:

It did not appear that there was appreciable bioaccumulation of the test compound or its radioactive metabolites. The levels in blood, gonads, brain, and muscle were only slightly above the limit of detection. Liver and fat had detectable levels (Table 4); the level in lung, heart, and kidneys was approximately two-fold above the upper limit of detection. In an attempt to characterize the residues in liver, 5 grams of liver from 3 males or 3 females was homogenized in 20 ml of phosphate buffer pH 7.4 and precipitated with 10% trichloroacetic acid (1 ml). Approximately 26-32% of the radioactivity was soluble; the amount in the proteinaceous fraction averaged 0.108-0.124 µg equivalents/g tissue. Hexane extraction of liver homogenates followed by gas-liquid chromatography failed to recover any radioactive ^{14}[C]-chlorophenyl-SD 92459. Samples of fat from 3 males or 3 females were homogenized, extracted with hexane and analyzed by GLC. Approximately 54% and 65% of the radioactivity in males and females was recovered as the administered material (SD 43775), respectively; the average of bound residues in males was 0.643 µg equivalents/g and in females 0.133 µg equivalents/g (Table 4).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. After a single oral dose of 8.4 mg/kg ^{14}[C]-chlorophenyl-SD 43775 was administered to male and female rats, there was rapid elimination of radioactivity in the urine and feces. Most of the radioactivity was eliminated in the first 24 hours. No exhaled radioactivity was detected. Excretion was similar in males and females. The examination of urinary metabolites by chromatography indicated metabolic cleavage of the ester linkage followed by further oxidation to an acid moiety and conjugation with sulfate or glucuronide. Undegraded SD 43775 was found only in the feces, accounting for approximately 30 and 46% of the administered dose in males and females, respectively. Tissue residue distribution data showed a lack of bioconcentration of radioactive residues in blood, lung, heart, kidney, gonad, muscle, and brain. Detectable levels of radioactive residues were found in the liver (approximately 0.16 ppm equivalents) and fat (1.67 and 0.81 ppm equivalents/g in males and females, respectively). Greater than 50% of the residues in body fat were undegraded SD 43775.

B. A quality assurance statement was not present.

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TABLE 3. Radioactive Metabolites in Feces of Rats after Administration of ^{14}[C]-Chlorophenyl-SD 43775

	Percent of Administered Radioactivity[a]	
	Males	Females
Organic Extractable Metabolites	45.4	44.2
SD 43775[b]	30.5	46.2
SD 44064	3.0	3.1
SD 48838	3.8	2.2
Others[c]	6.0	2.5
Unextracted	3.1	3.5
Water Soluble Conjugates	0.9	1.4

a Mean value for males and females combined.

b Unchanged test material. The structures of the metabolites are given in Appendix B:
SD 44064: 4-chloro-α-(1-methylethyl)benzeneacetic acid
SD 48838: 4-chloro-α-(1-methylethyl)-cyano-(3-phenoxy-4-hydroxy-phenyl)benzeneacetic acid, methyl ester.

c Radioactivity remaining at the origin of TLC plate and minor metabolites.

TABLE 4. Residue Level in Tissues of Male and Female Rats 5 Days after Oral Administration of ^{14}[C]-Chlorophenyl-SD 43775

	µg Equivalents/g Wet Tissue		
	UDL[a]	Males	Females
Lung[b]	0.043	0.089	0.083
Heart[b]	0.038	0.087	0.073
Kidney[b]	0.040	0.087	0.059
Liver (total)[b]	0.042	0.157	0.150
Liver (proteinaceous)[c]	0.042	0.124	0.108
Fat (total)[b]	0.040	1.668	0.806
Fat (unextractable)[c]	0.040	0.643	0.133

[a] UDL - upper detection limit.

[b] Average of 5 animals/sex.

[c] Average of 3 animals/sex.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors' conclusions were supported by individual animal data. The protocol was adequate to determine the metabolism, tissue distribution, and excretion of the test material and its metabolites, and the study was well conducted and reported; we assess that the study was acceptable for showing the metabolism of SF 43775. Recoveries of radioactivity were high. The levels of specific activity of the test compound were sufficiently high to detect equivalent residues in animal tissue at a level of 0.005 to 0.009 of the administered dose using 100 mg samples of tissue. Combustion efficiency ranged from 89.8-96.2%. Radioactive samples of urine and feces were corrected for quenching and counting efficiency. Although there was some variability from animal to animal in percent of radioactivity in various fractions or metabolites, this is to be expected and sufficient animals were used to calculate mean values for all parameters. The test material and metabolites were well characterized by chromatographic means and the data presented. Data on autoradiograms of the two dimensionally chromatographed plates were presented. Data were also presented confirming identification of the metabolites by mass spectrometry.

Items 15 - see footnote 1.

16. APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp. 4-17.

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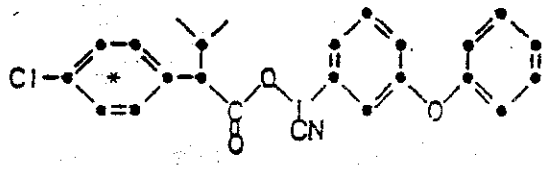
APPENDIX A

MATERIALS and METHODS (Protocol)

III. Description of the Conduct of Experiment

A. Test Compound

SD 43775 labeled with carbon-14 at the chlorophenyl-postion was used.



SD 43775

*Denotes carbon-14.

The specific activity of the ^{14}C-chlorophenyl-SD 43775 is 43.7 microCuries/mg as determined by capillary gas-liquid chromatography (GLC) and liquid scintillation counting (LSC). Detailed calculations of the specific activity of the ^{14}C-chlorophenyl-SD 43775 is presented in Appendix II. Based on the sensitivity of detection, the radiochemical purity of ^{14}-chlorophenyl-SD 43775 is greater than 99.5% as determined by two-dimensional thin-layer chromatography (TLC) (Figure 1) and LSC. Concentrated ^{14}C-chlorophenyl-SD 43775 stock solution (6.57 mg/ml in dichloromethane) is stored at -4°C. Unlabeled SD 43775 (analytical standard code 10-1-0-0) was also used in this study and its chemical purity was greater than 99.4% (Appendix III).

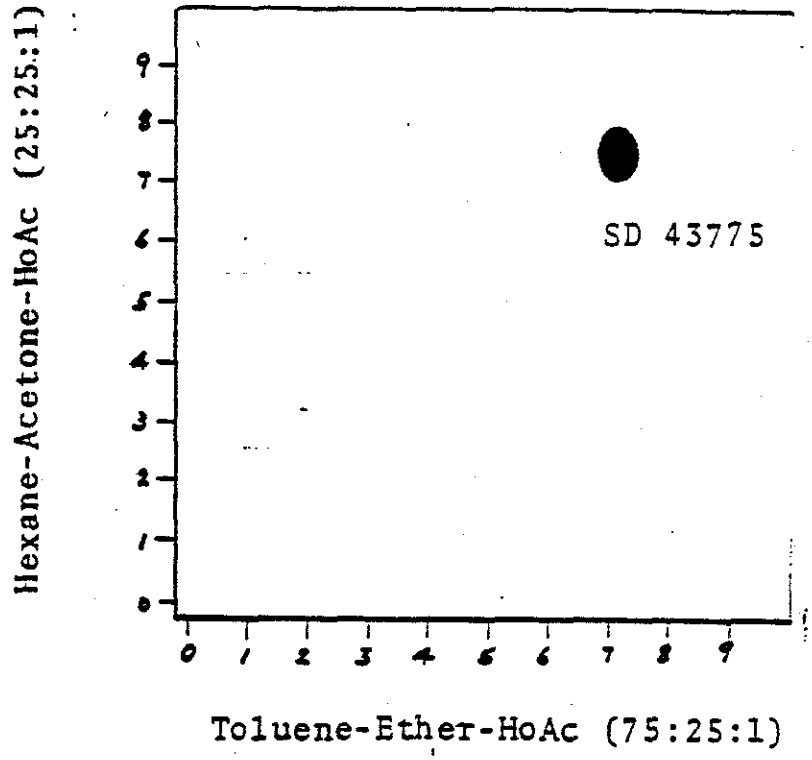
B. Test Animals

Male and female Sprague-Dawley albino rats (SIM:SD f1 strain, 7 weeks old, weighing from 175-200 grams each) were obtained from Simonsen's Laboratories, Gilroy, California. The number of total test animals, their experimental identification number, sexes, arrival date, initial body weight, experimental treatment date and their corresponding body weights are summarized in the Animal Resource Record (Appendix IV).

C. Test Animals Housing and Caring

All newly received animals were identified by ear code, weighed and housed individually in a suspended cage system (Lab Products, Rochelle Park, NJ) equipped with an automatic water feeding system (System Engineering, Napa, CA) and were given free access to food (Purina Rodent Chow) and water. The chemical analysis of the lab chows and the water used in this study are presented in Appendix V A and B. The physiological conditions and the behavioral patterns of the test animals were observed and recorded twice daily. Daily observations were recorded in the raw data file submitted for storage at BSRC Chemical Archives under Protocol No. PPL-22-009-80. The daily food intake, water consumption, urine volume and feces weight for each experimental animal were recorded and presented in the Animal Physiological Record (Appendix VI).

Figure 1. Autoradiogram of ^{14}C-chlorophenyl-SD 43775 used in this study



D. Route of Administration

^{14}C-chlorophenyl-SD 43775 was formulated in corn oil immediately prior to treatment and the appropriate dose (8.4 mg/kg) was administered to the test animals by stomach intubation at a constant volume dose of 5 ml/kg. ^{14}C-chlorophenyl-SD 43775 treatment solution (50 mg) was prepared by diluting ^{14}C-chlorophenyl-SD 43775 stock (5.5 mg, specific activity 43.7 microCi/mg) with unlabeled SD 43775 (44.5 mg) in the final volume of 29.8 ml of corn oil. The final concentration of SD 43775 in this treatment solution was 1.68 mg/ml and had the final specific activity of 4.8 microCi/mg. This level of specific activity allowed the detection of ^{14}C-chlorophenyl-SD 43775 equivalent residues in the animal tissues at the 0.5 to 1% level of the applied radioactivity. Animals were fasted for 16 hours prior to dosing with free access to water. Control animals were treated with corn oil only. The actual dose of ^{14}C-chlorophenyl-SD 43775 for each individual animal received is summarized and presented in the Animal Physiological Record (Appendix VI). Food was returned to all control and treated animals one hour after dosing.

E. Animal Treatment Groups

Experimental animals were organized into the following treatment groups.

1. Control Treatment Groups

Five male and 5 female (80R001-80R010) (single oral, corn oil, 5 ml/kg). Animals were treated on June 18, 1980 and were maintained individually in a Nalgene Metabolism Cage unit (4 males and 4 females) and an all glass Stanford metabolism chamber (1 male and 1 female) for 7 days. The purposes of this contol treatment group are to provide the basic animal physiological parameters (such as daily food and water intake, urine and feces excretion profile, etc) of the treated animals maintained under laboratory experimental conditions. Individual body organs and tissues of the animal in the control treatment group were also used to establish the normal background level, sensitivity of the radioanalysis and the limit of determination of ^{14}C-chlorophenyl-SD 43775 equivalent residues by analytical procedures used in this study.

2. ^{14}C-Chlorophenyl-SD 43775 Treatment Group I

1 male (80R048) and 1 female (80R011) (single oral ^{14}C-chlorophenyl-SD 43775, 8.4 mg/kg). Animals were treated on July 16, 1980 and were maintained individually in an all-glass metabolism chamber for 7 days. The purposes of this treatment group was to provide basic information concerning:

a) The rate of carbon-14 dioxide generation in the respired air. If no $^{14}CO_2$ or other volatile radioactive materials were exhaled during the period from zero to 24 hours immediately after dosing, the requirement for the monitoring of the exhaled air from the treated animal is waived.

b) The rate of loss of applied radioactivity via urine and feces. This data will determine the length of test period of the actual ^{14}C-chlorophenyl-SD 43775 treatment group. The experimental protocol specified that the treated animal will be sacrificed 7 days after administration of the radioactive dose or until 95% of the administered dose was excreted.

c) To establish basic animal physiological parameters. Daily monitoring of the food and water intake, urine and fecal excretion profiles, etc. of the treated animals in the all-glass metabolism chamber under laboratory experimental conditions was carried out.

3. ^{14}C-Chlorophenyl-SD 43775 Treatment Group II

Five male (80R012,14,18,20 and 22) and 5 female (80R015, 17,19,21 and 23) (single oral, ^{14}C-chlorophenyl-SD 43775, 8.4 mg/kg); 1 male (80R024) and 1 female (80R025) (control, corn oil, single oral, 5 ml/kg). Animals were treated on July 22, 1980 and were maintained individually in Nalgene metabolism cage units for 5 days. Animal physiological parameters (daily food and water intake, urine and feces excretion profile) were recorded daily. The rates of excretion of ^{14}C-chlorophenyl-SD 43775 equivalent residues in the urine and feces were also monitored daily. At the end of the Day-5 holding period, animals were sacrificed and individual organ tissues were collected and radioassayed for ^{14}C-chlorophenyl-SD 43775 equivalent residues.

The actual dose of ^{14}C-chlorophenyl-SD 43775 and control corn oil carrier administered to each individual test animal are summarized in Table 1.

TABLE 1 SUMMARY OF TEST ANIMALS AND THEIR TREATMENT SCHEDULE

	ANIMAL ID	SEX	DOSE	HOLDING CONDITION
Control Treatment Group (corn oil)				
	80R001	F	0.94 ml[a)]	PC[b)]
	80R002	M	1.07 ml	PC
	80R003	F	0.91 ml	PC
	80R004	M	1.14 ml	PC
	80R005	F	0.93 ml	PC
	80R006	M	0.93 ml	PC
	80R007	F	0.92 ml	PC
	80R008	M	1.10 ml	PC
	80R009	F	1.02 ml	GC[c)]
	80R010	M	1.12 ml	GC
^{14}C-Chlorophenyl-SD 43775 Treatment Group I				
	80R011	F	1.72 mg[d)]	GC
	80R048	M	1.63 mg	GC
^{14}C-Chlorophenyl-SD 43775 Treatment Group II				
	80R012	M	1.97 mg[d)]	PC
	80R014	M	2.05 mg	PC
	80R015	F	1.65 mg	PC
	80R017	F	1.75 mg	PC
	80R018	M	2.08 mg	PC
	80R019	F	1.73 mg	PC
	80R020	M	2.03 mg	PC
	80R021	F	1.75 mg	PC
	80R022	M	1.95 mg	PC
	80R023	F	1.70 mg	PC
	80R024[e)]	M	1.16 ml[a)]	PC
	80R025[e)]	F	1.04 ml	PC

a) Dosage of corn oil (5 ml/kg)
b) Nalgene Metabolism cage unit
c) All-Glass Metabolism chamber
d) Dosgae of ^{14}C-chlorophenyl-SD 43775 (8.4 mg/kg)
e) Control animal

F. Preliminary All-Glass Metabolism Chamber Holding Study

A preliminary range-finding experiment was carried out using 1 female (80R011) and 1 male (80R048) rat treated with a single oral dosage of 8.4 mg/kg of ^{14}C-chlorophenyl-SD 43775. Animals were maintained in individual Stanford all-glass metabolism chamber (Model MC 3000, Stanford Glassblowing Laboratories, Palo Alto, CA) and allowed free access to food (one hour after dosing) and water. A schematic diagram of this all-glass metabolism chamber unit is presented in Appendix VII. This is an air-tight structure that allows a continuous monitoring of $^{14}CO_2$ generation and the separate collection of urine and feces excreta. $^{14}CO_2$ was collected over a CO_2 absorption tower which contained 250 ml of ethanolamine. Several minor modifications were added to this existing unit. A manifold system was used which provided an independent fine control of the amount of air passing through each individual metabolism chamber. Gas washing bottles (Laboratory Glass Apparatus, Berkeley, CA) were modified to provide a continuous monitoring of the generation of $^{14}CO_2$ during the entire holding periods. A detailed description of this experimental setup is presented in Figure 2.

G. Nalgene Metabolism Cages Holding Study

A control treatment group (5 male and 5 female) and the ^{14}C-chlorophenyl-SD 43775 treatment group II (5 male and 5 female) plus 2 control animals (1 male and 1 female) were maintained individually in Nalgene metabolism cage units (Sybron/Nalge Co., Rochester, NY). A description of the assembly of the Nalgene metabolism cage unit is presented in Appendix VIII. Although these metabolism units are not designed for the collection of $^{14}CO_2$ from the exhaled air of the treated animals, they were excellent and efficient in the separate collection of urine and feces excreta. A group of 10-12 metabolism cages were used in each experiment. Animals were allowed free access to food and water. Because of the poor design of the food container, animals in control treatment group were able to carry extra food pellets back into the main metabolism chamber, thus resulting in the contamination of both the urine and feces excreta. Modification of the food container on these Nalgene metabolism cages was carried out prior to the initiation of the ^{14}C-chlorophenyl-SD 43775 treatment group II study and resulted in the elimination of the cross contamination of the urine and feces excreta by the food diet (Figures 3 and 3A). A Detailed description of this modification is presented in a separate report (RIR-22-018-80).[a)]

a) RIR-22-018-80 Modification of the Diet Feeder in the Nalgene Metabolic Cages for Rats to Minimize Contamination of Excreta.

