Metabolism of Triallate in Sprague-Dawley Rats. 1. Material Balance, Tissue Distribution, and Elimination Kinetics

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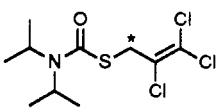
Triallate isotopically labeled with ^{14}C and ^{13}C in the C-1 allylic carbon position was administered orally to male and female Sprague-Dawley rats at dose levels of 5 and 500 mg/kg of body weight. Most of the dose was rapidly eliminated within 72 h after administration, and dose recoveries averaged 92% and 96% for the low-dose and high-dose animals, respectively. Approximately 42–52% of the dose was excreted via the urine and 33–46% via the feces. Expired $^{14}CO_2$ accounted for approximately 4% of the dose, while the residual carcass at sacrifice contained approximately 2%. Radioactivity in blood was associated principally with hemoglobin. Kinetics for whole-body elimination of radioactivity were consistent with a two-compartment open model. The half-life of the α phase varied from 5.9 to 22.8 h, and that of the β phase from 171 to 265 h.

INTRODUCTION

Triallate (1) [*N*,*N*-bis(1-methylethyl)-*S*-(2,3,3-trichloro-2-propenyl)thiocarbamate] is the active ingredient in the preemergence Monsanto herbicides Avadex BW and Fargo and has been widely used since 1962 for the control of



triallate (1, * = C-1)

wild oats in a variety of crops. Previous papers have provided limited information on the metabolism of triallate in sugar beets (Ebing and Schuphan, 1979) and in laboratory rats (Marsden and Casida, 1982). The latter study reported only chloroacrylic acid and trichloroacrylic acid as trace urinary metabolites, which together accounted for less than 0.10% of the dose. We were interested in obtaining a detailed understanding of the metabolism of triallate in laboratory rats dosed orally with [^{14}C]triallate, and we report our findings herein.

The present paper describes the material balance, tissue distribution, and elimination kinetics in Sprague-Dawley rats orally dosed with isotopically labeled triallate. The following paper in this issue (Nadeau et al., 1993) describes the chemical identification of urinary and fecal metabolites excreted by these animals and outlines possible metabolic pathways accounting for metabolite formation. The final paper in this series (Hackett et al., 1993) describes an in vitro study of triallate metabolism using microsomal enzyme preparations obtained from Sprague-Dawley rats and provides a detailed mechanistic understanding of the formation and reactions of metabolic intermediates giving rise to observed in vivo rat metabolites.

MATERIALS AND METHODS

Chemicals. The triallate used for this study was a mixture of [1-^{14}C]- and [1-^{13}C]-*N*,*N*-bis(1-methylethyl)-*S*-(2,3,3-trichloro-2-propenyl)thiocarbamate. The radiolabel location took into account previous studies of the metabolic fate of the amine, carbonyl, and thiol moieties of other thiocarbamates, demonstrating the lability of the thiocarbamate amine moiety (Fang, 1969) and carbonyl group (Hubbell and Casida, 1977). The [^{14}C]-triallate had a specific activity of 9.87 mCi/mmol and chemical and radiochemical purities of 98% and 99%, respectively. [^{13}C]-Triallate, admixed to facilitate metabolite mass spectral identification, was enriched at a ^{13}C level of 94.4% and had a chemical purity of 98.4%. Unlabeled triallate used for dilution to the desired specific activity had a purity of 99%. Test materials were synthesized in the laboratories of Monsanto Agricultural Co., St. Louis, MO (Nadeau at al., 1993). Purchased chemicals were of reagent or scintillation grade. Fluoralloy scintillation cocktail was obtained from Beckman Co. (Irvine, CA). Soluene 350, Dimilume 30, and Instagel scintillation cocktails were products of Packard Instrument Co. (Downers Grove, IL).

Animal Husbandry. Sprague-Dawley rats (120–230 g) were pruchased from Charles River Laboratories (Wilmington, MA) and quarantined for at least 6 days prior to the study. Study animals were housed in glass Roth-type metabolism units for 3 days before dosing. An airflow of 400–500 mL/min was maintained through the system, and a 12-h light–dark cycle was provided. The rats were supplied Certified Purina Rat Chow pellets (Ralston Purina, St. Louis, MO) and water ad libitum.

