Retention, Distribution, and Excretion of Ametryne (2-Methylmercapto-4-ethylamino-6-isopropylamino-*s*-triazine) in the Rat

William H. Oliver, Gordon S. Born, and Paul L. Ziemer

The fate of ^{14}C-Ametryne, a methylmercapto derivative of the *s*-triazine herbicides, was determined following administration of a single oral dose in rats. The results of respiration studies showed that both alkyl side chains were cleaved from the *s*-triazine ring and metabolized to CO_2 which was excreted via the lungs primarily in the first 12 hours. After 48 hours, 42% and 18% of the dose of ^{14}C-isopropyl Ametryne (I*A) and ^{14}C-ethyl chain Ametryne (E*A), respectively, had been excreted as $^{14}CO_2$. No $^{14}CO_2$ was detected in respired air of ^{14}C-ring Ametryne (R*A) dosed rats. The ^{14}C-ring Ametryne was excreted primarily via the urine (58%) and feces (32%), and less than 2% remained after 72 hours. Eleven tissues were analyzed from each of six rats at 6, 48, and 72 hours after dosing. The tissues highest in activity at all times for both sexes were blood, liver, lung, spleen, and kidney.

Ametryne, a member of the *s*-triazine family of herbicides, has been used as a pre- and post-emergent herbicide since 1960 to selectively kill weeds in food crops (Gysin, 1962). Although the literature reflects toxicity studies of Ametryne (Knusli, 1964) and metabolic studies of related *s*-triazine compounds (Böhme and Bär, 1967; Bakke *et al.*, 1967; Robbins *et al.*, 1968), no reports were found in the literature concerning the metabolic fate of methylmercapto derivatives of *s*-triazine in mammalian systems. This paper reports on studies which were conducted to determine the persistence, tissue accumulation, and excretion patterns resulting from acute dosage of ^{14}C-labeled Ametryne in rats.

MATERIALS AND METHODS

2 - Methylmercapto - 4 - ethylamino - 6 - isopropylamino - *s*-triazine-2,4,6-^{14}C, uniform ring labeled Ametryne (R*A) 7.2 μCi/mg.; 2-methylmercapto-4-ethyl-1-^{14}C-amino-6-isopropylamino-*s*-triazine, ethyl chain labeled Ametryne (E*A) 6.7 μCi/mg.; and 2-methylmercapto-4-ethylamino-6-isopropyl-1-^{14}C-amino-*s*-triazine, isopropyl chain labeled Ametryne (I*A) 5.2 μCi/mg. were provided courtesy of Geigy Agricultural Chemicals (Division of Geigy Chemical Corp., Ardsley, N. Y. 10502). Each of the above compounds was shown to have a radiochemical purity of greater than 99% by thin-layer chromatography, autoradiography, and liquid scintillation counting techniques. The chemical purity of the three labeled Ametrynes compared satisfactorily with authentic Ametryne in melting point determination and ultraviolet absorption spectra which were conducted on the four compounds. All other chemicals or reagents used in these studies were of analytical grade or in the case of chromatographic procedures were redistilled technical grade. R*A, I*A, and E*A were dissolved in 50% ethanol and these stock solutions were calibrated against ^{14}C-benzoic acid internal standard in a liquid scintillation detector. The results showed the concentration of R*A, I*A, and E*A to be 32.0 μCi. per ml., 43.6 μCi. per ml., and 36.7 μCi. per ml., respectively. The herbicide was administered orally by stomach tube to 44 male and female Sprague-Dawley strain rats weighing 195 to 265 gm. at dosage levels from 1 mg. (6.8 μCi.) to 4 mg. (18.2 μCi.) per animal. The oral LD_{50} for Ametryne is 1110 mg. per kg. of body weight (Knusli, 1964). After dosing, the animals used in the respiration studies were placed individually in metabolism cages (Roth *et al.*, 1948) and fasted; in the tissue distribution studies, they were placed individually in metal metabolism cages and allowed food and water *ad libitum*. Respired CO_2, urine, and feces were collected periodically. Expired CO_2 was trapped in a solution of one part monoethanolamine and two parts 2-ethoxyethanol which was changed at 1- and 12-hour intervals and analyzed for radioactivity for two days following ^{14}C-Ametryne administration. Collection efficiency was 100% for CO_2.