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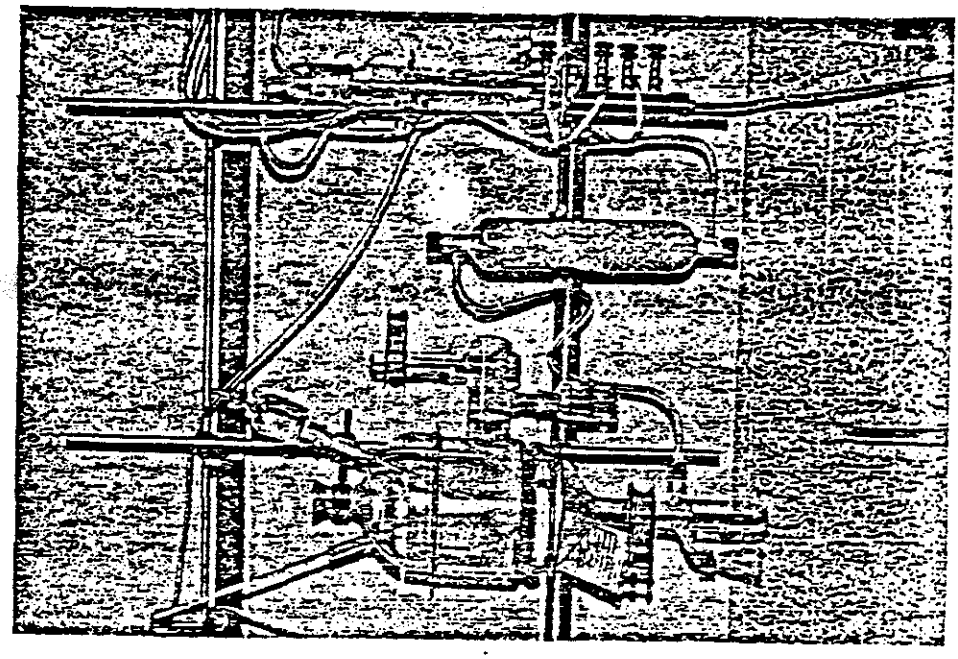


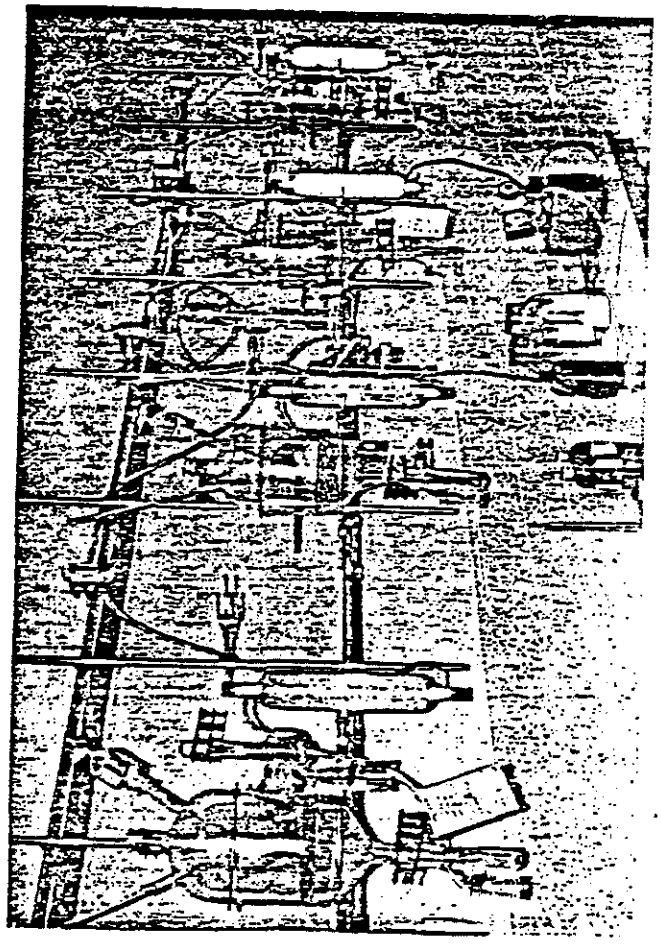


Figure 2 Experimental setup for the all glass metabolism chamber holding study

Figure 3 Experimental setup for the Nalgene plastic metabolism chamber unit

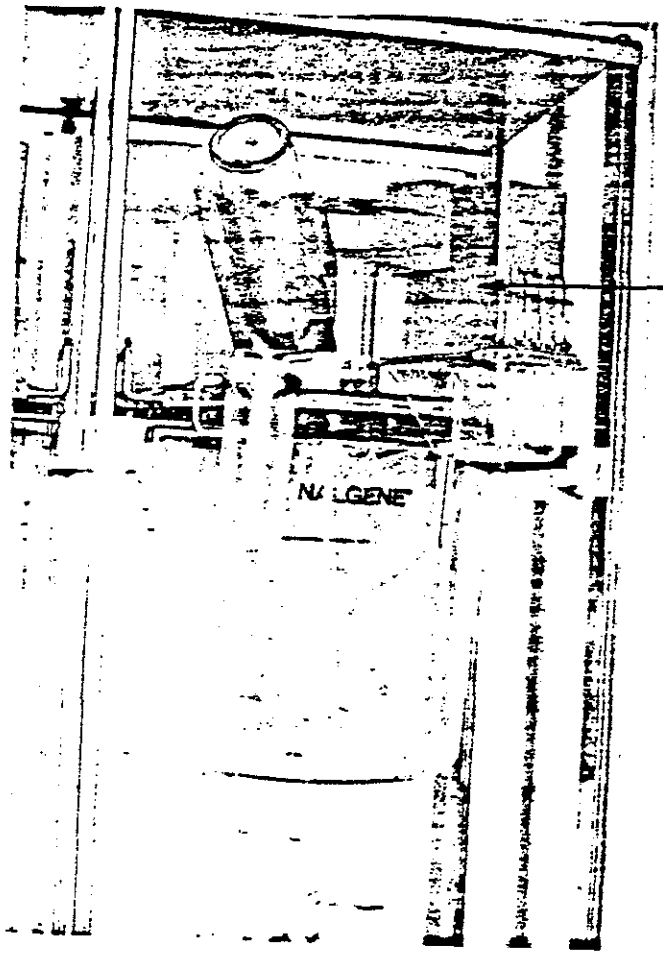
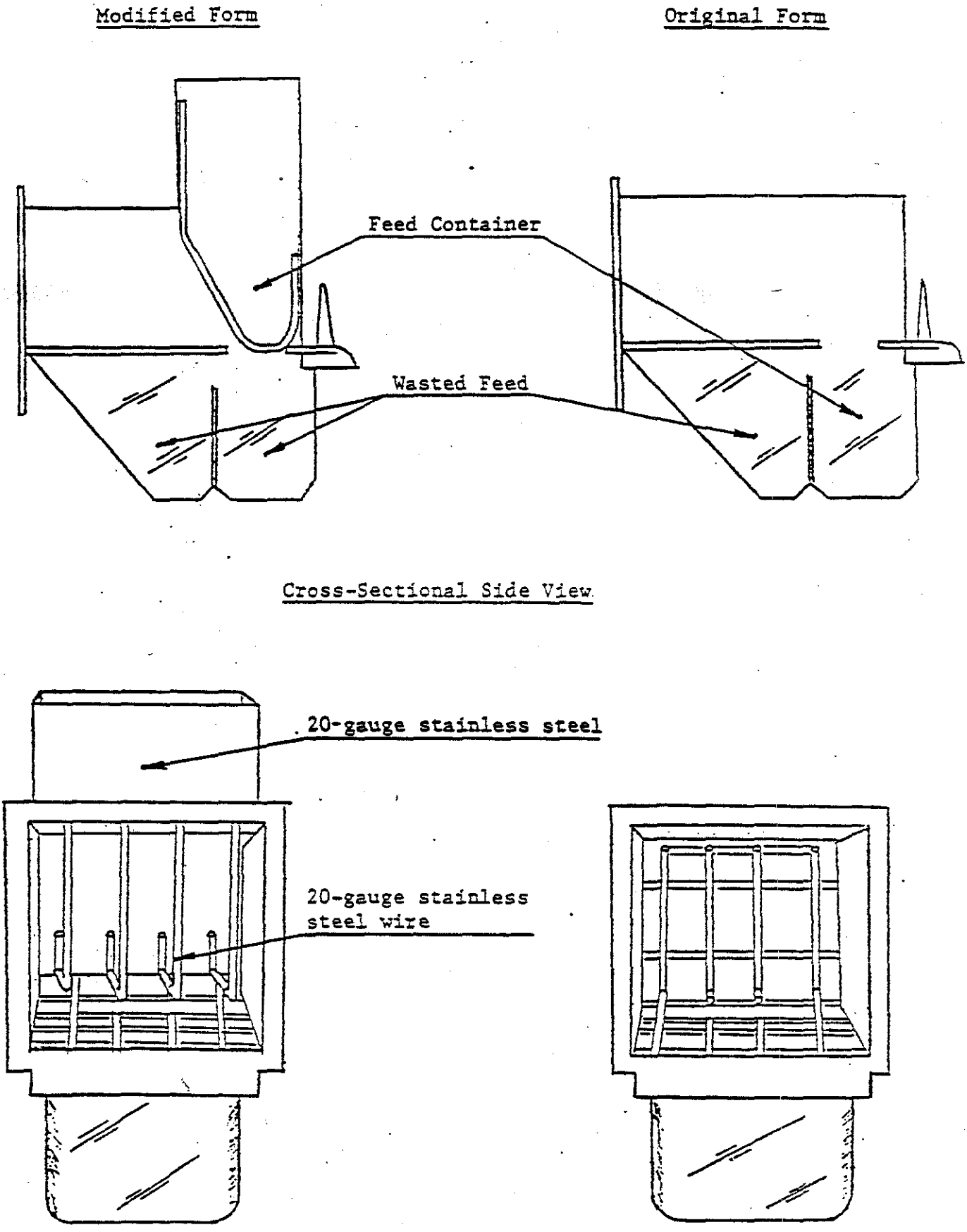


Figure 3A MODIFICATION OF METABOLISM FEED CHAMBER FOR RATS



H. Urine Sampling and Analysis

Urine excreta from all the control and ^{14}C-chlorophenyl-SD 43775 treated animals were collected daily and the total urine volume from each individual animal was recorded into the Animal Physiology Record (Appendix VI). The entire daily volume of urine of each individual animal was adjusted to a final volume of 25 ml with 0.01 M phosphate buffer (pH 7.4). From this buffered volume, triplicate 100-µl aliquots were quantitatively analyzed by LSC for calculating the total daily ^{14}C-chlorophenyl-SD 43775 equivalent residues present in the urine. All samples were analyzed immediately after collection. All urine samples were stored at 4°C prior to further qualitative analysis.

For the quantitative and qualitative examination of the excreted urinary metabolites, 15 ml aliquots of the Day-1 and Day-2 urine samples from each treated animal were combined for analysis. Analysis were carried out immediately after the termination of the animal holding period. Triplicate 100-µl aliquots were subjected to LSC quantitation. The pH of the combined urine sample was adjusted to pH 3 with 1 ml of 6N hydrochloric acid, and extracted three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate extraction were subjected to enzyme hydrolysis. Enzyme hydrolysis was carried out at 35 ± 1°C for 12 hours using approximately 1x10^4 units of the sulfatase/β-glucuronidase enzyme (No. S-9126, Sigma Chemical Co., one enzyme unit will hydrolyze 1 mole of nitrocatechol sulfate per hour at pH 5 at 37°C). Water-soluble conjugates released during enzyme hydrolysis were recovered by extracting the aqueous phase three times with equal volumes of ethyl acetate. Organic solvents were dried over anhydrous magnesium sulfate, concentrated and further analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after enzyme hydrolysis was quantitated by LSC. If the amount of the radioactivity remaining in this aqueous fraction represented greater than 5% of the initial applied radioactivity, this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by the acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the urine sample after the initial organic solvent extraction, enzyme hydrolysis and acid hydrolysis was considered as unextractable materials, and was not further qualitatively analyzed.

I. Sampling and Analysis of Fecal Material

Fecal excreta from all the control and ^{14}C-chlorophenyl-SD 43775 treated animals were collected daily and the total fecal wet weight from each animal was recorded into the Animal Physiological Record (Appendix VI). Daily fecal excretion was freeze dried in a freeze dryer (Thermovac Industries Corp.) for 24 hours and the final resultant dry weight was also recorded. Dried fecal material was pulverized by using a microanalytical mill (Tekmar Company). Percent of the applied radioactivity recovered in the daily fecal excreta was

quantitatively analyzed by oxygen combustion of triplicate fecal subsamples (approximately 100 mg each) by using a Packard Model 306B sample oxidizer and subsequent LSC quantitation. All samples were analyzed immediately after collection. All fecal samples were stored at 4°C prior to further qualitative analysis.

For the qualitative and quantitative examination of the excreted fecal metabolites, a 3-gram subsample of the Day-1 and Day-2 combined fecal excreta from each treated animal was analyzed. Analysis was carried out immediately after the termination of the animal holding period. Fecal excreta was first extracted three times (15 minutes each) using 30 ml of methanol-water (9:1) solvent mixture. The solvent extract and solid fecal materials were separated by centrifugation (2000 rpm for 10 minutes). The combined methanol-water solvent extract was quantitatively analyzed by LSC for the total amount of solvent extractable metabolites. The volume of the methanol-water solvent extract was then concentrated to approximately 15 ml by rotor evaporation. The final volume of this concentrated fecal solvent extract was adjusted to 30 ml using 0.01M phosphate buffer (pH 7.4) and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate solvent extract was dried over anhydrous magnesium sulfate, concentrated and the organo-soluble fecal metabolites of ^{14}C-chlorophenyl-SD 43775 were analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after organic solvent extraction was considered as fecal water-soluble conjugates and was quantitated by LSC. If the amount of radioactivity remaining in the aqueous fraction represented greater than 5% of the initial applied radioactivity, then this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the solid fecal residues after the initial methanol-water solvent extraction was considered as unextractable materials and was not further qualitatively analyzed.

J. Animal Sacrifice and Tissue Sampling Procedures

Control and treated animals were sacrificed 5 days after the administration of ^{14}C-chlorophenyl-SD 43775. Each animal was removed from its respective metabolism cage and its final body weight was recorded. Sacrifice was carried out by decapitation. Control animals were sacrificed prior to the ^{14}C-chlorophenyl-SD 43775 treated animals to minimize cross contamination.

The required tissues were excised from each experimental animal in the order that follows. Special care was taken to prevent contamination between individual tissues.

Whole blood, approximately 2 ml anticoagulated with ethylenediamine tetraacetate (EDTA, 10 mg), was collected from the severed carotid arteries in a 15-ml centrifuge tube immediately after sacrifice. Lung, heart, liver, kidney, gonads, inguinal fat, back fat, muscle (from the hind leg), and brain were obtained. All organs and tissue samples, except blood, were individually weighed, placed in prelabeled vials and stored frozen prior to analysis. All organ and tissue weights were recorded in the Animal Tissue Record (Appendix

IX). The remaining carcasses were labeled accordingly and sealed in polyester bags and stored frozen (-10°C).

Tissues were subsampled (triplicate subsamples approximately 100 mg) and quantitatively analyzed for total ^{14}C-chlorophenyl-SD 43775 equivalent residues by oxygen combustion techniques and LSC.

K. Radioassay

Radioactivity was quantitated by using a Packard Model 2660 Liquid Scintillation System. Counting efficiency determination was carried out by using the external standard ratio (ESR) technique. The actual quench curve was determined at a monthly interval to insure its validity. An example of the instrument print-out for the counting efficiency correlation with ESR is presented in Appendix X. Radioactivity was analyzed in 15 ml of Aquasol-2 scintillation solution. Radioactive areas on the TLC plate after solvent development were removed by scraping and analyzed for radioactivity in an Aquasol-2/water (11:4 ml) gel system.

Radioactive residues associated with animal tissues and fecal excreta were analyzed by weighing approximately 100 mg each of the subsample into a Combusto-Cone sample holder (Packard Instrument Co.) and combusted in a Packard Model 306B TriCarb sample oxidizer. Combustion efficiency of the individual tissue was determined using control animals and calibrated ^{14}C-chlorophenyl-SD 43775 solution as internal recovery standard. Counting solution included Carbo-Sorb and Permafluor V (10:12 ml) mixture.

L. Chromatographic Procedures

The chemical nature of the radioactivity recovered in the urine, feces and other selected organ tissues was qualitatively and quantitatively analyzed by TLC. Two-dimensional TLC (silica gel F-254, 0.25mm thickness, E. Merck) was performed in the following solvent system combinations.

(A) Hexane-acetone-HoAc (25:25:1)

(B) Toluene-ether-HoAc (75:25:1)

The R_f values of SD 43775 and other model metabolites are presented in Table 2. Reference standards were visualized under UV light. TLC plates were scanned for radioactivity by either using a Berthold LB 2760 TLC scanner or a Berthold LB 292 Beta Camera (Beta Analytical Inc.). Final visual confirmation of the distribution of ^{14}C-chlorophenyl-SD 43775 and its metabolites was carried out by autoradiography on Kodak SB-54 single-coated blue sensitive x-ray film (Eastman Kodak Co.).

Capillary gas-liquid chromatography was carried out using a 25m x 0.37mm I.D. SE-30 WCOT glass column in a Varian 3700 gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector. Isothermal analysis was carried out at injector, column and detector temperatures of 280, 245 and 320°C, respectively. The helium carrier gas and nitrogen make up gas flow rate through the detector were 3 and 36 ml/minute, respectively. On-column split ratio was controlled at 10:1 ratio.

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TABLE 2 R_f values of SD 43775 and its metabolites on Silica gel F 254 plates using two solvent systems

	R_f	
	System 1	System 2
SD 43775	0.78	0.72
SD 52667	0.73	0.68
SD 44064	0.70	0.38
SD 53065	0.62	0.23
SD 53919	0.58	0.13
SD 52666	0.48	0.09
SD 90930	0.24	0.03
SD 48838	0.62	0.42

Solvent system 1 Hexane-acetone-HoAc (25:25:1)
Solvent system 2 Toluene-ether-HoAc (75:25:1)

Mass spectroscopy in election impact mode (EIMS) was carried out on the Finnigan 3200 mass spectrometer with the instrument settings as follows:

GC setting

Stationary phase	SE-30
Solid support	WCOT column
Column size and material	1.2m x 0.3 mm ID, glass
Carrier	He approximately 3 ml/min
Column temp	100°C for 2 min; program @ 15°C/min. to 280°C
Transfer line	Approximately 250°C
Solvent Vent	at 2 min

MS setting

Electron energy	70 eV
Electron multiplier voltage	1800 V
Emission current	1.0 ma
Preamplifier sensitivity	10^{-7} a/v

Radio-gas-liquid chromatography (RGLC) was carried out using a Varian 1440 gas-liquid chromatograph equipped with a flame ionization detector and a Packard Model 894 gas-proportional counter. The column used was a 1m x 2mm I.D. glass column packed with 3% OV-101 on 80/100 mesh Supelcoport (Supelco Co.). The air, hydrogen and helium flow rates for RGLC were 210, 30 and 30 ml/minute, respectively. The helium and propane quench gas flow rates for the gas-proportional counter were 120 and 15 ml/minute, respectively.

M. Data Handling and Presentation

All raw data obtained from this metabolism study were recorded directly and promptly into laboratory notebooks designated for this study. Raw data are records of any original experimental observation which are necessary for the reconstruction of this experiment and the evaluation of this report. The following experimental records were also established as a permanent file on the HP-3000 computer system. Such files include the Animal Resource Record (Appendix IV), and the Animal Physiology Record (Appendix VI).

All experimental observations (urine volume, feces weight, tissue weight, subsample volume and weight and the radioactivity associated with these samples) were also presented as the Animal Tissue Record (Appendix IX) and the Excretion and Tissue Distribution Profile (Appendix XI). The percent of applied ^{14}C-chlorophenyl-SD 43775 equivalent residues recovered in the urine and fecal excreta of the treated animals were calculated by using Computer Program-2 (Appendix XII). Standard student's t-test was used to calculate the significant level between experimental and control animals.

All raw data from this study were submitted to the BSRC Archives under designated Protocol PPL-22-009-80 code number for permanent storage.

N. Biological Samples Storage and Retention

All biological samples generated from this study (animal excreta, organ tissues, animal carcasses, etc.) were packaged and stored in the Building 16 walk-in freezer at BSRC at -10°C under the designated Protocol PPL-22-009-80 code number for a minimum of five years.

O. Good Laboratory Practice Compliance Program

This metabolism study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guideline (CFR, vol. 43, no. 247, page 59986-60020, December 22, 1978). A Standard Operation Procedures (SOP) package designated for this metabolism study under the code number of Protocol PPL-22-009-80 was prepared and submitted as raw data to the BSRC Archives for permanent storage. The table of contents of this SOP package is presented in Appendix XIII.

EPA: 68-01-6561
TASK: 112
August 14, 1985

004631

109301

DATA EVALUATION RECORD

FENVALERATE

Metabolism Study in Rats

STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of [^{14}C]-phenoxyphenyl-SD 43775 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-020-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254117.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 8-13-85

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1. CHEMICAL: SD 43775, Pydrin insecticide, benzeneacetic acid, (S,R-(±)-)-4-chloro-α-(1-methylethyl)-,(S,R-(±)-)-cyano-(3-phenoxy-phenyl) methyl ester.

2. TEST MATERIAL: The test material was [^{14}C]-phenoxyphenyl]-SD 43775 with a specific activity of 52.7 μCi/mg and a radiochemical purity greater than 99.5 percent. A stock solution of [^{14}C]-phenoxy phenyl]-SD 43775 in dichloromethane was stored at -4°C prior to use. Unlabeled SD 43775 with a chemical purity of greater than 99.4 percent was also used.

3. STUDY/ACTION TYPE: Metabolism study in rats.

4. STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of [^{14}C]-phenoxyphenyl-SD 43775 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-020-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254117.