Animal Dosing. The triallate test substance was labeled at a final specific activity of 4.7 mCi/mmol for the 5 mg/kg groups and at a specific activity of 0.24 mCi/mmol for the 500 mg/kg group. The dosing solutions were prepared by dissolving isotopically labeled triallate in laboratory grade corn oil (Fisher Scientific Co., St. Louis, MO) to give a dose volume of 0.2 mL/100 g of body weight. Rats were given a single oral dose by intubation using a straight Perfektum 16-gauge 3-in animal feeding needle (Popper and Sons, Inc., New Hyde Park, NY) connected to a 1-mL syringe. The amount of solution administered was determined gravimetrically. Males each received an average of 17.4 (1.14 mg) and 10.4 μCi (130 mg) of radiolabeled triallate for the low- and high-dose groups, respectively, while females in the low- and high-dose groups each received 15.5 (1.01 mg) and 8.2 μCi (102 mg), respectively.

Sample Collection and Analysis. Effluent air from Roth metabolism cages was passed through a primary scrubbing tower containing 100 mL of 5 M ethanolamine in methoxyethanol and a secondary trap containing 50 mL of the same trapping solution. Two cylindrical polyurethane trapping plugs (Fisher Scientific; 1-cm diameter × 5-cm length) were also inserted in series in the air effluent line between the Roth cages and the primary CO_2 trap. Urine was collected in tubes maintained at 4 °C using a constant-temperature bath, and feces were frozen with dry ice at collection. Trapping solutions, urine, and feces were collected at 6-, 12- and 24-h intervals and then daily for 10 days after dosing. At sacrifice the cage was thoroughly rinsed with water and acetone, and rinses were measured for radioactivity.

Expired gas traps were analyzed for radioactivity by adding 1-mL samples of trapping solution to 15 mL of counting solution consisting of a 7/2 (v/v) mixture of Fluoralloy and methanol. Polyurethane plugs were extracted with hexane, and 0.5-mL aliquots of extract were added to 15 mL of Instagel for counting.

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Urine and other aqueous samples were counted by adding 0.2–1.0 mL of sample to 15 mL of Instagel. Fecal samples were frozen, lyophilized to dryness, and ground to a fine powder. Powdered fecal material was combusted in a Tricarb B306 sample oxidizer (Packard Instrument) using a 2-min burn cycle. All samples were combusted in duplicate. A ^{14}C standard was combusted after every 20 samples, and the average recovery was used to correct fecal combustion data.

Rats were anesthetized 10 days after dosing and sacrificed by exsanguination; blood was collected in vacutainers containing sodium heparin. Organs, tissue samples, and residual carcasses were stored at −20 °C. Minced organ and tissue samples were combusted in duplicate, as were feces. Carcasses were frozen at −70 °C, fractured into small pieces, and ground to a fine powder in a blender. Five replicate samples were combusted in the same manner as other tissue samples. Ovary samples were solubilized by suspension in a 10-fold (w/v) excess of Soluene 350, followed by heating at 50–60 °C for 4–5 h in a sand bath. The solubilized samples were counted in Dimilume 30.

All samples were counted with a Mark III liquid scintillation spectrometer (Model 81, TM Analytic, Elk Grove Vilalge, IL) using variable quench and external standardization. The standard error of the mean (SEM) and Student's t-test were used to determine the statistical significance of differences in excreta and tissue values between sexes.

Blood Analysis. Pooled blood samples were prepared from the four study groups by combining 1-mL portions of the whole blood from the male and female rats at each dose level. Portions (1 mL) of pooled samples were mixed with 2 mL of phosphate buffer (pH 7) and were lysed by a cycle of vigorous shaking, heating at 70 °C for 5 min, and freezing in dry ice. The freeze–thaw cycle was repeated, samples were centrifuged, and supernatants were removed. Pellets were further processed by a second lysis cycle, and supernatants from both cycles were combined. Protein in the combined supernatants was precipitated with 7% trichloroacetic acid (TCA) and centrifuged, and the supernatants were discarded. The precipitated proteins were solubilized using 5% sodium dodecyl sulfate (SDS) and mild heating at 50–60 °C. Combustion was used to determine radioactivity in the original pooled blood, TCA supernatants, and the final solubilized proteins.