Radioanalysis. The radioactivity in all samples except for tissue samples was measured in a Packard Model 3003 Tricarb liquid scintillation spectrometer. The radioactivity in tissue was analyzed using the Beckman Model LS 100 liquid scintillation counter. Three counting formulations were used: solution A for counting CO_2 trapping solution consisted of one part toluene, one part 2-ethoxyethanol, 0.8% PPO (2,5-diphenyloxazole) and 0.01% dimethyl POPOP [1, 4-bis-(4-methyl-5-phenyloxazole)-benzene]; solution B for counting urine samples consisted of one part xylene, three parts *p*-dioxane, three parts 2-ethoxyethanol, 1.0% PPO, 0.05% POPOP, and 8% naphthalene; solution C used for TLC plate scrapings, feces, and tissue samples consisted of one part toluene, one part 2-ethoxyethanol, and 0.3% PPO. Low potassium counting vials were used throughout the studies. All counted samples were fortified with ^{14}C-benzoic acid internal standard and recounted to determine the counting efficiency. For all samples giving statistically significant count rates, the counting efficiency was greater than 50%. Fifteen milliliters of solution A and 3 ml. of the CO_2 trapping solution comprised the counting solution for CO_2 samples. Appropriate aliquots of urine in duplicate were counted in vials containing 15 ml. of solution B. Thin-layer chromatogram plate scrapings were added to 15 ml. of solution C for counting with no evidence for the need of a suspending agent. The radioactivity of the feces was determined in duplicate by dissolving representative 20 to 40 mg. aliquots of dried and crushed excreta. The aliquots were placed in counting vials and digested at 14° C. with 0.25 ml. of perchloric acid 60% (Mahin and Lofberg, 1966) and $^1/_2$-ml. hydrogen peroxide 30% which was added for decoloring purposes. After solubilization was complete and the samples were cooled, they were counted with 15 ml. of solution C. Rat tissue was analyzed for radioactivity by solubilizing duplicate 100- to 200-mg. samples by the same procedure as was used for feces. The rat carcass was homogenized to obtain representative samples. Perchloric

Bionucleonics Department, Purdue University, Lafayette, Ind. 47907

Table I. Average Per Cent Recovery of Radioactivity as $^{14}CO_2$ in Respired Air of Rats Fed I*A

Hours after Dose	Recovery, % 6 Males	6 Females	Student "t"[a]	Recovery, % 12 Rats	Cumulative Recovery %
0–1	0.06	0.47	1.35	0.27	0.27
1–2	0.44	1.04	1.86	0.74	1.01
2–3	1.98	3.45	1.40	2.72	3.73
3–4	3.28	4.48	0.95	3.88	7.61
4–5	4.48	4.51	0.02	4.50	12.11
5–6	5.43	4.18	1.10	4.81	16.92
6–7	4.82	3.65	1.15	4.24	21.16
7–8	3.24	3.92	0.62	3.58	24.74
8–9	2.52	3.00	0.67	2.76	27.50
9–10	1.92	2.80	1.41	2.36	29.86
10–11	1.74	2.34	1.14	2.04	31.90
11–12	1.24	1.65	0.66	1.45	33.35
12–24	4.57	6.53	1.32	5.55	38.90
24–36	1.99	1.91	0.15	1.95	40.85
36–48	1.01	1.08	0.50	1.05	41.90
0–48	38.72	45.01	2.31	41.9	41.9

[a] $tp \leq 0.05 = 2.228$.

Table II. Average Per Cent Recovery of Radioactivity as $^{14}CO_2$ in Respired Air of Rats Fed E*A

Hours after Dose	Recovery, % 6 Males	6 Females	Student "t"[a]	Recovery, % 12 Rats	Cumulative Recovery %
0–1	0.11	0.25	3.69	0.18	0.18
1–2	0.66	1.04	1.99	0.85	1.03
2–3	1.24	1.86	1.68	1.55	2.58
3–4	1.27	1.97	2.06	1.62	4.20
4–5	1.38	2.13	2.57	1.76	5.96
5–6	1.40	1.86	2.32	1.63	7.59
6–7	1.27	1.45	0.88	1.36	8.95
7–8	1.30	1.26	0.13	1.28	10.23
8–9	1.21	1.19	0.10	1.20	11.43
9–10	0.97	0.79	1.10	0.88	12.31
10–11	0.87	0.69	1.09	0.78	13.09
11–12	0.69	0.60	0.60	0.65	13.74
12–24	3.43	2.83	0.56	3.13	16.87
24–36	0.74	0.70	0.65	0.72	17.59
36–48	0.49	0.50	0.24	0.50	18.09
0–48	17.03	19.12	1.43	18.1	18.1

[a] $tp \leq 0.05 = 2.228$.

acid digestion proved equally efficient for feces and tissue analysis to Hyamine treatment. No evidence of loss of radioactivity by oxidation to volatile products was found in a preliminary study designed to determine if such losses occurred when feces and tissue samples were prepared by the above methods.