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7. CONCLUSIONS:

When Pydrin, [^{14}C]-phenoxyphenyl-SD 43775, was administered to male and female rats in a single dose at 8.4 mg/kg, radioactive material was rapidly eliminated in the feces and urine. Greater than 95 percent of the radioactivity was recovered in the excreta within five days after dosing, with almost all radioactivity being eliminated by 24 hours. [^{14}C]O_2 was not detected in the expired air of the dosed animals. There was no observable difference in the total elimination of radioactivity between male and female rats; the major route of elimination was fecal excretion (58-67%). The quantities of the recovered SD 43775 metabolites varied among individual test animals. There was no apparent sex difference in the chemical nature of these metabolic products. The primary urinary metabolic pathway was via cleavage of the ester linkage of SD 43775, after which the metabolite underwent further oxidation and conjugation. The alcohol moiety of SD 43775 was rapidly excreted as free molecules or as glucuronide or sulfate conjugates. SD 44607 and SD 46114 were identified as the primary urinary degradation products. SD 48838 and SD 46114 were identified as the primary fecal degradation products, with undegraded SD 43775 accounting for approximately 38 and 44 percent of the administered radioactivity in males and females, respectively. Tissue residue distribution data indicated the lack of bioconcentration of the SD 43775 equivalent residues in the blood, lung, heart, kidney, gonad, muscle, and brain tissue of the test animals. Significant levels of the SD 43775 equivalent residues were detected in liver (approximately 0.11 ppm) and fat tissues (approximately 1.66 and 1.17 ppm) in the male and female test animals, respectively. Further analysis indicated that greater than 50 percent of these residues in the body fat was undegraded SD 43775.

Under the conditions in which the study was conducted, it is acceptable.

Items 8 through 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test animals were male and female Sprague-Dawley rats (Simonsen's Laboratories, Gilroy, CA), 7 weeks of age, and weighing 175-200 g.

2. Control groups of 5 males and 5 females received a single dose of 5 ml/kg corn oil and were maintained to monitor basic

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[1]Only items appropriate to this DER have been included.

physiological parameters under sham conditions of the experimental groups. Four/sex were maintained in Nalgene metabolism cages and one/sex in Stanford all glass metabolism cages. Daily food and water consumption and urine and feces elimination were measured.

3. Treatment group 1 consisted of 1 male and 1 female given single oral doses of 8.4 mg/kg [^{14}C]-phenoxyphenyl-SD 43775. The animals were housed for two days in Stanford metabolism cages to monitor for radioactivity expired as [^{14}C]O_2 and/or excreted in urine and feces.

4. Treatment group 2 consisted of 5 rats of each sex given a single oral dose of 8.4 mg/kg test material and maintained individually for 5 days in Nalgene metabolism cages with slightly modified feeders that eliminated food contamination of collected urine and feces. Food and water intake were measured daily and urine and feces collected daily.

5. Urine volume was measured, the volume then brought to 25 ml with a 0.01M phosphate buffer (pH 7.2) and aliquots radioassayed in triplicate by liquid scintillation counting (LSC). For metabolite identification, 15 ml aliquots of urine samples for days 1 and 2 were combined, adjusted to pH 3 with 1 ml 6N HCl, and extracted 3 times with equal volumes of ethyl acetate. The resulting aqueous phase was adjusted to pH 5.0, enzymatically hydrolyzed with sulfatase and β-glucuronidase, and then extracted 3 times with equal volumes of ethyl acetate. If more than 5 percent of the initial radioactivity remained in the aqueous phase, the sample was subjected to acid hydrolysis for 4 hours at 90°C at pH 1.0 and then extracted with solvent. The organic phases were concentrated and subjected to thin layer chromatography (TLC).

6. Feces were weighed wet, freeze dried, and reweighed. Radioactivity was determined by LSC after combusting a 100 mg sample. For identification of metabolites, 3 g samples of feces collected on days 1 and 2 were combined, extracted 3 times with 30 ml MeOH:H_2O (9:1), and an aliquot of the extract radioassayed to determine total radioactivity. The extracts were concentrated, adjusted to pH 7.4 with 0.01 M phosphate buffer, extracted 3 times with equal volumes of ethyl acetate, and the organic phase brought to dryness. If the aqueous phases contained greater than 5 percent of the radioactivity, they were hydrolyzed at pH 1.0 (90°C) for 4 hours and extracted with ethyl acetate. The organic phases were subjected to two-dimensional TLC. Radioactivity remaining in the solid fecal residues after the initial MeOH-H_2O extraction was considered as unextractable; hence, it was not further qualitatively analyzed.

Because of the possible low level of radioactivity (the residual level of SD 43775 equivalent) in the fecal excreta and other biological tissues associated with these samples (1.5 to 2 times above the background level), the normal background in each of the control urine, fecal, and tissue samples were individually determined in order to establish a valid limit and sensitivity of detection. From these control data, a 99 percent upper confidence limit (UCL) was calculated. This 99 percent UCL was chosen as the concentration which must be exceeded before a treated tissue was assessed to have significant radioactive residue.

7. Animals were sacrificed at day 5, 2 ml blood collected in EDTA, and the following tissues trimmed and weighed: lung, liver, heart, kidneys, gonads, inguinal and back fat, muscle, and brain. Radioactivity was determined in triplicate 100 mg subsamples by combustion and LSC.

8. Radioactivity was determined for liquid samples in Aquasol-2 and for CO_2 from combusted samples in Carbosorb-Permafluor by liquid scintillation counting. Quench correction was determined monthly, and efficiency was determined by the external standard counting method. Combustion efficiency was determined on tissue samples of control animals to which an internal standard [^{14}C]-test material was added.

9. Thin layer chromatography solvents were: hexane/acetone/HOAc (25/25/1) and toluene/ether/HOAc (75/25/1). Reference standards were visualized with UV light, and areas containing [^{14}C] located by a Beta scanner and confirmed using autoradiography. Capillary GLC was performed with a Varian chromatograph equipped with an electron capture detector; radio-gas-liquid chromatography used a Varian equipped with flame ionization detector and a Packard gas proportional counter. Mass spectrometry used the electron impact mode.

B. Protocol: Materials and Methods are given in Appendix A in lieu of protocol.

12. REPORTED RESULTS:

A. Determination of the Limit and Sensitivity of Selection:

The combustion efficiency on all tissues ranged from 90-96 percent. The sensitivity level of detection for all control biological tissues ranged from 0.006 to 0.010 ppm. This indicated the uniformity of the test samples and the efficiency of the analytical procedures used in this study.

Based on the results obtained from the control treatment group, the specific activity of the SD 43775 treatment solution was adjusted to approximately 5 µCi/mg to allow the detection of a significant level of 0.5 to 1.0 percent of the applied radioactivity.

B. Preliminary All-Glass Metabolism Chamber Study: By monitoring an ethanolamine carbon dioxide trap at the 24 hour post treatment time intervals, it was determined that no $[^{14}C]O_2$ or other volatile radioactive material was generated in the respired air of the treated animals. Rapid elimination of radiolabel was noted in the urine and feces with about 92.9 and 98.7 percent of the administered dose eliminated during the initial 48 hours in male and female, respectively. Excreta were not further analyzed.

C. Animal Physiological Parameters: Under the experimental conditions of the study, there were no observable behavioral or toxicological abnormalities among the control and treated animals. There were also no differences in body weight gain, water or food intake, and fecal and urine excretion.

D. Urine and Feces Excretion Rate Profile (Treatment Group II): Most of the applied radioactivity in the urine was recovered from the test animals during the initial 24 hours post treatment (Table 1); females, approximately 31 percent (range 21-41 percent); males, 37 percent (range 26-42 percent). Radioactivity recovered in the day-2 urine accounted for approximately 2 percent of the administered dose in the males and females. Less than 2 percent of the radioactivity was recovered in the combined day 3, 4, and 5 urines. The total radioactivities detected in the urine of dosed male and female animals were approximately 41 percent (range 28-47 percent) and 34 percent (range 24-44 percent) of the administered dose, respectively.

In the feces, approximately 56 percent (range 43-72 percent) and 63 percent (range 51-73 percent) of the total administered radioactivity was recovered in the males and females, respectively, during the initial 24 hours post treatment (Table 1). Radioactivity recovered in the day-2 feces accounted for approximately 1 to 9 percent of the administered dose to male or female animals. Approximately 2 percent of the radioactivity was recovered in the combined day 3, 4, and 5 feces. The total radioactivity detected in the urine and feces of dosed male and female animals was approximately 99 percent (range 94-103) and 101 percent (range 96-107 percent), respectively.

E. Distribution of Urinary Metabolites: In males, approximately 39.2±7.5 percent of the administered radioactivity was eliminated in the urine during the initial 48 hours (combined day 1 and day 2 urinary excreta). Approximately 4.5±0.7 percent of the administered radioactivity (i.e., 11 percent of the recovered radioactivity in the urine) was extractable by ethyl acetate. Of the remaining radioactivity, 31.5±7.5 percent (80 percent) was water-soluble materials recovered by organic solvent extraction after enzyme hydrolysis with sulfatase and β-glucuronidase. Approximately 3.3 percent of the administered radioactivity remained in the aqueous fraction after the initial organic solvent extraction and enzyme hydrolysis. This aqueous fraction was not further analyzed.

TABLE 1. Distribution of the Administered [^{14}C]-Phenoxyphenyl-SD 43775 Equivalent Radioactivity in the Urine and Feces of Male and Female Animals following a Single Oral Administration of 8.4 mg/kg of the Test Compound

	Percent Recovery of the Administered Radioactivity on Days[a]					
	1	2	3	4	5	Total
Urine						
Female	30.6 ± 7.4	2.1 ± 0.5	0.7 ± 0.4	0.4 ± 0.2	0.2 ± 0.0	34.0 ± 7.7
Male	36.9 ± 6.5	2.4 ± 1.2	0.8 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	40.8 ± 7.9
Feces						
Female	62.8 ± 1.0	3.5 ± 3.0	0.4 ± 0.2	0.2 ± 0.2	0.1 ± 0.0	67.0 ± 11.7
Male	56.0 ±11.3	1.6 ± 0.7	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	58.2 ± 10.6

[a]Mean value ± standard deviation.

Undegraded SD 43775 was not detected in the ethyl acetate extract. The following degradation products of [^{14}C]-phenoxyphenyl-SD 43775 were detected: SD 44607 (2-3 percent of the administered radioactivity), SD 46114 (approximately 1-2 percent), and 3-4 other unknown minor urinary degradation products (Table 2; Figure 1).

Analysis of water-soluble conjugates after enzyme hydrolysis with sulfatase and β-glucuronidase showed that SD 46114 (18-35 percent of the administered radioactivity) was the major SD 43775 metabolite detected; there were also several other unknown minor urinary metabolites (Table 2). Radioactive material remaining in the aqueous phase after the initial ethyl acetate extraction and enzyme hydrolysis was not analyzed further.

In females, approximately 32.7±7.4 percent of the administered radioactivity was eliminated in the urine during the initial 48 hours. Approximately 6.9±3.3 percent of the administered radioactivity (or 21 percent of the recovered radioactivity in the urine) was extractable in ethyl acetate; 23.6±5.3 percent was water-soluble materials that were released by enzyme hydrolysis, and 2.3±0.6 percent was unextractable residues. There was no qualitative difference in the chemical nature or distribution of SD 43775 urinary metabolites between the male and females. SD 44607 (1-3 percent of the administered radioactivity) and SD 46114 (2-7 percent) were identified as the primary degradation products of SD 43775 in the organic extractable fraction of the female urine. There were several other unknown minor metabolites which constituted about 1-3 percent of the total administered radioactivity (Table 2; Figure 1). In the water-soluble material, SD 46114 (15-26 percent of the administered radioactivity) was recovered as the major degradation product of SD 43775 (Table 2). There were several other unidentified minor products detected that accounted for about 1-3 percent of the total administered radioactivity.

F. Distribution of Fecal Metabolites: In males, approximately 57.6±10.7 percent of the administered radioactivity (or 99 percent of the recovered radioactivity in the combined day 1 and day 2 feces) was extractable with the methanol water solvent system. Approximately 7.2 ± 4.9 percent of the administered radioactivity (or 12 percent of the radioactivity recovered from the feces) was unextractable residues, and no attempt was made to further qualify these materials.

The methanol-water extract was then partitioned with ethyl acetate. The majority of the extractable residues were recovered in the ethyl acetate phase with less than 3 percent of the administered radioactivity remaining in the aqueous fraction as water-soluble materials.

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TABLE 2. Distribution of [^{14}C]-Phenoxyphenyl-SD 43775 Metabolites in the Urinary Excreta of Male and Female Animals

	Percent Recovery of Radioactivity Administered	
	Male	Female
Organic Extractable Fraction		
SD 44607	**2.0 ±0.39[b]**	**1.58±0.89**
SD 46114	1.16±0.44	3.06±2.10
Other[a]	1.36±0.38	2.24±1.21
Total	4.52±0.69	6.88±3.27
Water Soluble Fraction		
Released by Enzyme Hydrolysis	31.48±7.53	23.56±5.30
SD 46114	28.88±6.86	21.34±5.23
Other[a]	2.60±0.85	2.22±0.30
Unextractable	3.26±0.38	2.26±0.61
Total	**34.72±7.66**	**25.82±5.47**

[a] Radioactivity associated with the origin of the TLC plate and other unidentified minor metabolites.

[b] Mean value ± standard deviation.

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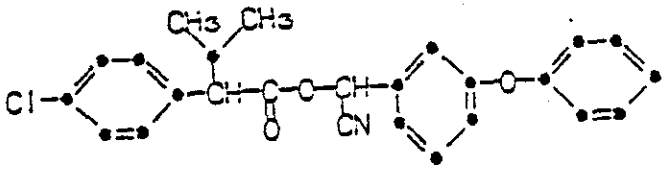
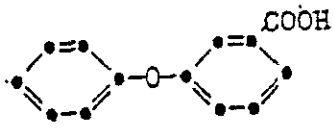
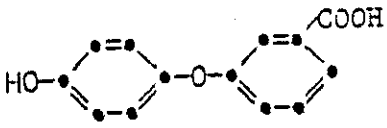
Figure 1. TABLE OF COMPOUNDS



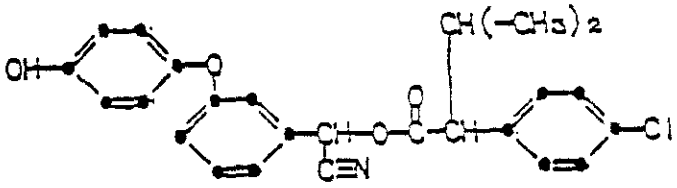
SD 43775: Benzeneacetic acid, 4-chloro--α-(1-methylethyl)-, cyano-(3-phenoxyphenyl)methyl ester.



SD 44607: 3-Phenoxy-benzoic acid



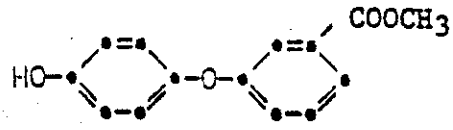
SD 46114: 3-(4-Hydroxyphenoxy)-benzoic acid



SD 48838: Benzeneacetic acid, 4--chloro-α-(1-methylethyl)-, cyano--(3-phenoxy-4-hydroxyphenyl)methyl ester.

C032-1

TABLE OF COMPOUNDS



SD 48389: 3-(4-Hydroxyphenoxy)-benzoic acid methyl ester

The SD 43775 metabolites were identified by TLC and subsequently by MS analysis. In addition to the undegraded SD 43775, SD 48838 (3-5 percent of the administered radioactivity), was identified as the primary fecal degradation product. Undegraded SD 43775 accounted for approximately 37.7±7.2 percent of the administered radioactivity (range 30-48 percent). There were 3 or 4 other minor metabolites observed (Table 3; Figure 1).

In the females, approximately 58.4±12.5 percent of the administered radioactivity (or 88 percent of the radioactivity recovered in the combined day 1 and day 2 feces) was extractable by the methanol-water (9:1) solvent system. The remaining 7.9±3.0 percent of the administered radioactivity (or 12 percent of the recovered radioactivity in the feces) was unextractable residues, and no attempt was made to further qualify these materials.

The methanol-water extract was then partitioned with ethyl acetate Most of the radioactivity was recovered in the ethyl acetate phase with less than 4 percent of the radioactivity remaining in the aqueous fraction. There were no observable qualitative differences in the chemical nature of the SD 43775 fecal metabolites between male and female animals. Undegraded SD 43775 accounted for 44.0±12.0 percent of the radioactivity administered (range of 30-58 percent), and SD 48838 (3-5 percent) was identified as the primary fecal metabolite (Table 3; Figure 1).

G. Tissue Distribution of SD 43775 Equivalent Residues: A summary of the distribution of SD 43775 equivalent residues in various organ tissues of male and female test animals is presented in Table 4. The concentrations of SD 43775-equivalent residues detected in blood, lung, heart, gonads, and muscle were slightly above the level of detection. In the liver, where the SD 43775 equivalent residues were approximately 3 times the upper limit of detection, their distributions were determined in whole tissue, the soluble fraction or the proteinaceous fraction. The data showed that 40 percent of the residues were present in the soluble fraction and that the remaining 60 percent of the equivalent residues were present in the proteinaceous fraction. The SD 43775 equivalent residues detected in the kidney were approximately 2 times the upper limit of detection.

The SD 43775-equivalent residues in the fat were approximately 31-44 times the upper limit of detection. The distribution of radiolabeled residues was determined in whole tissue, in the hexane extract following homogenization, and in the non-extractable residues. In males, approximately 67-83 percent of the radioactivity associated with fat tissues was extracted by hexane, and approximately 34-48 percent of the total tissue residues (0.72-0.9 ppm) was identified as unchanged SD 43775. In females, approximately 57-61 percent of the radioactivity associated with fat tissues was extracted, and approximately 49-54 percent of the total tissue residues (0.86-0.77 ppm) was unchanged SD 43775. The level of SD 43775-equivalent residues in the brain were below the limit of detection.

TABLE 3. Distribution of [^{14}C]-Phenoxyphenyl-SD 43775 Fecal Metabolites in Male and Female Rats

	Percent Recovery of Administered Radioactivity	
	Male	Female
Organic Extractable	48.12± 7.12	54.70±11.70
SD 43775	**37.74± 7.22**	**43.98±12.03**
SD 48838	3.78± 0.97	4.02± 0.91
SD 46114	1.52± 0.27	3.52± 1.12
Other[a]	5.08± 1.63	3.22± 1.43
Water Soluble	2.28± 0.40	3.72± 1.49
Unextractable Residues	7.18± 4.86	7.86± 3.00
Total	57.58±10.71	66.28±11.60

[a] Radioactivity remaining in the origin of TLC plate and minor metabolites.

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TABLE 4. A Summary of the Residue Level of [^{14}C]-Phenoxyphenyl-SD 43775 Equivalent in the Various Organ Tissues of Male and Female Test Animals[a]

	µg SD 43775 Equivalent/ g wet tissue (ppm)	
	Male	Female
Blood	0.051±0.005	0.054±0.006
Lung	0.052±0.002	0.047±0.006
Heart	0.042±0.001	0.041±0.001
Liver	0.111±0.023	0.113±0.023
Kidney	0.069±0.008	0.079±0.012
Fat	1.655±0.428	1.174±0.397
Gonad	0.043±0.004	0.135±0.047
Muscle	0.042±0.007	0.043±0.010
Brain	< 0.038	< 0.038

[a] Five male and five female test animals.