For analysis by electrophoresis, 0.45 mL of the solubilized blood proteins from the 500 mg/kg groups was mixed with 0.05 mL of 2-mercaptoethanol and 0.5 mL of a buffer consisting of 62.5 mM Tris (pH 6.8), 2.3% SDS, and 20% glycerol. Electrophoresis was performed using a Hoefer Series 600 unit (Hoefer Scientific Instruments Inc., San Francisco, CA) on 20 × 20 cm polyacrylamide gels containing a gradient of 10–20% SDS. Rat hemoglobin (50 μg) was electrophoresed concurrently as a standard. Electrophoresed gels were cut into 1-cm strips, which were transferred to scintillation vials, mixed with 1 mL of water, and digested with 1 mL of 30% hydrogen peroxide at 70 °C for 8 h. Gel solutions were cooled and mixed with Instagel for liquid scintillation counting.

Kinetic Analysis. Excretion data were analyzed by the "sigma-minus" plotting method (Wagner, 1975) used to characterize the kinetics of whole-body elimination; i.e., the difference between the percentage of dose recovered and the cumulative percentage eliminated (urine plus feces) was plotted on a logarithmic scale vs time on a linear scale. Sigma-minus plots obtained for triallate elimination could not be approximated by a straight line, and the data were therefore fit to a typical biexponential function (eq 1).

$$y(t) = Ae^{-k_\alpha t} + Be^{-k_\beta t} \quad (1)$$

$$t_{1/2} = 0.693/k_\alpha \text{ or } k_\beta \quad (2)$$

Percentage of dose recovered minus the cumulative percentage eliminated in urine and feces (percentage of dose remaining in the body) is expressed as y, and the terms k_α and k_β correspond to the rate constants of the initial rapid α phase and the secondary (slower) β phase, respectively. The constants A, k_α, B, and k_β were determined from the experimental data by resolving the biexponential function into individual components using a nonlinear regression analysis based on the method of residuals (Tuey, 1980). The half-life values for the α and β phases were calculated using eq 2.

Table I. Radioactivity Recovered from Rats Dosed with Triallate[a]

	5 mg/kg dose		500 mg/kg dose	
fraction	males	females	males	females
urine	42.3 ± 7.1	51.5 ± 5.7	44.1 ± 2.7	51.8 ± 3.6
feces	42.0 ± 4.1	32.6 ± 3.9	45.6 ± 4.7	36.2 ± 4.1
$^{14}CO_2$	4.30 ± 0.31	4.89 ± 0.14	3.42 ± 0.44	4.55 ± 0.39
carcass[b]	2.10 ± 0.13	2.19 ± 0.10	1.72 ± 0.11	1.94 ± 0.18
total[c]	91.9 ± 4.7	92.5 ± 2.2	95.9 ± 4.9	95.8 ± 1.4

[a] Mean percentage of dose ± SEM from five animals for 0–10 days after dosing. [b] Whole rat minus organs/tissues removed at necropsy. [c] Includes cage washes (0.3–0.8%) and removed organs/tissues (0.4–0.5%).

Table II. Radioactivity Distribution in Tissues of Rats Dosed with Triallate[a]

	5 mg/kg dose		500 mg/kg dose	
tissue[b]	males	females[c]	males	females[d]
blood	1.41 ± 0.07	1.94 ± 0.23	155 ± 15.7	239 ± 23.5
spleen	0.31 ± 0.01	0.49 ± 0.004	29 ± 1.0	54 ± 3.6
kidney	0.30 ± 0.01	0.47 ± 0.02	26 ± 2.7	39 ± 2.2
liver	0.21 ± 0.01	0.29 ± 0.02	24 ± 2.2	36 ± 2.2
heart	0.14 ± 0.01	0.20 ± 0.03	15 ± 0.3	24 ± 1.1
muscle[e]	0.13 ± 0.01	0.16 ± 0.01	10 ± 0.8	14 ± 1.1
fat[e]	0.08 ± 0.01	0.10 ± 0.01	4.9 ± 0.6	6.8 ± 0.5
femur	0.08 ± 0.01	0.11 ± 0.003	8.6 ± 0.7	12 ± 0.9
brain	0.07 ± 0.004	0.10 ± 0.003	8.7 ± 0.9	14 ± 1.4

[a] Mean triallate equivalents (ppm) ± SEM for five animals sacrificed 10 days after dosing. [b] Colon, stomach, and small intestine (not shown) contained less than 0.07 ppm at 5 mg/kg and less than 7.3 ppm at 500 mg/kg. [c] Females were statistically different from males ($p \leq 0.05$) for all tissues except blood. [d] Females were statistically different from males ($p \leq 0.05$) for all tissues. [e] Abdominal fat and muscle were sampled.