Thin-Layer Chromatography and Autoradiography. To determine radiochemical purity of the labeled compounds, ^{14}C-Ametrynes (in 50% ethanol) and authentic unlabeled Ametryne were spotted on activated (1 hour at 100° C.) 250-micron coated plates. The slurry used to coat the plates consisted of one part Adsorbosil-1P and two parts of Adsorbosil-1 (Applied Science Laboratories, Inc., State College, Pa.). The chromatographic plates were then developed in each of the following developing solvent systems: toluene to methyl alcohol (135 to 15); *n*-hexane to *n*-butyl acetate (95 to 55); cyclohexane to nitromethane (95 to 55); and chloroform to acetone (135 to 15). Autoradiograms were made from the developed chromatograms by exposing medical X-ray film for a period of time calculated to allow 10^8 radioactive disintegrations in order to observe the presence of radioactive spots as small as 0.5% of the total radioactivity placed on the chromatograms. To further quantitate, the adsorbant was scraped from chromatograms in sections and the radioactivity determined with a liquid scintillation counter.

RESULTS AND DISCUSSION

Excretion Patterns of Ametryne. The mean excretion of expired $^{14}CO_2$ of six male and six female rats orally dosed with I*A is shown in Table I, and the results of an identical study with E*A is shown in Table II. Results of a Student's "*t*" test for comparing the means of males and females at each time interval showed no significant difference in the I*A respiration study between groups. However, the females did respire slightly more $^{14}CO_2$ during the 48-hour time period. Both male and female respired approximately 80% of the total exhaled $^{14}CO_2$ in the first 12 hours after dosing. The males excreted 38.7% and the females respired 45.0% of the total dose after 48 hours.

In the E*A respiration study, the female group respired significantly more $^{14}CO_2$ during several time periods (0–1, 4–5, and 5–6 hours), but at the end of the 48-hour study total elimination for both groups was not significantly different. Males and females excreted approximately 75% of the total expired $^{14}CO_2$ in the first 12 hours after dosing with E*A. The males respired 17.0% and the females respired 19.1% of the total E*A dosage. A study on one male and one female rat showed 0.01% and 0.05% of a 15-μCi. oral dose of ^{14}C-ring labeled dose was expired as $^{14}CO_2$ during a 2-day period. This could have been the result of radioimpurities since the amount of $^{14}CO_2$ detected was within purity limits established for R*A.

In the case of I*A, in addition to the 42% of the radioactive dose recovered as respired $^{14}CO_2$, 20% was recovered as Ametryne and/or metabolites in the urine, 2% in the feces, and 7% remained in the carcass after 48 hours. With E*A, 18%, 45%, 5%, and 9% of the administered dose were accounted for in the respired CO_2, urine, feces, and carcass, respectively. On the average, a total of about 88% of the dose was recovered from both the I*A- and E*A-dosed animals. Excretion of Ametryne and/or metabolites in the urine and feces was studied by collecting appropriate samples from the 18 R*A dosed rats (nine males and nine females) which were used in the tissue distribution study. Three male and three female rats were removed from the original group at six, 48, and 72 hours for determining tissue concentrations. Table III shows the data for the urinary and fecal excretions. A rapid elimination of ^{14}C-ring Ametryne and/or metabolites was observed. Approximately 75% of the urinary elimination of activity was in the first 24 hours. Within 72 hours, 58% of the dosage had been eliminated by way of the urine. Approximately 32% of the R*A activity was recovered in the feces after three days with 50% of this amount occurring in the first 24 hours. No significant differences between males and females in the over-all excretion of R*A and/or metabolites through the urine and feces was observed. About 4% of the administered dose remained in the carcass at the end of 48 hours and less than 2% remained at the end of the 72-hour time period.

Tissue Distribution of Ametryne. The mean percentage of radioactivity per gram of tissue (average for three male and three female rats at each of the time periods) at three time periods after oral administration of R*A is shown in Table IV. The tissues where differences in concentration of activity

Table III. Average Per Cent Recovery of Radioactivity in Urine and Feces of Rats Fed R*A

Hours after Dose	Recovery, % Male	Recovery, % Female	Student "t"[a]	Average Recovery, Male and Female	Cumulative Recovery, %
Urine					
0–6	18.75[b]	16.81[b]	0.51	17.78	17.78
6–12	21.28[c]	23.90[c]	0.43	22.59	40.37
12–24	12.06[c]	11.58[c]	0.17	11.82	52.19
24–36	3.07[c]	3.10[c]	0.64	3.40	55.59
36–48	1.03[c]	1.17[c]	0.28	1.10	56.69
48–60	0.46[d]	0.65[d]	1.15	0.56	57.25
60–72	0.27[d]	0.40[d]	8.14	0.34	57.59
0–72	57.55	57.61	0.43	57.6	57.6
Feces					
0–6[e]	0.18	0.07	0.89	0.13	0.13
6–12[f]	3.25	0.09	2.26	1.67	1.80
12–24	17.91	13.92	0.69	15.92	17.73
24–36	7.43	10.36	0.78	8.90	26.62
36–48[g]	2.58	4.45	1.04	3.52	30.14
48–60	2.13	1.07	1.05	1.60	31.74
60–72	0.36	0.37	0.04	0.37	32.11
0–72	33.84	30.33	1.24	32.1	32.1