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13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. After a single oral dose of 8.4 mg/kg [^{14}C]-phenoxyphenyl-SD 43775 was administered to male and female rats, there was rapid elimination of radioactivity in the urine and feces. Most of the radioactivity was eliminated in the first 24 hours, and no exhaled radioactivity was detected. Excretion was similar in males and females. The major route of elimination was the feces.

The examination of urinary metabolites by chromatography and GC-MS indicated that the major metabolic activity resulted in cleavage of the ester linkage in the parent molecule which was followed by further oxidation. The alcohol moiety of SD 43775 was rapidly excreted as free molecules or as glucuronide or sulfate conjugates. SD 44607 and SD 46114 were the primary urinary degradation products; SD 48838 and SD 46114 were the primary fecal degradation products. Approximately 38 and 44 percent of the test material's total radioactivity was recovered as undegraded SD 43775 in the feces of males and females, respectively.

Tissue residue distribution data showed a lack of bioconcentration of radioactive residues in blood, lung, heart, kidney, gonad, muscle, and brain. Detectable levels of radioactive residues were found in the liver (approximately 0.11 ppm equivalents) and fat (1.66 and 1.17 ppm equivalents/g in males and females, respectively). Further analysis indicated that greater than 50 percent of the residues in body fat were undegraded SD 43775.

B. A quality assurance statement was not included in the report. However, this study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guidelines.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors' conclusions were supported by individual animal data. The protocol was adequate to determine the metabolism, tissue distribution, and excretion of the test material and its metabolites, and the study was well conducted and reported; we assess that the study was acceptable for showing the metabolism of SD 43775.

Although there was variability among animals in percent of radioactivity in the various fractions or metabolites, this is not unusual in metabolism studies and sufficient animals were used to calculate mean values for all parameters. The data indicate rapid elimination of radiolabeled material in urine and feces with most of the radioactivity being eliminated during the first 24 hours. The compound is hydrolyzed at the ester linkage followed by oxidation and/or conjugation and elimination in the urine; the alcohol moiety is also excreted as free molecules. Several metabolites were also found in the feces, but the undegraded parent compound was the major component. Tissue residues were very low except for the liver (0.11 ppm) and fat tissues (1.5 ppm) which were relatively higher. There were no major differences in the metabolic pattern among females and males.

Item 15 - see footnote 1.

16. APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-17.

APPENDIX A

Materials and Methods

Appendix # 12 A
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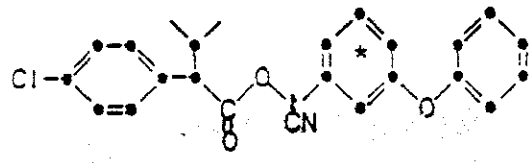
RIR-22-020-80

III. Description of the Conduct of Experiment

A. Test Compound

SD 43775 labeled with carbon-14 at the phenoxyphenyl-postion was used.



SD 43775

*Denotes carbon-14.

The specific activity of the ^{14}C-phenoxyphenyl-SD 43775 was 52.7 microCuries/mg as determined by capillary gas-liquid chromatography (GLC) and liquid scintillation counting (LSC). Detailed calculations of the specific activity of the ^{14}C-phenoxyphenyl-SD 43775 is presented in Appendix II. Based on the sensitivity of detection, the radiochemical purity of ^{14}C-phenoxyphenyl-SD 43775 was greater than 99.5% as determined by two-dimensional thin-layer chromatography (TLC) (Figure 1) and LSC. Concentrated ^{14}C-phenoxyphenyl-SD 43775 stock solution (2.85 mg/ml in dichloromethane) was stored at -4°C. Unlabeled SD 43775 (analytical standard code 10-1-0-0) was also used in this study and its chemical purity was greater than 99.4% (Appendix III).

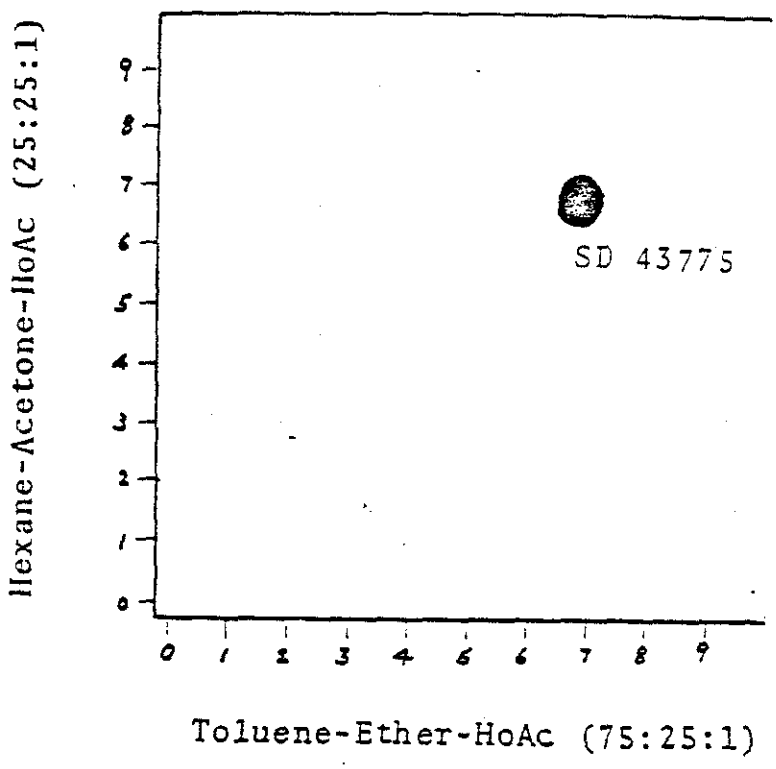
B. Test Animals

Male and female Sprague-Dawley albino rats (SIM:SD f1 strain, 7 weeks old, weighing from 175-200 grams each) were obtained from Simonsen's Laboratories, Gilroy, California. The number of total test animals, their experimental identification number, sexes, arrival date, initial body weight, experimental treatment date and their corresponding body weights are summarized in the Animal Resource Record (Appendix IV).

C. Test Animals Housing and Caring

All newly received animals were identified by ear code, weighed and housed individually in a suspended cage system (Lab Products, Rochelle Park, NJ) equipped with an automatic water feeding system (System Engineering, Napa, CA) and were given free access to food (Purina Rodent Chow) and water. The chemical analysis of the lab chows and the water used in this study are presented in Appendix V A and B. The physiological conditions and the behavioral patterns of the test animals were observed and recorded twice daily. Daily observations were recorded in the raw data file submitted for storage at the BSRC Chemical Archives under Protocol Number PPL-22-009-80. The daily food intake, water consumption, urine volume and feces weight for each experimental animal were recorded and presented in the Animal Physiological Record (Appendix VI).

Figure 1 Autoradiogram of ^{14}C-phenoxyphenyl-SD 43775 used in this study



D. Route of Administration

^{14}C-phenoxyphenyl-SD 43775 was formulated in corn oil immediately prior to treatment and the appropriate dose (8.4 mg/kg) was administered to the test animals by stomach intubation at a constant volume dose of 5 ml/kg. ^{14}C-phenoxyphenyl-SD 43775 treatment solution (50 mg) was prepared by diluting ^{14}C-phenoxyphenyl-SD 43775 stock (4.6 mg, specific activity 52.7 microCi/mg) with unlabeled SD 43775 (45.4 mg) in the final volume of 29.8 ml of corn oil. The final concentration of SD 43775 in this treatment solution was 1.68 mg/ml and had the final specific activity of 5.1 microCi/mg. This level of specific activity allowed the detection of ^{14}C-phenoxyphenyl-SD 43775 equivalent residues in the animal tissues at the 0.5 to 1% level of the applied radioactivity. Animals were fasted for 16 hours prior to dosing with free access to water. Control animals were treated with corn oil only. The actual dose of ^{14}C-phenoxyphenyl-SD 43775 each individual animal received is summarized and presented in the Animal Physiological Record (Appendix VI). Food was returned to all control and treated animals one hour after dosing.

E. Animal Treatment Groups

Experimental animals were organized into the following treatment groups.

1. Control Treatment Groups

Five male and 5 female (80R001-80R010) (single oral, corn oil, 5 ml/kg). Animals were treated on June 18, 1980 and were maintained individually in a Nalgene Metabolism Cage unit (4 males and 4 females) and an all glass Stanford metabolism chamber (1 male and 1 female) for 7 days. The purposes of this contol treatment group are to provide the basic animal physiological parameters (such as daily food and water intake, urine and feces excretion profile, etc) of the treated animals maintained under laboratory experimental conditions. Individual body organs and tissues of the animal in the control treatment group were also used to establish the normal background level, sensitivity of the radioanalysis and the limit of determination of ^{14}C-phenoxyphenyl-SD 43775 equivalent residues by analytical procedures used in this study.

2. ^{14}C-Phenoxyphenyl-SD 43775 Treatment Group I

One male (80R050) and 1 female (80R013) (single oral ^{14}C-phenoxyphenyl-SD 43775, 8.4 mg/kg). Animals were treated on July 16, 1980 and were maintained individually in an all-glass metabolism chamber for 7 days. The purpose of this treatment group was to provide basic information concerning:

a) The rate of carbon-14 dioxide generation in the respired air. If no $^{14}CO_2$ or other volatile radioactive materials were exhaled during the period from zero to 24 hours immediately after dosing, the requirement for the monitoring of the exhaled air from the treated animal is waived.

b) The rate of loss of applied radioactivity via urine and feces. This data will determine the length of test period of the actual ^{14}C-phenoxyphenyl-SD 43775 treatment group. The experimental protocol specified that the treated animal will be sacrificed 7 days after administration of the radioactive dose or until 95% of the administered dose was excreted.

c) To establish basic animal physiological parameters. Daily monitoring of the food and water intake, urine and fecal excretion profiles, etc., of the treated animals in the all-glass metabolism chamber under laboratory experimental conditions was carried out.

3. ^{14}C-Phenoxyphenyl-SD 43775 Treatment Group II

Five male (80R056,58,60,62 and 64) and 5 female (80R045, 47,49,53 and 55) (single oral, ^{14}C-phenoxyphenyl-SD 43775, 8.4 mg/kg); 1 male (80R054) and 1 female (80R051) (control, corn oil, single oral, 5 ml/kg). Animals were treated on August 13, 1980 and were maintained individually in a Nalgene metabolism cage unit for 5 days. Animal physiological parameters (daily food and water intake, urine and feces excretion profile) were recorded daily. The rates of excretion of ^{14}C-phenoxyphenyl-SD 43775 equivalent residues in the urine and feces were also monitored daily. At the end of the Day-5 holding period, animals were sacrificed and individual organ tissues were collected and radioassayed for ^{14}C-phenoxyphenyl-SD 43775 equivalent residues.

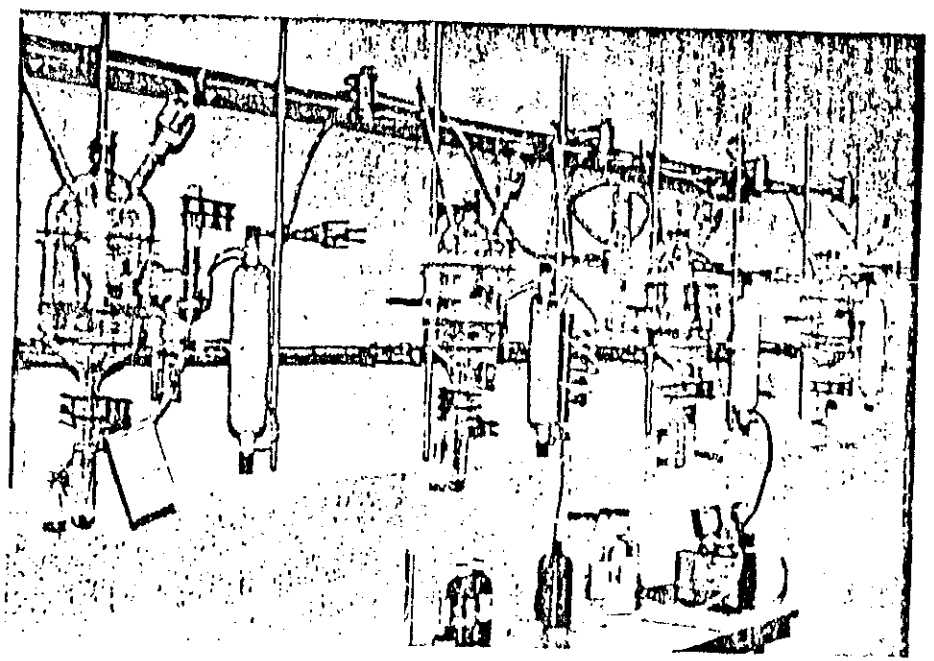
The actual dose of ^{14}C-phenoxyphenyl-SD 43775 and control corn oil carrier administered to each individual test animal are summarized in Table 1.

F. Preliminary All-Glass Metabolism Chamber Holding Study

A preliminary range-finding experiment was carried out using 1 female (80R013) and 1 male (80R050) rat treated with a single oral dosage of 8.4 mg/kg of ^{14}C-phenoxyphenyl-SD 43775. Animals were maintained in individual Stanford all-glass metabolism chamber (Model MC 3000, Stanford Glassblowing Laboratories, Palo Alto, CA) and allowed free access to food (one hour after dosing) and water. A schematic diagram of this all-glass metabolism chamber unit is presented in Appendix VII. This is an air-tight structure that allows a continuous monitoring of $^{14}CO_2$ generation and the separate collection of urine and feces excreta. $^{14}CO_2$ was collected over a CO_2 absorption tower which contained 250 ml of ethanolamine. Several minor modifications were added to this existing unit. A manifold system was used which provided an independent fine control of the amount of air passing through each individual metabolism chamber. Gas washing bottles (Laboratory Glass Apparatus, Berkeley, CA) were modified to provide a continuous monitoring of the generation of $^{14}CO_2$ during the entire holding periods. A detailed description of this experimental setup is presented in Figure 2.

	ANIMAL ID	SEX	DOSE	HOLDING CONDITION
Control Treatment Group (corn oil)	80R001	F	0.94 ml[a]	PC [b]
	80R002	M	1.07 ml	PC
	80R003	F	0.91 ml	PC
	80R004	M	1.14 ml	PC
	80R005	F	0.93 ml	PC
	80R006	M	0.93 ml	PC
	80R007	F	0.92 ml	PC
	80R008	M	1.10 ml	PC
	80R009	F	1.02 ml	GC [c]
	80R010	M	1.12 ml	GC
^{14}C-Phenoxyphenyl-SD 43775 Treatment Group I				
	80R013	F	1.53 mg[d]	GC
	80R050	M	1.71 mg	GC
^{14}C-Phenoxyphenyl-SD 43775 Treatment Group II				
	80R045	F	1.51 mg[d]	PC
	80R047	F	1.50 mg	PC
	80R049	F	1.57 mg	PC
	80R053	F	1.52 mg	PC
	80R055	F	1.50 mg	PC
	80R056	M	1.70 mg	PC
	80R058	M	1.73 mg	PC
	80R060	M	1.69 mg	PC
	80R062	M	1.63 mg	PC
	80R064	M	1.71 mg	PC
	80R051[e]	F	0.86 ml[a]	PC
	80R054[e]	M	1.00 ml	PC

a) Dosage of corn oil (5 ml/kg)
b) Nalgene Metabolism cage unit
c) All-glass metabolism chamber
d) Dosage of ^{14}C-phenoxyphenyl-SD 43775 (8.4 mg/kg)
e) Control animal



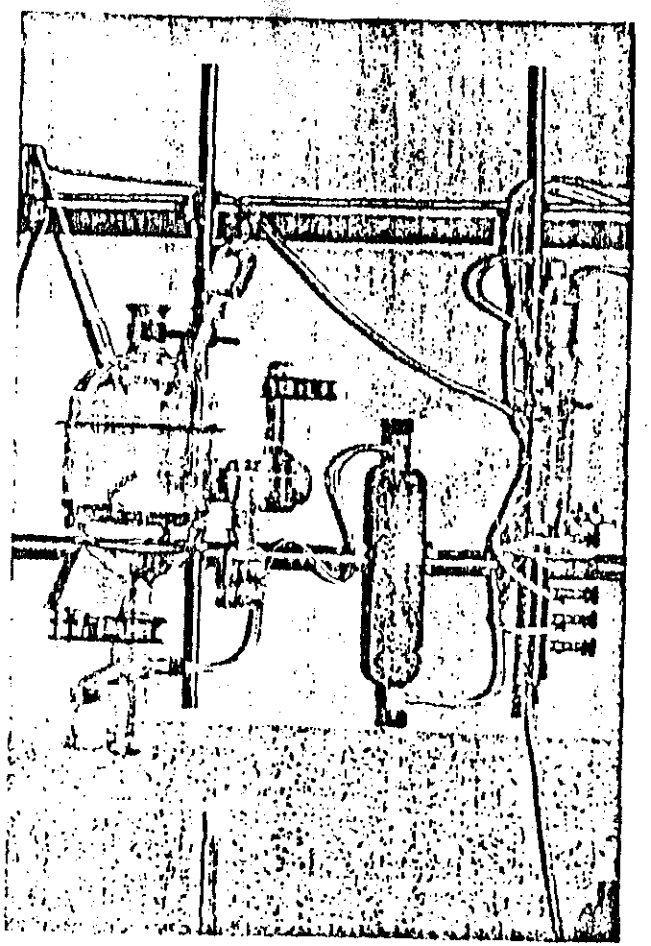


Figure 2 Experimental setup for the all glass metabolism chamber holding study

G. Nalgene Metabolism Cages Holding Study

A control treatment group (5 male and 5 female) plus 2 control animals (1 male and 1 female) were maintained individually in Nalgene metabolism cage units (Sybron/Nalge Co., Rochester, NY). A description of the assembly of the Nalgene metabolism cage unit is presented in Appendix VIII. Although these metabolism units are not designed for the collection of $^{14}CO_2$ from the exhaled air of the treated animals, they were excellent and efficient in the separate collection of urine and feces excreta. A group of 10-12 metabolism cages were used in each experiment. Animals were allowed free access to food and water. Because of the poor design of the food container, animals (in control treatment group) were able to carry extra food pellets back into the main metabolism chamber, thus resulting in the contamination of both the urine and feces excreta. Modification of the food container on these Nalgene metabolism cages was carried out prior to the initiation of the ^{14}C-phenoxyphenyl-SD 43775 treatment group II study and resulted in the elimination of the cross contamination of the urine and feces excreta by the food diet (Figures 3 and 3A). Detailed description of this modification is presented in a separate report (RIR-22-018-80).[a)]

H. Urine Sampling and Analysis

Urine excreta from all the control and ^{14}C-phenoxyphenyl-SD 43775 treated animals were collected daily and the total urine volume from each individual animal was recorded into the Animal Physiology Record (Appendix VI). The total volume of each individual daily urine excreta was then adjusted to the final volume of 25 ml with 0.01M phosphate buffer (pH 7.4). Triplicate 100-µl aliquots were sampled and the total daily ^{14}C-phenoxyphenyl-SD 43775 equivalent residues present in the urine excreta was quantitatively analyzed by LSC. All samples were analyzed immediately after collection. All urine samples were stored at 4°C prior to further qualitative analysis.

For the quantitative and qualitative examination of the excreted urinary metabolites, 15 ml aliquots of the Day-1 and Day-2 urine samples from each treated animal were combined for analysis. Analysis was carried out immediately after the termination of the animal holding period. Triplicate 100-µl aliquots were subjected to LSC quantitation. The pH of the combined urine sample was adjusted to pH 3 with 1 ml of 6N hydrochloric acid, and extracted three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

a) RIR-22-018-80 Modification of the Diet Feeder in the Nalgene Metabolic Cages for Rats to Minimize Contamination of Excreta.