RESULTS AND DISCUSSION

Material Balance. The triallate dose was substantially eliminated during the 10 days after dosing (Table I). There was no statistically significant difference in overall elimination between sexes or dose levels, although females consistently excreted more of the dose in the urine and less in the feces than males. Approximately 4.6% and 4.0% of the dose was expired at the 5 and 500 mg/kg levels, respectively. Polyurethane trapping plugs contained less than 0.02% of the dose, indicating that unmetabolized triallate was not exhaled by the rats. The expired gases consisted of $^{14}CO_2$ derived from the [1-^{14}C]-2,3,3-trichloro-2-propenethiol moiety of [^{14}C]triallate. Approximately 2% of the oral dose remained in the residual carcass. Total radioactive accountability was excellent, averaging between 91.9% and 95.9%.

Tissue Distribution. At sacrifice the highest levels of radioactivity were found in blood (Table II). Activity was also observed in highly perfused tissues such as spleen, kidney, liver, and heart, at levels at least 4-fold lower than blood. Tissue residue levels were approximately proportional to dose level, and statistically higher levels of triallate-derived tissue activity were observed in females at both dose levels, as compared with males. Since tissues were not perfused with saline at sacrifice, the measured tissue radioactivity is at least partially due to the presence of blood in addition to tissue-specific accumulation. Some tissue accumulation via label incorporation into natural constituents is also possible in view of the observed label turnover as expired $^{14}CO_2$.

Blood-Associated Radioactivity. Approximately 2% of the dose was calculated as being associated with blood,

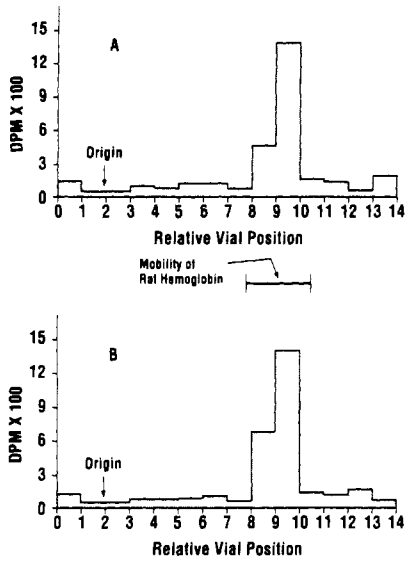

Figure 1. Gel electrophoresis of solubilized protein from red blood cells of rats dosed with triallate: (A) males; (B) females.

assuming that the latter approaches 6% of the final body weight (Wang, 1959) and using the blood radioactivity level determined by combustion (Table II). This calculated blood-associated percentage of the dose correlates well with the percentage actually found in the carcass at sacrifice (Table I). Centrifugation of isolated whole blood demonstrated that the cellular fraction contained levels of triallate equivalents 60–100-fold higher than that of the blood plasma. Lysis of the blood cells and precipitation of protein resulted in association of 88–105% of the radioactivity with the protein fraction. The precipitated protein for the high-dose group was solubilized with SDS and subjected to polyacrylamide slab gel electrophoresis. Gel histograms (Figure 1) demonstrate that a single major radioactive peak was found, suggesting that triallate-derived residues were covalently associated with hemoglobin. Blood-associated radioactivity has also been observed for another thiocarbamate herbicide, ordram (Debaun et al., 1978a,b), but has not been further characterized.