[a] No sign differences except 60–72 hr. for urine. [b] Mean of 9 animals. [c] Mean of 6 animals. [d] Mean of 3 animals. [e] 6 male and 2 female contributing. [f] 5 male and 3 female contributing. [g] 1 female did not contribute.

Table IV. Average Per Cent of Radioactivity in Tissues of Rats Fed R*A

Organ	Recovery, %/Gram[a] 6 hr.	48 hr.	72 hr.
Blood	0.16[b]	0.15	0.19
Brain	0.04[b]	0.01	0.01
Carcass	0.06	0.01	0.01
Fat	0.07	0.01	0.01
Heart	0.07[b]	0.04	0.02
Kidney	0.34[b]	0.05	0.04[b]
Liver	0.29	0.11[b]	0.07[b]
Lungs	0.11[b]	0.06	0.05
Muscle	0.04	0.01	0.01
Spleen	0.31	0.06[b]	0.05
Stomach	2.38	0.03	0.01

[a] Average for three male and three female rats at each time interval. [b] Indicates significant difference in concentration of tissue radioactivity between males and females.

occurred between males and females are marked. The highest concentration was present in females in all cases and probably reflects hormonal differences. The blood, liver, lung, spleen, and kidney were the tissues highest in activity at all time intervals for both male and female animals. The tissues lowest in activity were the heart, fat, muscle, brain, and carcass. The activity in the blood appeared to remain about constant with time, whereas the activity in all other tissue decreased with time. The experiment was terminated prior to the observation of a decrease in blood activity, but no evidence was found which would conflict with the hypothesis that the blood activity would decrease when Ametryne and/or metabolites were cleared from the tissue. The average per cent recovery of R*A at the 72-hour period was 98%.

SUMMARY AND DISCUSSION

Ametryne, a pre- and post-emergent herbicide, was rapidly metabolized and excreted from the rat. It appeared that both alkylamino side chains of Ametryne were metabolized by de-alkylation and conversion to CO_2, which was excreted via the lungs. The parent compound, and/or metabolites, were excreted by way of the urine and feces with less than 4% and 2% remaining in the carcass at 48 to 72 hours, respectively.

Total elimination is not pragmatically different for the I*A, E*A, and R*A compounds from the rat. But, the pathways of elimination for the radioactivity vary, depending upon the labeling position in the compound. The pathways of elimination, indicated by the data, imply that the isopropyl chain of Ametryne is more easily metabolized to CO_2 in the rat than is the ethyl chain.

With I*A and E*A, 7% and 9%, respectively, of the administered dose remained in the carcass, while less than 4% of the R*A remained in the carcass after 48 hours. This difference in carcass retention may be the consequence of observing the metabolism of two- and three-carbon atom compounds which resulted from side chain cleavage. In general, no significant differences were observed between male and female groups in the excretion of Ametryne and/or metabolites.

As a result of the above findings, it was concluded that Ametryne would not be stored or accumulated in any tissue or body fluid of the rat's body for extended periods of time. Also, it was concluded that an ingested dose would be rapidly eliminated, to the extent of 75% within 24 hours; and that less than 2% of the dose would remain in the animal body after 72 hours.

LITERATURE CITED

Bakke, J. E., Robbins, J. D., Feil, V. J., J. AGR. FOOD CHEM. **15**, 628–31 (1967).
Böhme, C., Bär, F., *Food Cosmet. Toxicol.* **5**, 23–8 (1967).
Gysin, H., *Chem. Ind.* (London), **31**, 1393–1400 (1962).
Knusli, E., "Ametryne," in "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," G. Zweig, Ed., Vol. 4, Academic Press, N. Y., 1964.
Mahin, D. T., Lofberg, R. T., *Anal. Biochem.* **16**, 500–9 (1966).
Robbins, J. D., Bakke, J. E., Feil, V. J., J. AGR. FOOD CHEM. **16**, 698–700 (1968).
Roth, L. J., Leifer, E., Hogness, J. R., Langham, W. H., *J. Biol. Chem.*, **176**, 249–57 (1948).

Received for review March 3, 1969. Accepted July 14, 1969. Research conducted under the auspices of the Institute for Environmental Health Studies and supported in part by the National Center for Radiological Health, United States Public Health Service.