Figure 3 Experimental setup for the Nalgene plastic metabolism chamber unit

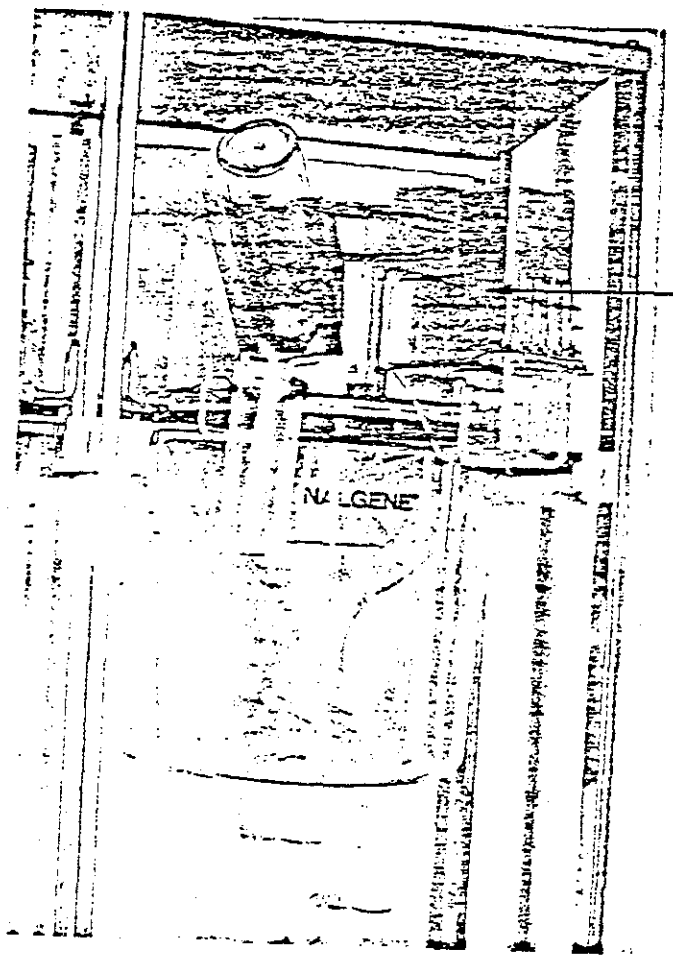
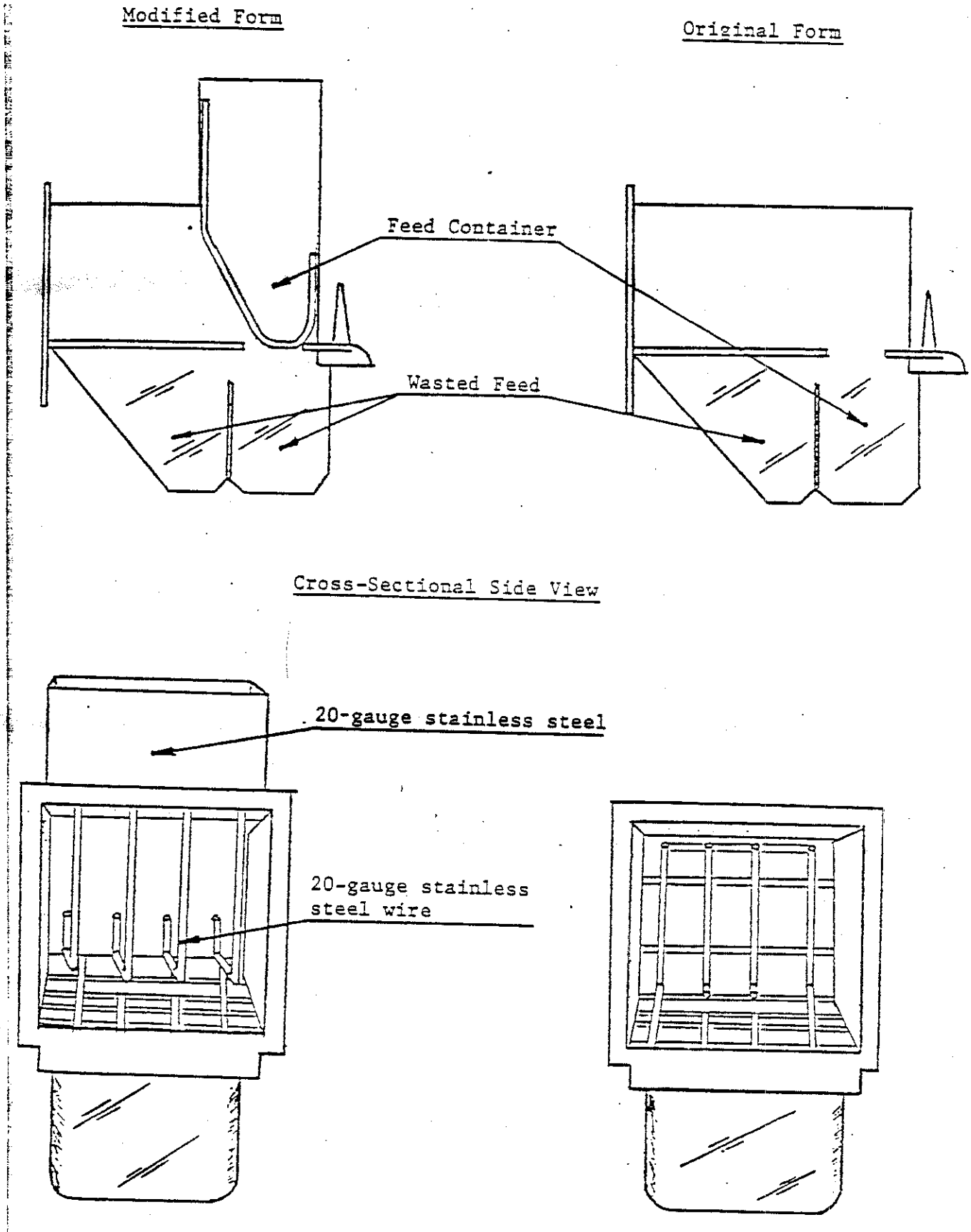


Figure 3A MODIFICATION OF METABOLISM FEED CHAMBER FOR RATS



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Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate xtraction were subjected to enzyme hydrolysis. Enzyme hydrolysis was carried out at 35 ± 1°C for 12 hours using approximately $1x10^4$ units of the sulfatase/β-glucuronidase enzyme (No. S-9126, Sigma Chemical Co., one enzyme unit will hydrolyze 1 µmole of nitrocatechol sulfate per hour at pH 5 at 37°C). Water-soluble conjugates released during enzyme hydrolysis were recovered by extracting the aqueous phase three times with equal volumes of ethyl acetate. Organic solvents were dried over anhydrous magnesium sulfate, concentrated and further analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after enzyme hydrolysis was quantitated by LSC. If the amount of the radioactivity remaining in this aqueous fraction represented greater than 5% of the initial applied radioactivity, this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by the acid hydrolysis was recovered by organic solvent extraction (three times with an equal volume of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the urine sample after the initial organic solvent extraction, enzyme hydrolysis and acid hydrolysis was considered as unextractable materials, and was not further qualitatively analyzed.

I. Sampling and Analysis and Fecal Material

Fecal excreta from all the control and ^{14}C-phenoxyphenyl-SD 43775 treated animals were collected daily and the total fecal wet weight from each animal was recorded into the Animal Physiological Record (Appendix VI). Daily fecal excretion was freeze dried in a freeze dryer (Thermovac Industries Corp.) for 24 hours and the final resultant dry weight was also recorded. Dried fecal material was pulverized by using a microanalytical mill (Tekmar Company). Percent of the applied radioactivity recovered in the daily fecal excreta was quantitatively analyzed by oxygen combustion of triplicate fecal subsamples (approximately 100 mg each) by using a Packard Model 306B sample oxidizer and subsequent LSC quantitation. All samples were analyzed immediately after collection. All fecal samples were stored at 4°C prior to further qualitative analysis.

For the qualitative and quantitative examination of the excreted fecal metabolites, a 3-gram subsample of the Day-1 and Day-2 combined fecal excreta from each treated animal was analyzed. Analysis was carried out immediately after the termination of the animal holding period. Fecal excreta was first extracted three times (15 minutes each) using 30 ml of methanol-water (9:1) solvent mixture. The solvent extract and solid fecal materials were separated by centrifugation (2000 rpm for 10 minutes). The combined methanol-water solvent extract was quantitatively analyzed by LSC for the total amount of solvent extractable metabolites. The volume of the methanol-water solvent extract was then concentrated to approximately 15 ml by rotor evaporation. The final volume of this concentrated fecal solvent extract was adjusted to 30 ml using 0.01M phosphate buffer (pH 7.4) and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate solvent extract was dried over anhydrous magnesium sulfate, concentrated and the organo-soluble fecal metabolites of ^{14}C-phenoxyphenyl-SD 43775 were analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after organic solvent extraction was considered as fecal water-soluble conjugates and was quantitated by LSC. If the amount of radioactivity remaining in the aqueous fraction represented greater than 5% of the initial applied radioactivity, then this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the solid fecal residues after the initial methanol-water solvent extraction was considered as unextractable materials and was not further qualitatively analyzed.

J. Animal Sacrifice and Tissue Sampling Procedures

Control and treated animals were sacrificed 5 days after the administration of ^{14}C-phenoxyphenyl-SD 43775. Each animal was removed from its respective metabolism cage and its final body weight was recorded. Sacrifice was carried out by decapitation. Control animals were sacrificed prior to the ^{14}C-phenoxyphenyl-SD 43775 treated animals to minimize cross contamination.

The required tissues were excised from each experimental animal in the order that follows. Special care was taken to prevent contamination between individual tissues.

Whole blood, approximately 2 ml anticoagulated with ethylenediamine tetraacetate (EDTA, 10 mg), was collected from the severed carotid arteries in a 15-ml centrifuge tube immediately after sacrifice. Lung, heart, liver, kidney, gonads, inguinal fat, back fat, muscle (from the hind leg), and brain were obtained. All organs and tissue samples, except blood, were individually weighed, placed in prelabeled vials and stored frozen prior to analysis. All organ and tissue weights were recorded in the Animal Tissue Record (Appendix IX). The remaining carcasses were labeled accordingly and sealed in polyester bags and stored frozen (-10°C).

Tissues were subsampled (triplicate subsamples approximately 100 mg) and quantitatively analyzed for total ^{14}C-phenoxyphenyl-SD 43775 equivalent residues by oxygen combustion techniques and LSC.

K. Radioassay

Radioactivity was quantitated by using a Packard Model 2660 Liquid Scintillation System. Counting efficiency determination was carried out by using the external standard ratio (ESR) technique. The actual quench curve was determined at a monthly interval to insure its validity. An example of the instrument print-out for the counting efficiency correlation with ESR is presented in Appendix X. Radioactivity was analyzed in 15 ml of Aquasol-2 scintillation solution. Radioactive areas on the TLC plate after solvent development were removed by scraping and analyzed for radioactivity in an Aquasol-2/water (11:4 ml) gel system.

Radioactive residues associated with animal tissues and fecal excreta were analyzed by weighing approximately 100 mg each of the subsample into a Combusto-Cone sample holder (Packard Instrument Co.) and combusted in a Packard

Model 306B TriCarb sample oxidizer. Combustion efficiency of the individual tissue was determined using control animals and calibrated ^{14}C-chlorophenyl-SD 43775 solution as internal recovery standard. Counting solution included Carbo-Sorb and Permafluor V (10:12 ml) mixture.

L. Chromatographic Procedures

The chemical nature of the radioactivity recovered in the urine, feces and other selected organ tissues was qualitatively and quantitatively analyzed by TLC. Two-dimensional TLC (silica gel F-254, 0.25mm thickness, E. Merck) was performed in the following solvent system combinations.

(A) Hexane-acetone-HoAc (25:25:1)

(B) Toluene-ether-HoAc (75:25:1)

The R_f values of SD 43775 and other model metabolites are presented in Table 2. Reference standards were visualized under UV light. TLC plates were scanned for radioactivity by either using a Berthold LB 2760 TLC scanner or a Berthold LB 292 Beta Camera (Beta Analytical Inc.). Final visual confirmation of the distribution of ^{14}C-phenoxyphenyl-SD 43775 and its metabolites was carried out by autoradiography on Kodak SB-54 single-coated blue sensitive x-ray film (Eastman Kodak Co.).

Capillary gas-liquid chromatography was carried out using a 25m x 0.37mm I.D. SE-30 WCOT glass column in a Varian 3700 gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector. Isothermal analysis was carried out at injector, column and detector temperatures of 280, 245 and 320°C, respectively. The helium carrier gas and nitrogen make up gas flow rate through the detector were 3 and 36 ml/minute, respectively. On-column split ratio was controlled at 10:1 ratio.

Radio-gas-liquid chromatography (RGLC) was carried out using a Varian 1440 gas-liquid chromatograph equipped with a flame ionization detector and a Packard Model 894 gas-proportional counter. The column used was a 1m x 2mm I.D. glass column packed with 3% OV-101 on 80/100 mesh Supelcoport (Supelco Co.). The air, hydrogen and helium flow rates for RGLC were 210, 30 and 30 ml/minute, respectively. The helium and propane quench gas flow rates for the gas-proportional counter were 120 and 15 ml/minute, respectively.

TABLE 2 R_f values of SD 43775 and its metabolites on Silica gel F 254 plates using two solvent systems

	R_f	
	System 1	System 2
SD 43775	0.78	0.72
SD 48838	0.60	0.40
SD 44607	0.49	0.38
SD 46114	0.41	0.16

Solvent system 1 Hexane-acetone-HoAc (25:25:1)

Solvent system 2 Toluene-ether-HoAc (75:25:1)

Mass spectroscopy in electron impact mode (EIMS) was carried out on the Finnigan 3200 mass spectrometer with the instrument settings as follows:

GC setting

Stationary phase	SE-30
Solid support	WCOT column
Column size and material	1.2m x 0.3 mm ID, glass
Carrier	He approximately 3 ml/min
Column temp	100°C for 2 min;program @ 15°C/min. to 280°C
Transfer line	Approximately 250°C
Solvent Vent	at 2 min

MS setting

Electron energy	70 eV
Electron multiplier voltage	1800 V
Emission current	1.0 ma
Preamplifier sensitivity	10^{-7} a/v

M. Data Handling and Presentation

All raw data obtained from this metabolism study were recorded directly and promptly into laboratory notebooks designated for this study. Raw data are records of any original experimental observation which are necessary for the reconstruction of this experiment and the evaluation of this report. The following experimental records were also established as a permanent file on the HP-3000 computer system. Such files include the Animal Resource Record (Appendix IV), and the Animal Physiology Record (Appendix VI).

All experimental observations (urine volume, feces weight, tissue weight, subsample volume and weight and the radioactivity associated with these samples) were also presented as the Animal Tissue Record (Appendix IX) and the Excretion and Tissue Distribution Profile (Appendix XI). The percent of applied ^{14}C-phenoxyphenyl-SD 43775 equivalent residues recovered in the urine and fecal excreta of the treated animals were calculated by using Computer Program-2 (Appendix XII). Standard student's t-test was used to calculate the significant level between experimental and control animals.

All raw data from this study were submitted to the BSRC Archives under designated Protocol PPL-22-009-80 code number for permanent storage.

N. Biological Samples Storage and Retention

All biological samples generated from this study (animal excreta, organ tissues, animal carcasses, etc.) were packaged and stored in the Building 16 walk-in freezer at BSRC at -10°C under the designated Protocol PPL-22-009-80 code number for a minimum of five years.

O. Good Laboratory Practice Compliance Program

This metabolism study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guideline (CFR, vol. 43, no. 247, page 59986-60020, December 22, 1978). A Standard Operation Procedures (SOP) package designated for this metabolism study under the code number of Protocol PPL-22-009-80 was prepared and submitted as raw data to the BSRC Archives for permanent storage. The table of contents of this SOP package is presented in Appendix XIII.

DOCUMENT SUMMARY

Document Id: 1232T
Document Name: Fenvalerate No. 4
Operator: PM
Author: Norvell [D90112A3]

Comments: Metabolism - Rats

STATISTICS

OPERATION	DATE	TIME	WORKTIME	KEYSTROKES
Created	07/03/85	10:50	1:35	4972
Last Revised	07/30/85	15:40	:40	1098
Last Printed	07/30/85	16:26		
Last Archived	07/10/85	10:32	onto Diskette 0094T	

Total Pages: 17 Total Worktime: 6:35
Total Lines: 604 Total Keystrokes: 14831

Pages to be printed 1

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EPA: 68-01-6561
TASK: 112
July 3, 1985

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PC 109301

DATA EVALUATION RECORD

FENVALERATE

Metabolism Study in Rats

STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of ^{14}[C]-chlorophenyl-SD 92459 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-022-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254118.

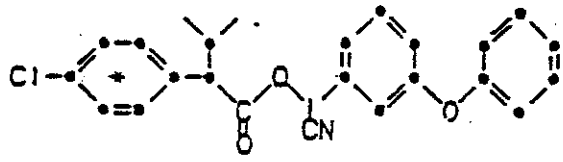
APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-3-85

1. CHEMICAL: Pydrin insecticide, benzeneacetic acid, 4-chloro-α-(1 methyl ethyl)-cyano-(3-phenoxyphenyl) methyl ester:



2. TEST MATERIAL: The test material was a preparation containing 85% of the Y isomers, SD 92459. It was prepared by repeated preparative thin layer chromatography (TLC) of ^{14}[C]-chlorophenyl-SD 43775 with hexane-tetrahydrofuran (97:3). The specific activity was 43.9 mCi/mg, the radiochemical purity greater than 99.5%, and the X/Y isomeric ratio 8/92.

3. STUDY/ACTION TYPE: Metabolism study in rats.

4. STUDY IDENTIFICATION: Lee, P. W., Stearns, S. M., and Powell, W. R. Metabolism of ^{14}[C]-chlorophenyl-SD92459 in male and female rats after a single oral dose (8.4 mg/kg) administration. (Unpublished study No. RIR-22-022-080 prepared and submitted by Shell Oil Co., Modesto, CA; dated August 25, 1981.) Accession No. 254118.

5. REVIEWED BY:

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Date: July 3, 1985

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7. CONCLUSIONS:

When the insecticidally active stereoisomer of Pydrin, ^{14}[C]-chlorophenyl-SD 92459, was administered to male and female rats in a single oral dose of 8.4 mg/kg, radioactivity was rapidly eliminated in the feces and urine; the majority of the radioactivity was eliminated in 24 hours. There was a lack of bioconcentration of radioactive residues in most tissues; detectable levels were found after 5 days only in the liver (0.11 ppm equivalent/g) and fat (1.37 ppm equivalent/g). Over 25% of the residues in fat were undegraded SD 92459. Examination of urinary metabolites indicated a metabolic cleavage of the ester linkage followed by further oxidation to an acid moiety which was excreted free or as a glucuronide or sulfate conjugate. Some unmetabolized SD 92459 (approximately 38% of the administered dose) was found in feces.

Under the conditions of the assay, the study is acceptable.

Item 8 - see footnote 1.

9. BACKGROUND:

Pydrin possesses two asymmetric carbon atoms, the α C of the α-cyano-3-phenoxybenzyl alcohol moiety and C_2 of the acid moiety. The most active isomer as an insecticide has 2S, αS configuration. SD 92459, which is Y rich, contains an excess ratio of the active stereoisomer. The excretion, bioconcentration, and metabolism of the active stereoisomer were studied after a single oral dose in male and female rats.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test animals were male and female Sprague-Dawley rats (Simonsen's Laboratories, Gilroy, CA), 7 weeks of age, and weighing 175-200 g.