Elimination Kinetics. The radiochemical whole-body elimination of triallate is compatible with a two-compartment, open pharmacokinetic model (Wagner, 1975). In this model, the central compartment is typically assumed to represent the blood and highly perfused tissues in rapid equilibrium with blood. The peripheral compartment generally corresponds to poorly perfused tissues such as fat. The association of a particular tissue with the central or peripheral compartment can depend on the individual characteristics of the compound being modeled. Such modeling assumes that elimination and intercompartmental transfer obey first-order kinetics and that elimination occurs only from the central compartment. Solution of the biexponential function described under Materials and Methods permits the calculation of half-lives for two phases of elimination, designated α and β phases. The α phase is assumed to reflect absorption and distribution, as well as elimination from the central compartment. The β or postdistributive phase is assumed to reflect primarily intercompartmental transfer between the central and peripheral compartments, as well as any continuing elimination from the central compartment.

Table III illustrates the results obtained for whole-body elimination of single 5 and 500 mg/kg doses of triallate. Differences in the α phase were observed as a function of gender and dose level. For both sexes the half-lives for

Table III. Whole-Body Elimination Kinetics in Rats Dosed with Triallate

group	dose, mg/kg	α phase[a]	β phase[a]
males	5	5.92 ± 0.31	171 ± 7.6
males	500	15.2 ± 0.64[b]	163 ± 17
females	5	7.64 ± 0.40[b]	179 ± 8.0
females	500	22.8 ● 2.10[c]	265 ± 99

[a] Half-life in hours. [b] Statistically different from 5 mg/kg males ($p \leq 0.05$). [c] Statistically different from 5 mg/kg females and 500 mg/kg males ($p \leq 0.05$).

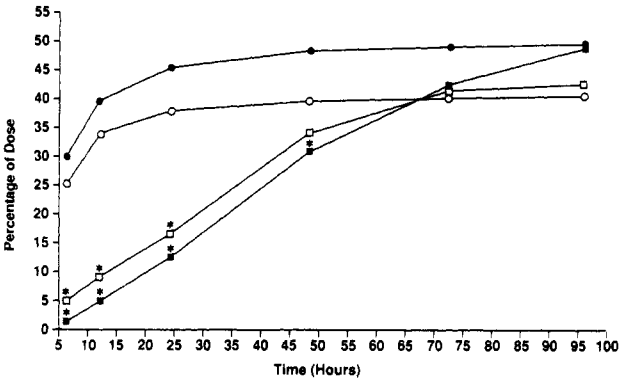

Figure 2. Elimination of radioactivity in urine of rats dosed with triallate: (■) 500 mg/kg, females; (□) 500 mg/kg, males; (●) 5 mg/kg, females; (○) 5 mg/kg, males. Asterisks (*) denote time intervals for which the 500 mg/kg dose group was statistically different from the 5 mg/kg group ($p \leq 0.05$).

the α phase at the high dose were 2.5–3-fold longer than the half-lives at the low dose. This longer half-life at the high-dose level suggests a major difference either in the rate of absorption of triallate from the gastrointestinal tract or in the rate of metabolism, or both, as compared with the corresponding processes at the low-dose level. At both dose levels females displayed a longer half-live for the α phase than did males.

Most of the triallate dose was eliminated in the urine and feces during the first 72 h. Typical elimination kinetics are illustrated in Figure 2, which plots the cumulative percentage of dose recovered in urine over the first 96 h after dosing. The rates of excretion for males and females in the 500 mg/kg groups were lower than the rates observed for the 5 mg/kg groups. The activity levels for urinary excretion at 500 mg/kg display an initial, statistically significant, lag period (Figure 2) consistent with a longer α phase half-life. The α phase reflects elimination as well as absorption, distribution, and metabolism, and changes in these processes at the high-dose level would be reflected in a longer initial half-life of elimination. Differences in triallate metabolite distribution are also observed at the 5 and 500 mg/kg dose levels (Nadeau et al., 1993). The β phase half-life is considerably longer than the α phase half-life for all groups (Table III). The β phase results are consistent with a peripheral compartment having a half-life of approximately 159–265 h. The tissue distribution data illustrated in Table II indicate a preference for retention of trialalte or its metabolites in red blood cells. Since the half-life of such cells in rats can be calculated to be approximately 253 h, on the basis of a measured mean cell lifetime of 70 days (Pereira and Chang, 1981), it appears that red cell turnover contributes significantly to the length of the half-life for the β phase.