2. Control groups of 5 males and 5 females received a single dose of 5 ml/kg corn oil and were maintained to monitor basic animal physiological parameters under sham conditions of the experimental groups. Four/sex were maintained in Nalgene metabolism cages and one/sex in Stanford all glass metabolism cages. Daily food and water consumption and urine and feces production were measured.

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[1]Only items appropriate to this DER have been included.

3. Treatment group 1 consisted of 1 male and 1 female given single oral doses of 8.4 mg/kg ^{14}[C]-chlorophenyl SD 92459 and housed for 7 days in Stanford metabolism cages to monitor for volatile radioactivity and exhaled $^{14}[CO_2]$. If no $^{14}[CO_2]$ was found in 48 hours, collection of expired air was discontinued.

4. Treatment group 2 consisted of 5 rats of each sex given a single oral dose of 8.4 mg/kg test material and maintained individually for 5 days in Nalgene metabolism cages with slightly modified feeders that eliminated food contaminating urine and feces. Food and water intake were measured daily and urine and feces collected daily.

5. Urine volume was measured, the volume then brought to 25 ml and an aliquot radioassayed by liquid scintillation counting (LSC). For metabolite identification, urine samples for days 1 and 2 were combined, adjusted to pH 3 and extracted 3 times with equal volumes of ethyl acetate. The resulting aqueous phase was adjusted to pH 5.0, enzymatically hydrolyzed with sulfatase and ß-glucuronidase, and then extracted 3 times with equal volumes of ethyl acetate. If more than 5% of the initial radioactivity remained in the aqueous phase, the sample was subjected to acid hydrolysis for 4 hours at 90°C at pH 1.0 and then extracted with solvent. The organic phases were concentrated and subjected to TLC.

6. Feces were weighed wet, then freeze dried and reweighed. An aliquot (100 mg) was combusted and radioactivity determined by LSC. For identification of metabolites a 3 g subsample of feces (days 1 and 2 collection) were combined, extracted 3 times with 30 ml MeOH:H_2O (9:1) and radioactivity determined on an aliquot. The extracts were concentrated, adjusted to pH 7.4, extracted with equal volumes of ethyl acetate, and the organic phase brought to dryness. If the aqueous phase contained greater than 5% of the radioactivity it was hydrolyzed at pH 1.0 and 90°C for 4 hours and extracted with ethyl acetate. The organic phases were subjected to 2 dimensional TLC.

7. Animals were sacrificed at 5 days, 2 ml blood collected in EDTA, and the following tissues trimmed and weighed: lung, liver, heart, kidneys, gonads, inguinal and back fat, muscle, and brain. Radioactivity was determined on triplicate 100 mg subsamples by combustion and LSC.

8. Radioactivity was determined by LSC in Aquasol (liquid samples) or Carbosorb-Permafluor (CO_2 from combusted samples). Quench correction was determined monthly, and efficiency was determined by the external standard counting method. Combustion efficiency was determined on tissue samples of control animals to which an internal standard ^{14}[C]-test material was added.

9. Thin layer chromatography solvents were: hexane/acetone/HOAc (25/25/1) and toluene/ether/HOAc (75/25/1). Reference samples chromatographed on thin layer plates were visualized by uv light and radioactivity located by radioautography. GLC was performed with a varian chromatograph with electron capture and radio-gas-liquid chromatography used a flame ionization detector and a Packard gas proportional counter. Mass spectrometry used the electron impact mode.

B. Protocol: Detailed methods are given in Appendix A.

12. REPORTED RESULTS:

There were no observable behavioral effects or toxic signs in dosed animals. Food consumption and water intake were similar in dosed and control rats.

In the preliminary study, there was no $^{14}[CO_2]$ or $^{14}[C]$-volatiles in exhaled air of one male and one female administered 8.4 mg/kg $^{14}[C]$-test material and maintained in an all glass metabolism chamber for 2 days. Further studies were carried out in Nalgene metabolism chambers without expired air collection.

Urinary and Fecal Excretion:

There was a rapid elimination of the administered radioactivity in the urine and feces (Table 1). Approximately 29 and 28 percent of the radioactivity was recovered in urine of males and females 24 hours after dosing, respectively. The second-day urine contained 4-6% of the administered label, and from urine collected on days 3-5 only 2-3% of the radioactive dose was recovered. Likewise, elimination of radioactivity in the feces in 24 hours was 52% and 51% of the administered dose in males and females, respectively. The total radioactivity recovered on day 2 was 4% in males and 8% in females, and approximately 1% was eliminated on days 3-5.

TABLE 1. Excretion of Radioactivity after a Single Oral Dose of $^{14}[C]$-Chlorophenyl-SD 92459 in Rats

	Percent of Administered Dose[a]	
	Males	Females
Urine	36.3±13.0	35.1±7.4
Feces	56.9± 9.2	59.9±8.3
Total	93.2± 4.5	95.0±8.8

[a] Mean ± SD for 5 animals

Urine samples were pooled for each animal for day 1 and day 2 and extracted with ethyl acetate. The water soluble conjugates were then enzymatically hydrolyzed and extracted into ethyl acetate. Table 2 shows the distribution of radioactivity. The organic fractions were then concentrated, and qualitatively and quantitatively analyzed by two dimensional TLC and liquid scintillation counting. The non-conjugated and conjugated metabolites were analyzed for each animal. There was no qualitative difference in the distribution of metabolites between males or females; however, there was some variability in the amounts of metabolites between individual animals. The major urinary metabolites were: 4-chloro-α-(1-methylethyl)benzeneacetic acid (SD 44064, 5-13%), 4-chloro-α-(2-hydroxy-1-methylethyl)benzeneacetic acid (SD 53919, 3-7%), and 4-chloro-α-hydroxy-α(2-hydroxy-1-methylethyl)benzeneacetic acid (SD 90930, 2-5%). Table 3 summarizes the mean percent of the administered radioactivity in urinary metabolites. Four to 5 minor metabolites were not identified.

TABLE 2. Distribution of the Eliminated ^{14}[C]-Chlorophenyl-SD 92459 Residues in the Urine of Male and Female Rats

	Percent of Administered Radioactivity Recovered	
	Male[a]	Female[a]
Organic Extractable	23.7±10.2[b]	28.0±5.3
Water Soluble Conjugates	10.4± 4.1	6.0±3.9
Released after Enzyme Hydrolysis	6.7± 2.7	3.4±2.6
Unextractable	3.7± 1.6	2.6±1.3
Total	34.1±14.2	34.0±9.2

[a] N = 5.
[b] Mean ± standard deviation.

TABLE 3. Radiolabeled Metabolites in Urine of Rats after Administration of ^{14}[C]-Chlorophenyl-SD 92459

Metabolite[b]	Sex	Percent of Administered[a] Dose		
		Nonconjugated	Conjugated	Total
SD 52667	**M**	**0.8**	**0.7**	**1.5**
	F	0.7	0.1	0.8
SD 44064	**M**	**7.5**	**1.3**	**8.8**
	F	8.6	1.0	9.7
SD 53065	M	0.1	0.40	0.5
	F	ND[c]	0.30	0.3
SD 53919	M	5.6	--[c]	5.6
	F	4.9	--	4.9
SD 52666	M	3.2	--	3.2
	F	2.2	--	2.2
SD 90930	M	3.1	2.0	5.1
	F	4.5	1.1	5.6
Other	M	4.6	1.5	9.8
	F	6.9	0.9	10.4
Total[d]	**M**	**23.8**	**6.7**	**33.9**
	F	24.2	3.0	34.0

[a] Mean of 5 males and 5 females combined.

[b] The structural formulas of the metabolites are given in Appendix B.
SD 52667 (α isomer): 3-(4-chlorophenyl)-dihydro-4-methyl-2(3H)-furanone
SD 44064: 4-chloro-α-(1 methylethyl)benzeneacetic acid
SD 53065: 4-chloro-α-hydroxy-α-(1 methylethyl)benzeneacetic acid
SD 53919 (b isomer): 4-chloro-α-(2 hydroxy-1-methylethyl)-benzene-acetic acid
SD 52666: alpha isomer of SD53919
SD 90930: 4-chloro-α-hydroxy-α-(2-hydroxy-1-methylethyl)benzene-acetic acid.

[c] (ND) = not detected; (--) not tabulated.

[d] Includes radioactivity remaining in the aqueous phase after initial organic extraction or after organic extraction following enzyme hydrolysis.

Most of the radioactivity in feces collected on days 1 and 2 was extracted by the organic solvent, a mean of 93.7% in males and 90.7% in females. Chromatographic analysis showed that approximately 64% of the radioactivity was unchanged ^{14}[C]-test material whereas, other major metabolites were the hydroxylated test compound (3 phenoxy-4-hydroxy derivative of SD 48838), and 4-chloro-α-(1 methylethyl)-benzeneacetic acid (SD 44064). The results are summarized in Table 4.

Tissue Distribution:

It did not appear that there was bioaccumulation of the test compound or its radioactive metabolites in the tissues. The levels in blood, lung, heart, kidney, and muscle ranged from 0.037-0.056 µg equivalents/g wet tissue, which is only slightly above the limit of detection (0.036 µg/g). In brain and testes, no radioactivity was detected. Liver and fat in both sexes and ovaries had detectable levels (Table 5). The level in the ovaries was attributed to contamination of the tissue with fat. In an attempt to characterize the residues in liver, 5 grams of liver from 2 males and 2 females were homogenized in 20 ml of phosphate buffer, pH 7.4, and precipitated with 10% trichloroacetic acid (1 ml). Approximately 75% of the radioactivity was soluble; the amount in the proteinaceous fraction averaged 0.035 and 0.031 µg equivalents/g tissue in males and females, respectively or about 0.02% of the administered radioactivity. Hexane extraction of liver extract followed by gas-liquid chromatography failed to recover any radioactive ^{14}[C]-chlorophenyl-SD 92459. Samples of fat from 2 males and 2 females were homogenized, extracted with hexane and analyzed by GLC. In males 27-38% and in females 24-16% of the radioactivity was extracted into hexane. Of the radioactivity 27.7 and 13.5% in males 15.7 and 15.6% in females was recovered as the administered material.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. After a single oral dose of 8.4 mg/kg ^{14}[C]-chlorophenyl-SD 92459 was administered to male and female rats, there was rapid elimination of radioactivity in the feces and urine; the majority of ^{14}[C]-labelled material eliminated in the first 24 hours. No exhaled radioactivity was detected. Excretion was similar in males and females. The examination of urinary metabolites by chromatography indicated metabolic cleavage of the ester linkage followed by further oxidation to an acid moiety and conjugation with sulfate or glucuronide. Undegraded SD 92459 was found only in the feces. Tissue residue distribution data showed a lack of bioconcentration of radioactive residues in blood, heart, kidney, gonad, and brain. Detectable levels of radioactive residues were found in the liver (approximately 0.11 ppm equivalents) and fat (approximately 1.37 ppm equivalents). No differences in tissue distribution were found between males and females. Greater than 25% of the ^{14}[C] residues found in fat were undegraded SD 92459.

TABLE 4. Radioactive Metabolites in Rats' Feces after Administration of ^{14}[C]-Chlorophenyl-SD 92459

	Percent of Administered Radioactivity[a]	
	Males	Females
Organic Extractable Metabolites		
SD 92459[b]	**35.1**	**38.6**
SD 44064	4.4	3.6
SD 48838	4.9	3.9
Others[c]	5.8	4.6
Unextracted	3.5	5.5
Water Soluble	2.2	3.5
Total	55.9	59.3

[a] Mean value for males and females combined.

[b] Unchanged test material. The structures of the metabolites are given in Appendix B:
SD 44064: 4-chloro-α-(1 methylethyl)benzeneacetic acid
SD 48838: 4-chloro-α-(1-methylethyl)-cyano-(3-phenoxy-4-hydroxyphenyl) methyl ester benzeneacetic acid.

[c] Radioactivity remaining at the origin of TLC plate and minor metabolites.

TABLE 5. Residue Level of ^{14}[C]-Chlorophenyl-SD 92459 in Male and Female Rat Tissues

	µg Equivalents/g Wet Tissue[a]	
	Males	Females
Liver	0.106±0.022	0.102±0.020
Fat	1.375±0.551	1.356±0.380
Ovaries	--	0.245±0.191

[a] Mean and standard deviation; 5 animals

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B. A quality assurance statement was not present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors' conclusions were supported by the individual animal data. The protocol was adequate and the study was well conducted and reported. We assess that the study is acceptable under the conditions it was conducted. Recoveries of radioactivity were high. The levels of specific activity of the test compound were sufficiently high to detect equivalent residues in animal tissue at a level of 0.005 to 0.009 of the administered dose using 100 mg samples of tissue. Combustion efficiencies ranged from 89.8-96.2%. Radioactive samples of urine and feces were corrected for quenching and counting efficiency in the SC system. Although there was some variability from animal to animal in percent of radioactivity in various fractions or metabolites, this is to be expected, and sufficient animals were used to calculate mean values for all parameters.

The test material was well characterized by chromatographic means from the data presented. Data on autoradiograms of the two dimensionally chromatographed plates were presented in the report. Data were not presented for the GLC/MS identification of the metabolites; however, detailed mass spectral analyses of the parent compound and metabolites were presented in Study RIR-22-021-080 (Accession No. 259117).

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI pp. 4-17, 58-59.

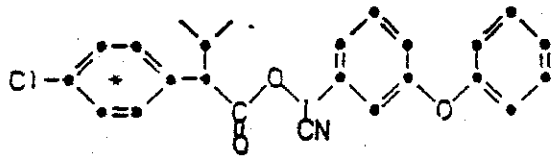
APPENDIX A

Materials and Methods (Protocol)

III. Description of the Conduct of Experiment

A. Test Compound

SD 43775 labeled with carbon-14 at the chlorophenyl-postion was used for the separation of SD 92459.



SD 43775

*Denotes carbon-14.

The specific activity of the ^{14}C-chlorophenyl-SD 43775 was 43.7 microCuries/mg as determined by capillary gas-liquid chromatography (GLC) and liquid scintillation counting (LSC). Detailed calculation of the specific activity of ^{14}C-chlorophenyl-SD 43775 is presented in Appendix II. Based on the sensitivity of detection, the radiochemical purity of ^{14}C-chlorophenyl-SD 43775 is greater than 99.5% as determined by two-dimensional thin-layer chromatography (TLC) and LSC.

^{14}C-chlorophenyl-SD 92459 was prepared by the TLC preparative separation of ^{14}C-chlorophenyl-SD 43775. ^{14}C-chlorophenyl-SD 43775 (72 mg, 2570 microCuries) was applied onto a 1.0mm preparative silica gel TLC plate and developed six consecutive times in the hexane-tetrahydrofuran (97:3) solvent system. SD 43775 was separated into two close bands and the bottom band corresponded to the SD 92459. The yield of ^{14}C-chlorophenyl-SD 92459 was 639 microCuries. The specific activity of ^{14}C-chlorophenyl-SD 92459 was 43.9 microCuries/mg as redetermined by GLC and LSC. A detailed calculation of the specific activity of ^{14}C-chlorophenyl-SD 92459 is presented in Appendix III. The X/Y isomeric ratio of ^{14}C-chlorophenyl-SD 92459 was 8/92 as determined by capillary-GLC (Figure 1). The radiochemical purity of ^{14}C-chlorophenyl-SD 92459 is greater than 99.5% as determined by autoradiography of two-dimensional TLC (Figure 2) and LSC. A stock solution of ^{14}C-chlorophenyl-SD 92459 in dichloromethane was stored at -4°C prior use.

B. Test Animals

Male and female Sprague-Dawley albino rats (SIM:SD f1 strain, 7 weeks old, weighing from 175-200 grams each) were obtained from Simonsen's Laboratories, Gilroy, California. The number of total test animals, their experimental identification number, sexes, arrival date, initial body weight, experimental treatment date and their corresponding bodyweights are summarized in the Animal Resource Record (Appendix IV).

Figure 1 Isomeric composition of SD 51142 (PYDRIN-Y), SD 51143 (PYDRIN-X) and ^{14}C-chlorophenyl-SD 92459 (PYDRIN-Y rich)

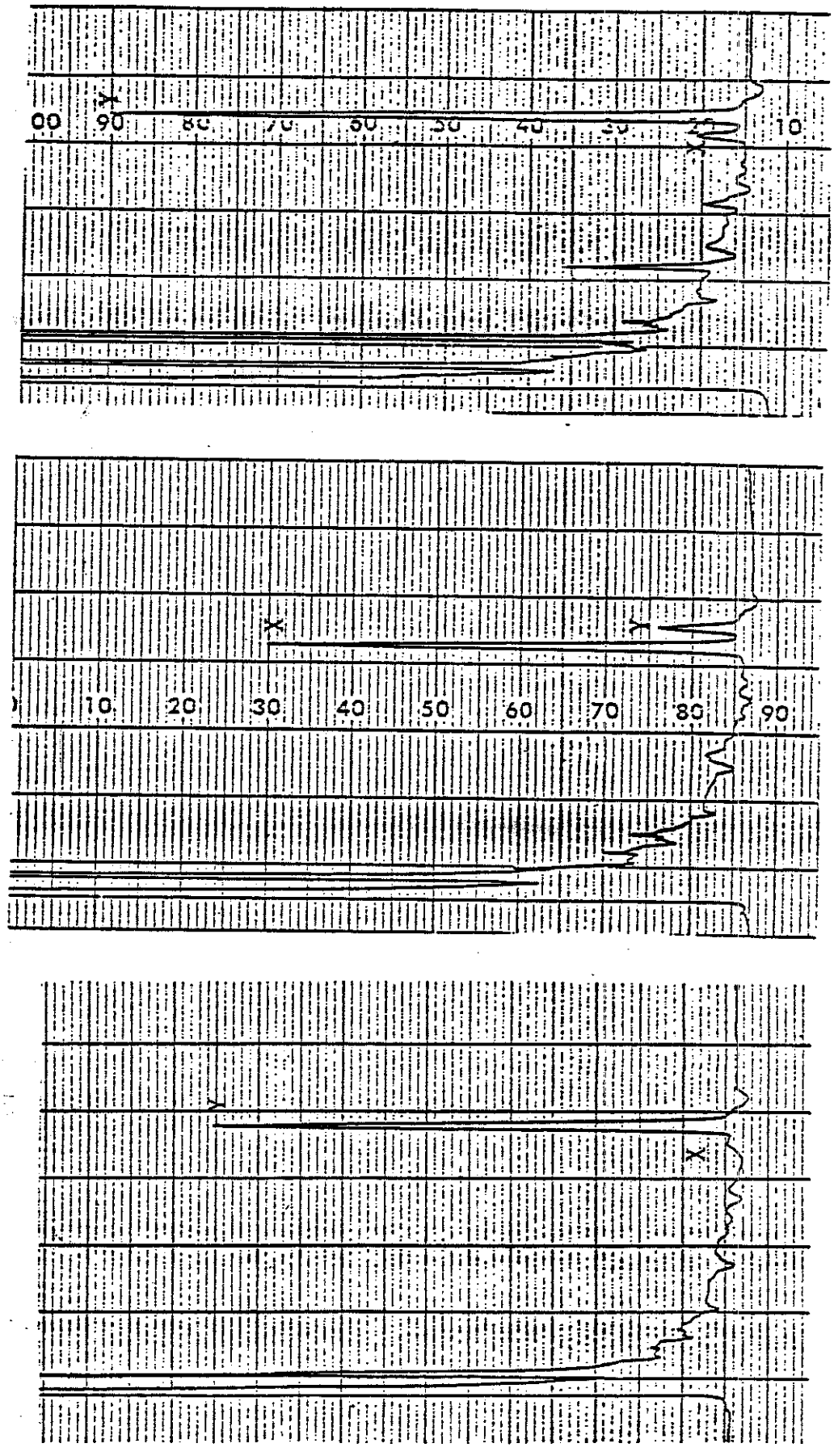
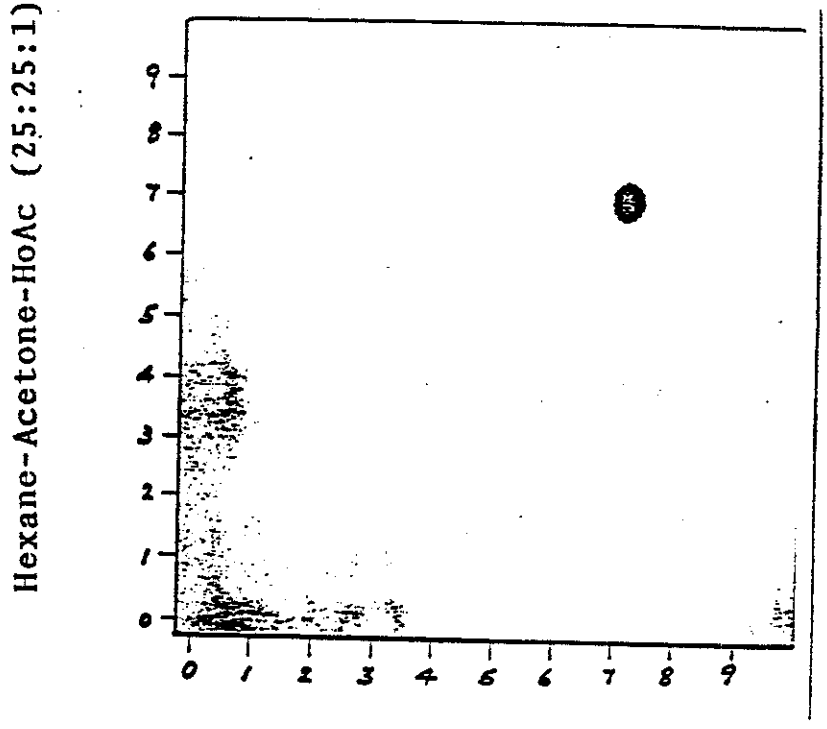


Figure 2. Autoradiogram of ^{14}C-chlorophenyl-SD 92459 used in this study



C. Test Animals Housing and Caring

All newly received animals were identified by ear code, weighed and housed individually in a suspended cage system (Lab Products, Rochelle Park, NJ) equipped with an automatic water feeding system (System Engineering, Napa, CA) and were given free access to food (Purina Rodent Chow) and water. The chemical analysis of the lab chows and the water used in this study are presented in Appendix V A and B. The physiological conditions and the behavioral patterns of the test animals were observed and recorded twice daily. Daily observations were recorded in the raw data file submitted for storage at BSRC Chemical Archives under Protocol No. PPL-22-009-80. The daily food intake, water consumption, urine volume and feces weight for each experimental animal were recorded and presented in the Animal Physiological Record (Appendix VI).

D. Route of Administration

^{14}C-chlorophenyl-SD 92459 was formulated in corn oil immediately prior to treatment and the appropriate dose (8.4 mg/kg) was administered to the test animals by stomach intubation at a constant volume dose of 5 ml/kg. ^{14}C-chlorophenyl-SD 92459 treatment solution (30 mg) was prepared by diluting ^{14}C-chlorophenyl-SD 92459 stock solution (3.5 mg, X/Y ratio 8/92, specific activity 43.9 microCuries/mg) with unlabeled SD 51142 (PYDRIN® Y, 25 mg, X/Y ratio 1/99, Figure 1) and SD 51143 (PYDRIN ® X, 1.5 mg, X/Y ratio 85/15, Figure 1) in the final volume of 17.8 ml of corn oil. The final concentration of SD 92459 in this treatment solution was 1.68 mg/ml and had the final specific activity of 5.76 microCuries/mg. The X/Y ratio of the ^{14}C-chlorophenyl-SD 92459 treatment compound was approximately 9/91 as determined by capillary GLC (Figure 3). The level of specific activity of ^{14}C-chlorophenyl-SD 92459 allowed the the detection of SD 92459 equivalent residues in the animal tissues at the level of 0.5 to 1% of the applied radioactivity. Animals were fasted for 16 hours prior to dosing with free access to water. Control animals were treated with corn oil only. The actual dose of ^{14}C-chlorophenyl-SD 92459 for each individual animal received is summarized and presented in the Animal Physiological Record (Appendix VI). Food was returned to all control and treated animals one hour after dosing.

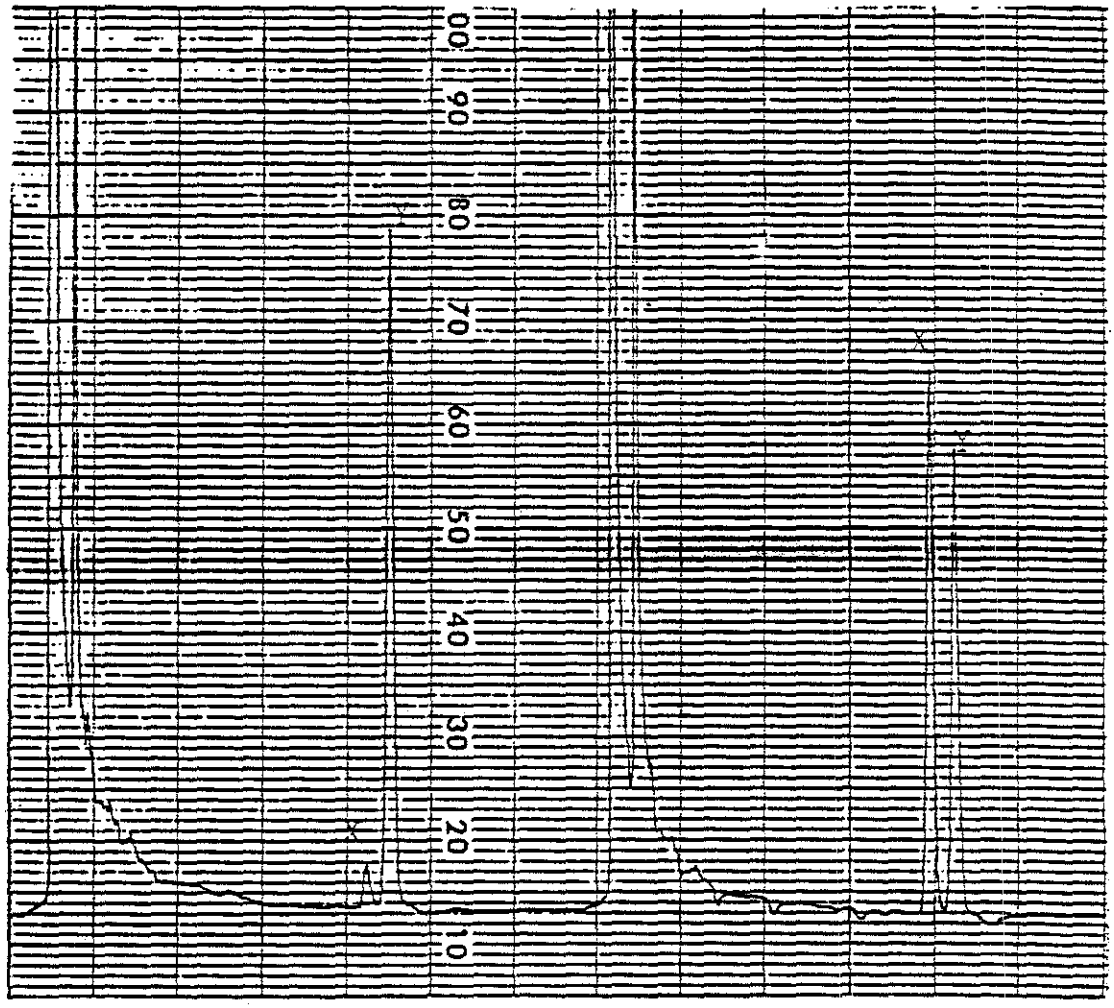
E. Animal Treatment Groups

Experimental animals were organized into the following treatment groups.

1. Control Treatment Groups

Five male and 5 female (80R001-80R010) (single oral, corn oil, 5 ml/kg). Animals were treated on June 18, 1980 and were maintained individually in a Nalgene Metabolism Cage unit (4 males and 4 females) and an all glass Stanford metabolism chamber (1 male and 1 female) for 7 days. The purpose of this contol treatment group was to provide the basic animal physiological parameters (such as daily food and water intake, urine and feces excretion profile, etc) of the treated animals maintained under laboratory

Figure 3 Isomeric composition of the dosing solution of ^{14}C-chlorophenyl-SD 92459 and ^{14}C-chlorophenyl-SD 43775



^{14}C-Chlorophenyl-SD 92459 X/Y=9:91

^{14}C-Chlorophenyl-SD 43775 X/Y=55:45

experimental conditions. Individual body organs and tissues of the animal in the control treatment group were also used to establish the normal background level, sensitivity of the radioanalysis and the limit of determination of ^{14}C-chlorophenyl-SD 92459 equivalent residues by analytical procedures used in this study.

2. ^{14}C-Chlorophenyl-SD 92459 Treatment Group I

1 male (80R082) and 1 female (80R077) (single oral, ^{14}C-chlorophenyl-SD 92459, 8.4 mg/kg). Animals were treated on September 18, 1980 and were maintained individually in an all-glass Stanford metabolism chamber for 2 days. The purpose of this treatment group was to demonstrate that no $^{14}CO_2$ or other volatile radioactive materials were exhaled in the respired air of the treated animals during the initial 48 hours immediately after dosing. Data from this study justified that the requirement for the monitoring of the exhaled air from the treatment group II is waived.

3. ^{14}C-Chlorophenyl-SD 92459 Treatment Group II

5 male (80R072,74,76,78 and 80) and 5 female (80R069, 73,75,79 and 81) (single oral, ^{14}C-chlorophenyl-SD 92459 8.4 mg/kg); 1 male (80R070) and 1 female (80R071) (control, corn oil, single oral, 5 ml/kg). Animals were treated on September 18, 1980 and were maintained individually in Nalgene metabolism cage units for 5 days. Animal physiological parameters (daily food and water intake, urine and feces excretion profile) were recorded daily. The rates of excretion of ^{14}C-chlorophenyl-SD 92459 equivalent residues in the urine and feces were also monitored daily. At the end of the Day-5 holding period, animals were sacrificed and individual organ tissues were collected and radioassayed for ^{14}C-chlorophenyl-SD 92459 equivalent residues.

The actual dose of ^{14}C-chlorophenyl-SD 92459 and control corn oil carrier administered to each individual test animals are summarized in Table 1.

TABLE 1 SUMMARY OF TEST ANIMALS AND THEIR TREATMENT SCHEDULE

	ANIMAL ID	SEX	DOSE	HOLDING CONDITION
Control Treatment Group (corn oil)	80R001	F	0.94 ml[a)]	PC[b)]
	80R002	M	1.07 ml	PC
	80R003	F	0.91 ml	PC
	80R004	M	1.14 ml	PC
	80R005	F	0.93 ml	PC
	80R006	M	0.93 ml	PC
	80R007	F	0.92 ml	PC
	80R008	M	1.10 ml	PC
	80R009	F	1.02 ml	GC[c)]
	80R010	M	1.12 ml	GC
^{14}C-chlorophenyl-SD 92459 Treatment Group I				
	80R077	F	1.51 mg[d)]	GC
	80R082	M	1.58 mg	GC
^{14}C-chlorophenyl-SD 92459 Treatment Group II				
	80R069	F	1.65 mg[d)]	PC
	80R072	M	1.73 mg	PC
	80R073	F	1.50 mg	PC
	80R074	M	1.73 mg	PC
	80R075	F	1.63 mg	PC
	80R076	M	1.71 mg	PC
	80R078	M	1.73 mg	PC
	80R079	F	1.71 mg	PC
	80R080	M	1.70 mg	PC
	80R081	F	1.50 mg	PC
	80R070[e)]	M	0.99 ml[a)]	PC
	80R071	F	0.96 ml	PC

a) Dosage of corn oil (5ml/kg)
b) Nalgene Metabolism Cage Unit
c) Stanford All-Glass Metabolism Chamber
d) Dosage of ^{14}C-chlorophenyl-SD 92459 (8.4 mg/kg)
e) Control animal

F. Preliminary All-Glass Metabolism Chamber Holding Study

A preliminary range-finding experiment was carried out using 1 female (80R077) and 1 male (80R082) rat treated with a single oral dosage of 8.4 mg/kg of ^{14}C-chlorophenyl-SD 92459. Animals were maintained in individual Stanford all-glass metabolism chamber (Model MC 3000, Stanford Glassblowing Laboratories, Palo Alto, CA) and allowed free access to food and water one hour after dosing. A schematic diagram of this all-glass metabolism chamber unit is presented in Appendix VII. This is an air-tight structure that allows a continuous monitoring of $^{14}CO_2$ generation and the separate collection of urine and feces excreta. $^{14}CO_2$ was collected over a CO_2 absorption tower which contained 250 ml of ethanolamine. Several minor modifications were added to this existing unit. A manifold system was used which provided an independent fine control of the amount of air passing through each individual metabolism chamber. Gas washing bottles (Laboratory Glass Apparatus, Berkeley, CA) were modified to provide a continuous monitoring of the generation of $^{14}CO_2$ during the entire holding periods. A detailed description of this experimental setup is presented in Figure 4.

G. Nalgene Metabolism Cages Holding Study

A control treatment group (5 male and 5 female) and the ^{14}C-chlorophenyl-SD 92459 treatment group II (5 male and 5 female) plus 2 control animals (1 male and 1 female) were maintained individually in Nalgene metabolism cage units (Sybron/Nalge Co., Rochester, NY). A description of the assembly of the Nalgene metabolism cage unit is presented in Appendix VIII. Although these metabolism units are not designed for the collection of $^{14}CO_2$ from the exhaled air of the treated animals, they were excellent and efficient in the separate collection of urine and feces excreta. A group of 10-12 metabolism cages were used in each experiment. Animals were allowed free access to food and water. Because of the poor design of the food container, animals in control treatment group were able to carry extra food pellets back into the main metabolism chamber, which resulted in the contamination of both the urine and feces excreta. Modification of the food container on these Nalgene metabolism cages was carried out prior to the initiation of the ^{14}C-chlorophenyl-SD 92459 treatment group II study and resulted in the elimination of the cross contamination of the urine and feces excreta by the food diet (Figures 5 and 6). A detailed description of this modification is presented in a separate report (RIR-22-018-80).[a)]

a) RIR-22-018-80 Modification of the Diet Feeder in the Nalgene Metabolic Cages for Rats to Minimize Contamination of Excreta.

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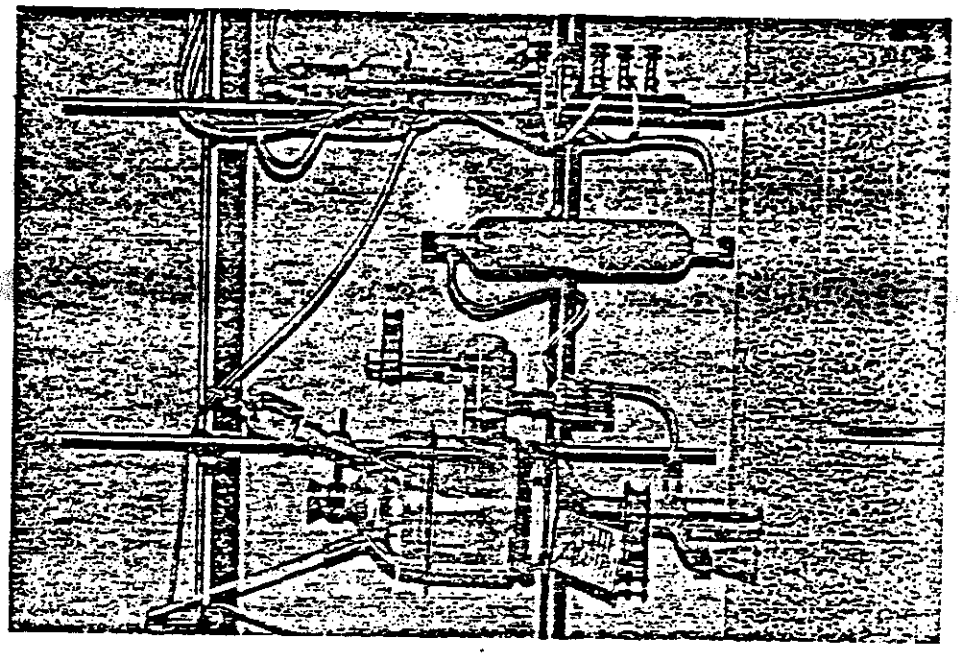