Conclusions. Rapid elimination of radioactivity was observed in Sprague-Dawley rats orally dosed with [^{14}C]-triallate at 5 and 500 mg/kg. At both dose levels males exhibited higher rates of elimination than did females, and excretion was more rapid for both sexes at the 5 mg/

kg dose level as compared with that at the 500 mg/kg dose level. Appreciable radioactivity was expired as $^{14}CO_2$, consistent with extensive breakdown of triallate to labile metabolic intermediates. Tissue residues remaining in the rat 10 days after dosing account for approximately 2% of the dose, and blood-contained radioactivity appears to be principally associated with hemoglobin in red blood cells.

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Supplementary Material Available: Plot of elimination of radioactivity in feces of rats dosed with triallate (2 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Chen, Y. S.; Schuphan, I.; Casida, J. E. S-Chloroallyl Thiocarbamate Herbicides: Mouse Hepatic Microsomal Oxygenase and Rat Metabolism of Cis and Trans-[^{14}C=O] Diallate. *J. Agric. Food Chem.* **1979**, *27*, 709–712.

Debaun, J. R.; Bova, D. L.; Finely, K. A.; Menn, J. J. Metabolism of [Ring-^{14}C]Ordram (Molinate) in the Rat. 1. Balance and Tissue Residue Study. *J. Agric. Food Chem.* **1978a**, *26*, 1096–1098.

Debaun, J. R.; Bova, D. L.; Tseng, C. K.; Menn, J. J. Metabolism of [Ring-^{14}C]Ordram (Molinate) in the Rat. 2. Urinary Metabolite Identification. *J. Agric. Food Chem.* **1978b**, *26*, 1098–1104.

Ebing, W.; Schuphan, I. In *Herbizide*; Boerner, H., Hietefuss, R., Hurle, K., Eds.; Harold Boldt Verlag: Boppard, Germany, 1979; pp 112–126.

Fang, S. C. Thiocarbamates. In *Herbicides: Chemistry, Degradation and Mode of Action*; Kearney, P.C., Kaufman, D. D., Eds.; Dekker: New York, 1969; Vol. 1, pp 323–348.

Hackett, A. G.; Kotyk, J. J.; Fujiwara, H.; Logusch, E. W. Metabolism of Triallate in Sprague-Dawley Rats. 3. In Vitro Metabolic Pathways. *J. Agric. Food Chem.* **1993**, third of three papers in this issue.

Hubbell, J. P.; Casida, J. E. Metabolic Fate of the N,N-Dialkyl Moiety of Thiocarbamate Herbicides in Rats and Corn. *J. Agric. Food Chem.* **1977**, *25*, 404–413.

Marsden, P. J.; Casida, J. E. 2-Haloacrylic Acids as Indicators of Mutagenic 2-Haloacrolein Intermediates in Mammalian Metabolism of Selected Promutagens and Carcinogens. *J. Agric. Food Chem.* **1982**, *30*, 627–631.

Nadeau, R. G.; Chott, R. C.; Fujiwara, H.; Shieh, H.-S.; Logusch, E. W. Metabolism of Triallate in Sprague-Dawley Rats. 2. Identification and Quantitation of Excreted Metabolites. *J. Agric. Food Chem.* **1993**, second of three papers in this issue.

Pereira, M. A.; Chang, L. W. Binding of Chemical Carcinogens and Mutagens to Rat Hemoglobin. *Chem. Biol. Interact.* **1981**, *33*, 301–305.

Schuphan, I.; Casida, J. E. S-Chloroallyl Thiocarbamate Herbicides: Chemical and Biological Formation and Rearrangements of Diallate and Triallate. *J. Agric. Food Chem.* **1979**, *27*, 1060–1066.

Tuey, D. B. Toxicokinetics. In *Introduction to Biochemical Toxicology*; Hodgson, E., Guthrie, F. C., Eds.; Elsevier: New York, 1980; pp 41–66.

Wagner, J. G. *Fundamentals of Clinical Pharmacokinetics*; Drug Intelligence Publications: Hamilton, IL, 1975.

Wang, L. Plasma Volume, Cell Volume, Total Blood Volume and Fcell Factor in the Normal and Splenectomized Rat. *Am. J. Physiol.* **1959**, *196*, 188–192.

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