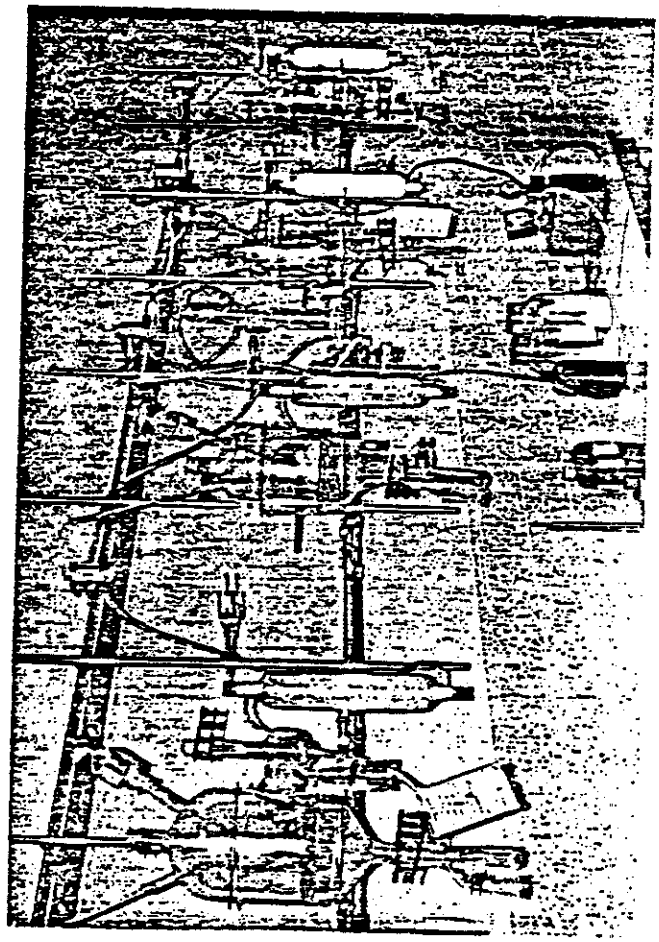


Figure 2 Experimental setup for the all glass metabolism chamber holding study

Figure 3 Experimental setup for the Nalgene plastic metabolism chamber unit

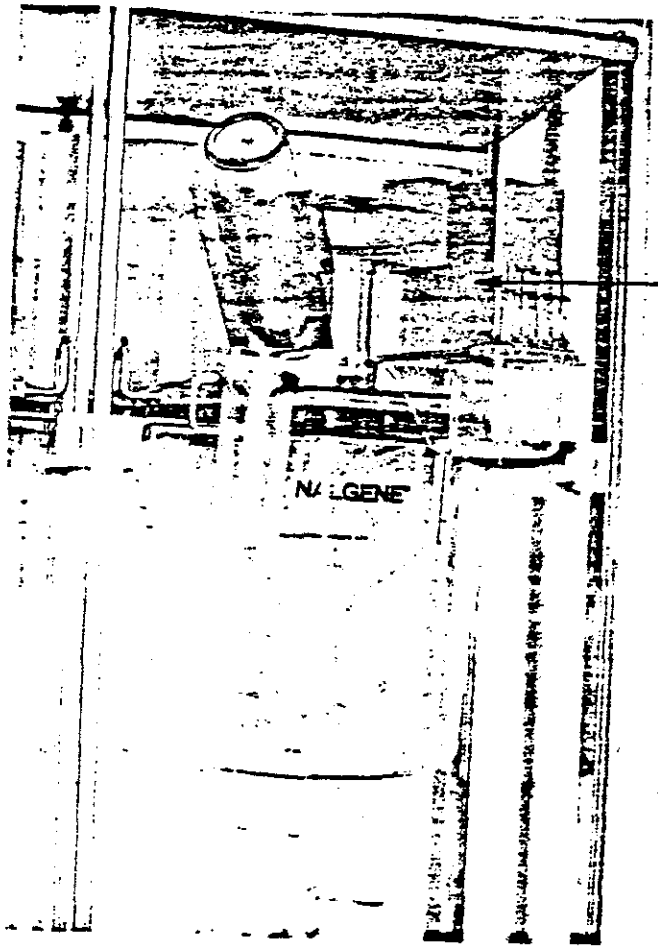
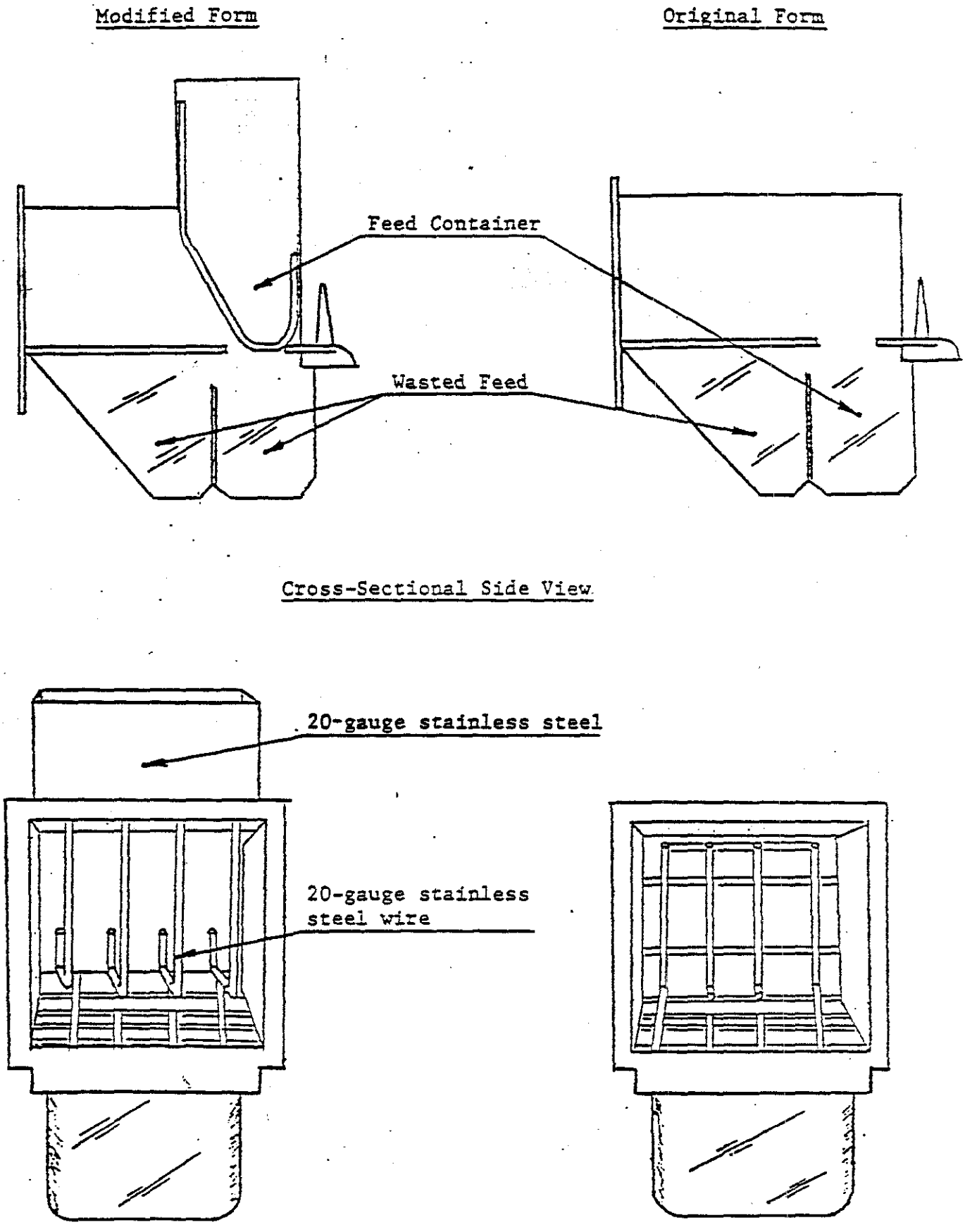


Figure 3A MODIFICATION OF METABOLISM FEED CHAMBER FOR RATS



H. Urine Sampling and Analysis

Urine excreta from all the control and ^{14}C-chlorophenyl-SD 92459 treated animals were collected daily and the total urine volume from each individual animal was recorded into the Animal Physiology Record (Appendix VI). The final volume of each individual daily urine excreta was then adjusted to a final volume of 25 ml with 0.01 M phosphate buffer (pH 7.4). Triplicate 100-µl aliquots were sampled and the total daily ^{14}C-chlorophenyl-SD 92459 equivalent residues present in the urine excreta was quantitatively analyzed by LSC. All samples were analyzed immediately after collection. All urine samples were stored at 4°C prior to further qualitative analysis.

For the quantitative and qualitative examination of the excreted urinary metabolites, 15 ml aliquots of the Day-1 and Day-2 urine samples from each treated animal were combined for analysis. Analysis was carried out immediately after the termination of the animal holding period. Triplicate 100-µl aliquots were subjected to LSC quantitation. The pH of the combined urine sample was adjusted to pH 3 with 1 ml of 6N hydrochloric acid, and extracted three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate extraction were subjected to enzyme hydrolysis. Enzyme hydrolysis was carried out at 35 ± 1°C for 12 hours using approximately 1×10^4 units of the sulfatase/ß-glucuronidase enzyme (No. S-9126, Sigma Chemical Co., one enzyme unit will hydrolyze 1 µmole of nitrocatechol sulfate per hour at pH 5 at 37°C). Water-soluble conjugates released during enzyme hydrolysis were recovered by extracting the aqueous phase three times with equal volumes of ethyl acetate. Organic solvents were dried over anhydrous magnesium sulfate, concentrated and further analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after enzyme hydrolysis was quantitated by LSC. If the amount of the radioactivity remaining in this aqueous fraction represented greater than 5% of the initial applied radioactivity, this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by the acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the urine sample after the initial organic solvent extraction, enzyme hydrolysis and acid hydrolysis were considered as unextractable materials and were not further qualitatively analyzed.

I. Sampling and Analysis of Fecal Material

Fecal excreta from all the control and ^{14}C-chlorophenyl-SD 92459 treated animals were collected daily and the total fecal wet weight from each animal was recorded into the Animal Physiological Record (Appendix VI). Daily fecal excretion was freeze dried in a freeze dryer (Thermovac Industries Corp.) for 24 hours and the final resultant dry weight was also recorded. Dried fecal materials were pulverized by using a microanalytical mill (Tekmar Company). Percent of the applied radioactivity recovered in the daily fecal excreta was

quantitatively analyzed by oxygen combustion of triplicate fecal subsamples (approximately 100 mg each) by using a Packard Model 306B sample oxidizer and subsequent LSC quantitation. All samples were analyzed immediately after collection. All fecal samples were stored at 4°C prior to further qualitative analysis.

For the qualitative and quantitative examination of the excreted fecal metabolites, a 3-gram subsample of the Day-1 and Day-2 combined fecal excreta from each treated animal was analyzed. Analysis was carried out immediately after the termination of the animal holding period. Fecal excreta was first extracted three times (15 minutes each) using 30 ml of methanol-water (9:1) solvent mixture. The solvent extract and solid fecal materials were separated by centrifugation (2000 rpm for 10 minutes). The combined methanol-water solvent extract was quantitatively analyzed by LSC for the total amount of solvent extractable metabolites. The volume of the methanol-water solvent extract was then concentrated to approximately 15 ml by rotor evaporation. The final volume of this concentrated fecal solvent extract was adjusted to 30 ml using 0.01M phosphate buffer (pH 7.4) and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate solvent extract was dried over anhydrous magnesium sulfate, concentrated and the organo-soluble fecal metabolites of ^{14}C-chlorophenyl-SD 92459 were analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after organic solvent extraction was considered as fecal water-soluble conjugates and was quantitated by LSC. If the amount of radioactivity remaining in the aqueous fraction represented greater than 5% of the initial applied radioactivity, then this aqueous phase was further subjected to acid hydrolysis at pH 1 at 90°C for 4 hours. Radioactivity released by acid hydrolysis was recovered by organic solvent extraction (three times with equal volumes of ethyl acetate), dried over anhydrous magnesium sulfate, concentrated and analyzed by two-dimensional TLC.

Radioactivity remaining in the solid fecal residues after the initial methanol-water solvent extraction was considered as unextractable materials and was not further qualitatively analyzed.

J. Animal Sacrifice and Tissue Sampling Procedures

Control and treated animals were sacrificed 5 days after the administration of ^{14}C-chlorophenyl-SD 92459. Each animal was removed from its respective metabolism cage and their final body weight was recorded. Sacrifice was carried out by decapitation. Control animals were sacrificed prior to the ^{14}C-chlorophenyl-SD 92459 treated animals to minimize cross contamination.

The required tissues were excised from each experimental animal in the order that follows. Special care was taken to prevent contamination between individual tissues.

Whole blood, approximately 2 ml anticoagulated with ethylenediamine tetraacetate (EDTA, 10 mg), was collected from the severed carotid arteries in a 15-ml centrifuge tube immediately after sacrifice. Lung, heart, liver, kidney, gonads, inguinal fat, back fat, muscle (from the hind leg), and brain were obtained. All organs and tissue samples, except blood, were individually weighed, placed in prelabeled vials and stored frozen prior to analysis. All organ and tissue weights were recorded in the Animal Tissue Record (Appendix

IX). The remaining carcasses were labeled accordingly and sealed in polyester bags and stored frozen (-10°C).

Tissues were subsampled (triplicate subsamples approximately 100 mg) and quantitatively analyzed for total ^{14}C-chlorophenyl-SD 92459 equivalent residues by oxygen combustion techniques and LSC.

K. Radioassay

Radioactivity was quantitated by using a Packard Model 2660 Liquid Scintillation System. Counting efficiency determination was carried out by using the external standard ratio (ESR) technique. The actual quench curve was determined at a monthly interval to insure its validity. An example of the instrument print-out for the counting efficiency correlation with ESR is presented in Appendix X. Radioactivity was analyzed in 15 ml of Aquasol-2 scintillation solution. Radioactive areas on the TLC plate after solvent development were removed by scraping and analyzed for radioactivity in an Aquasol-2/water (11:4 ml) gel system.

Radioactive residues associated with animal tissues and fecal excreta were analyzed by weighing approximately 100 mg each of the subsample into a Combusto-Cone sample holder (Packard Instrument Co.) and combusted in a Packard Model 306B TriCarb sample oxidizer. Combustion efficiency of the individual tissue was determined using control animals and calibrated ^{14}C-chlorophenyl-SD 43775 solution as internal recovery standard. Counting solution included Carbo-Sorb and Permafluor V (10:12 ml) mixture.

L. Chromatographic Procedures

The chemical nature of the radioactivity recovered in the urine, feces and other selected organ tissues was qualitatively and quantitatively analyzed by TLC. Two-dimensional TLC (silica gel F-254, 0.25mm thickness, E. Merck) was performed in the following solvent system combinations.

(A) Hexane-acetone-HoAc (25:25:1)

(B) Toluene-ether-HoAc (75:25:1)

The R_f values of SD 43775 and other model metabolites are presented in Table 2. Reference standards were visualized under UV light. TLC plates were scanned for radioactivity by either using a Berthold LB 2760 TLC scanner or a Berthold LB 292 Beta Camera (Beta Analytical Inc.). Final visual confirmation of the distribution of ^{14}C-chlorophenyl-SD 92459 and its metabolites were carried out by autoradiography on Kodak SB-54 single-coated blue sensitive x-ray film (Eastman Kodak Co.).

Capillary gas-liquid chromatography was carried out using a 25m x 0.37mm I.D. SE-30 WCOT glass column in a Varian 3700 gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector. Isothermal analysis was carried out at injector, column and detector temperatures of 280, 245 and 320°C, respectively. The helium carrier gas and nitrogen make up gas flow rate through the detector were 3 and 36 ml/minute, respectively. On-column split ratio was controlled at 10:1 ratio.

TABLE 2 R_f values of SD ~~43775~~ 92459 and its metabolites on Silica gel F 254 plates using two solvent systems

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	R_f	
	System 1	System 2
SD 43775	0.78	0.72
SD 52667	0.73	0.68
SD 44064	0.70	0.38
SD 53065	0.62	0.23
SD 53919	0.58	0.13
SD 52666	0.48	0.09
SD 90930	0.24	0.03
SD 48838	0.62	0.42

Solvent system 1 Hexane-acetone-HoAc (25:25:1)
Solvent system 2 Toluene-ether-HoAc (75:25:1)

Mass spectroscopy in election impact mode (EIMS) was carried out on the Finnigan 3200 mass spectrometer with the instrument settings as follows:

GC setting

Stationary phase	SE-30
Solid support	WCOT column
Column size and material	1.2m x 0.3 mm ID, glass
Carrier	He approximately 3 ml/min
Column temp	100°C for 2 min; program @ 15°C/min. to 280°C
Transfer line	Approximately 250°C
Solvent Vent	at 2 min

MS setting

Electron energy	70 eV
Electron multiplier voltage	1800 V
Emission current	1.0 ma
Preamplifier sensitivity	10^{-7} a/v

Radio-gas-liquid chromatography (RGLC) was carried out using a Varian 1440 gas-liquid chromatograph equipped with a flame ionization detector and a Packard Model 894 gas-proportional counter. The column used was a 1m x 2mm I.D. glass column packed with 3% OV-101 on 80/100 mesh Supelcoport (Supelco Co.). The air, hydrogen and helium flow rates for RGLC were 210, 30 and 30 ml/minute, respectively. The helium and propane quench gas flow rates for the gas-proportional counter were 120 and 15 ml/minute, respectively.

M. Data Handling and Presentation

All raw data obtained from this metabolism study were recorded directly and promptly into laboratory notebooks designated for this study. Raw data are records of any original experimental observation which are necessary for the reconstruction of this experiment and the evaluation of this report. The following experimental records were also established as a permanent file on the HP-3000 computer system. Such files include the Animal Resource Record (Appendix IV), and the Animal Physiology Record (Appendix VI).

All experimental observations (urine volume, feces weight, tissue weight, subsample volume and weight and the radioactivity associated with these samples) were also presented as the Animal Tissue Record (Appendix IX) and the Excretion and Tissue Distribution Profile (Appendix XI). The percent of applied ^{14}C-chlorophenyl-SD [redacted] equivalent residues recovered in the urine and fecal excreta of the treated animals were calculated by using Computer Program-2 (Appendix XII). Standard student's t-test was used to calculate the significant level between experimental and control animals.

All raw data from this study were submitted to the BSRC Archives under designated Protocol PPL-22-009-80 code number for permanent storage.

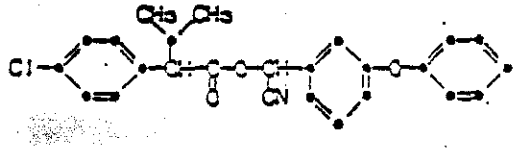
N. Biological Samples Storage and Retention

All biological samples generated from this study (animal excreta, organ tissues, animal carcasses, etc.) were packaged and stored in the Building 16 walk-in freezer at BSRC at -10°C under the designated Protocol PPL-22-009-80 code number for a minimum of five years.

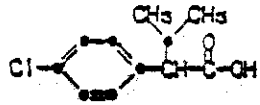
O. Good Laboratory Practice Compliance Program

This metabolism study was conducted in accordance with the guidelines specified under the Good Laboratory Practice Guideline (CFR, vol. 43, no. 247, page 59986-60020, December 22, 1978). A Standard Operation Procedures (SOP) package designated for this metabolism study under the code number of Protocol PPL-22-009-80 was prepared and submitted as raw data to the BSRC Archives for permanent storage. The table of contents of this SOP package is presented in Appendix XIII.

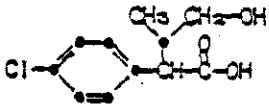
TABLE OF COMPOUNDS



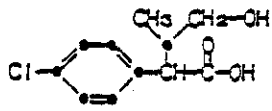
SD 43775: Benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, cyano(3-phenoxyphenyl)methyl ester.



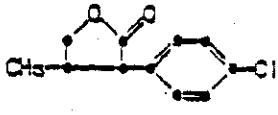
SD 44064: Benzeneacetic acid, 4-chloro-α-(1-methylethyl)-.



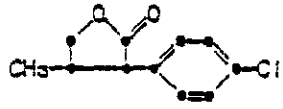
SD 52666 (*a isomer*): Benzeneacetic acid, 4-chloro-α-(2-hydroxy-1-methylethyl)-.



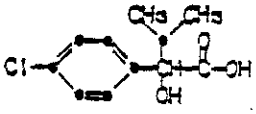
SD 53919 (*b isomer*): Benzeneacetic acid, 4-chloro-α-(2-hydroxy-1-methylethyl)-.



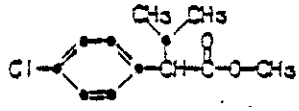
SD 52667 (*a isomer*): 2(3H)-Furanone, 3-(4-chlorophenyl)dihydro-4-methyl-.



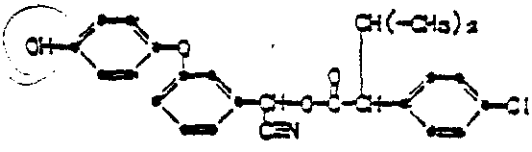
SD 53405 (*b isomer*): 2(3H)-Furanone, 3-(4-chlorophenyl)dihydro-4-methyl-,



SD 53065: Benzeneacetic acid, 4-chloro-α-hydroxy-α-(1-methylethyl)-.



SD 51889: Benzeneacetic acid, 4-chloro-α-(1-methylethyl-, methyl ester.



SD 48838: Benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, cyano-(3-phenoxy-4-hydroxyphenyl)methyl ester.

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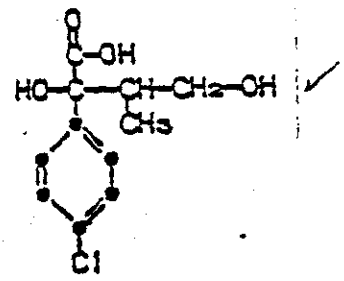
TABLE OF COMPOUNDS



SD 90930

Benzeneacetic acid, 4-chloro-alpha--hydroxy-alpha-(2-hydroxy-1-methylethyl)-, (Isomer A).

13544

026247

Chemical:	**Benzeneacetic acid, 4-chloro-.alpha.-(1-**
PC Code:	**109303**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/25/85**
File ID:	**TX004681**
Accession Number:	**412-03-0018**

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12/04/2002