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Fate of Radioactive Tetramine in Small Mammals and Its Possible Use as a Seedling Protectant

In spite of its extreme toxicity (Hagen, 1950), tetramethylenedisulphotetramine (tetramine) has been considered a potential chemical for protecting forest tree seedlings from animals (Kverno, 1960). An earlier investigation (Radwan, 1967) discussed absorption, translocation, and metabolism of tetramine in plants and questioned the usefulness of the chemical. In the present study, we evaluate tetramine further by investigating absorption, distribution, elimination, and metabolism in white mice (and a small number of snowshoe hares) after oral administration of C^{14} uniformly labeled tetramine (tetramine*).

Materials and Methods

Radioactive tetramine. The tetramine* (2.02 millicuries/millimole; prepared by Tracerlab,¹ Waltham, Mass.) used in this study was approximately 80 per cent pure, as determined earlier by paper chromatography (Radwan, 1967). To prepare the treatment solution, we dissolved the tagged chemical in a small amount of acetone and diluted it with water to a concentration of 15 parts per million (ppm).

Metabolic apparatus. Swiss white mice (*Mus musculus*), 20-40 days old, were used. Animals under test were individually housed in glass metabolism cages similar to those described by Comar (1955) to allow separate collection of urine and feces. Carbon dioxide-free air was passed through the cages at a rate of approximately 0.5 liter per minute, and respiratory CO_2 was trapped in carbonate-free 1N-NaOH solution. Water and pelleted food were available *ad libitum*, and animals were acclimated to the cages for two days before treatment.

Treatment of animals. Tetramine* was administered orally by stomach tube. The dose per mouse was 1.0 milliliter of the 15-ppm solution, or 15 micrograms tetramine* (35,300 counts per minute). This amount of tetramine* per kilogram of body weight was approximately equal to the oral LD_{50} value of 0.525 milligrams per kilogram.² At the end of each experiment, the mice were sacrificed and the desired samples collected and stored at $-15^{\circ}C$ until processed.

¹ Mention of commercial companies and their products does not represent endorsement by the Government.

² Unpublished data on file at the U.S. Bureau of Sport Fisheries and Wildlife laboratory, Olympia, Wash.

Gastrointestinal absorption of tetramine* was studied in a time series. Treated mice were sacrificed after 10 minutes, 30 minutes, 1 hour, 48 hours, and 72 hours, and their blood was drawn by heart puncture.

For determination of distribution and elimination of tetramine*, mice were held in the metabolism cages for 48 hours after dosing. At the end of the experiment, expired CO₂, feces, urine (including that drawn from the bladder), heart blood, lungs, brain, kidneys, liver, gluteal muscle, and gastrointestinal contents were collected, and the remaining "carcass" (skeletal muscle, skeleton, and skin) was ground and sampled.

Rates of fecal and urinary elimination of tetramine* were determined by collecting urine and feces from treated mice every 24 hours for a total of 96 hours. When these mice were sacrificed, the gastrointestinal contents were collected to determine residual radioactivity.

Sample preparation. Organs, tissues, and excreta collected in each experiment were rapidly dried in a stream of dry air, without loss of radioactivity. The dry material was homogenized and extracted with acetone several times until radioactivity in the extracting solvent became negligible. The combined extracts were then filtered and made to volume with acetone.

When CO₂ was collected for counting, the carbonate formed in the NaOH solution was precipitated as barium carbonate and the precipitate filtered on filter paper disks and washed (Comar, 1955).

In addition to collection and counting of expired CO₂, metabolism of tetramine* was studied by chromatography of the acetone extracts obtained from the distribution-excretion experiment. In this case, extracts of blood, lungs, brain, kidneys, liver, muscle, and carcass of each mouse were pooled to form "tissue extract." This extract and extracts of feces, urine, and gastrointestinal contents were concentrated and filtered for chromatography.

Separation of radioactive compounds. Radioactive compounds in the concentrated acetone extracts were separated by chromatography on Whatman No. 1 paper by the ascending technique. The paper was prewashed with 2N-HCl, distilled H₂O, and C₂H₅OH. After spotting, the paper was impregnated with 35 per cent formamide in methanol and developed in chloroform saturated with formamide after equilibration for 12 hours (Heftmann, 1961).

Assay methods. Measured portions of the acetone extracts were evaporated to dryness in planchets with a Picker sample drier. Radioactivity in the dried extracts and in the BaCO₃ collected from the expired CO₂ was quantitatively determined with a thin-window gas-flow Geiger-Müller tube and Tracerlab "Versa/Matic" scaler. In some cases, activity was also counted directly on the chromatograms containing the radioactive spots. All counts were appropriately corrected for background and self-absorption.

Radioactive compounds on the developed chromatograms were located by autoradiography with Kodak no-screen X-ray film.

Results and Discussion

All treated mice showed typical signs of mild to severe convulsions during the first 30 minutes after dosing, and some mice died. In most cases, however, convulsions disappeared and the mice resumed their normal appearance and behavior within a few

hours. No observable gross pathology was found in any of the surviving mice at time of sacrifice.

Although several extraction procedures were tried, it was not possible to extract all radioactivity from the collected samples. The average extraction efficiency with acetone was approximately 75 per cent. Radioactivity remaining in the samples was probably due to "fixation" of the label, which is known to occur with other chemicals (Klein *et al.*, 1959).

Absorption into the blood. The highest level of blood radioactivity was 0.11 per cent of that in the dose (Table 1). This level was reached in 10 minutes, remained essentially unchanged for 48 hours, and dropped to 0.03 per cent at 72 hours. Thus, the tracer was absorbed from the gastrointestinal tract into the blood rapidly. Apparently, levels of radioactivity in the blood were always low because (1) only small amounts of the tracer were absorbed, as evidenced by the large amounts that were not absorbed and were excreted in the feces, and (2) the absorbed activity continued to be distributed in the tissues and eliminated in the urine (see later).

Distribution and elimination. The tracer was detected in all the samples collected, as shown in Table 2. Only 5.76 per cent of the radioactivity remained in the gastrointestinal tract after 48 hours, and this activity would probably have been absorbed into the blood or eliminated in the feces in time.

TABLE 1. Absorption of radioactivity into the blood of white mice after a single oral dose of 15 micrograms tetramine-C¹⁴.

Time after dosing	Radioactivity in the collected blood	
	Counts/min ¹	% of dose ²
10 minutes	38 ± 4	0.11
30 minutes	31 ± 2	.09
1 hour	40 ± 5	.11
48 hours	33 ± 2	.09
72 hours	10 ± 2	.03

¹ Average of two mice and variation expressed as standard error of the mean.

² 35,300 counts per minute per dose.

TABLE 2. Distribution and elimination of radioactivity in white mice 48 hours after a single oral dose of 15 micrograms tetramine-C¹⁴.

Source of radioactivity	Recovered radioactivity	
	Counts/min ¹	% of dose ²
Heart and blood	63 ± 12	0.18
Lungs	23 ± 7	.06
Brain	81 ± 9	.23
Kidneys	94 ± 21	.27
Liver	482 ± 22	1.36
Gluteal muscle	352 ± 29	1.00
Gastrointestinal tract contents	2,032 ± 145	5.76
Carcass (remainder of body)	6,677 ± 180	18.92
Feces	14,401 ± 192	40.80
Urine	3,194 ± 95	9.05
Expired C ¹⁴ O ₂	339 ± 46	.96
Total animal	27,740	78.59

¹ Averages of three mice and variation expressed as standard error of the mean.

² 35,300 counts per minute per dose.

Radioactivity found in the tissues and organs amounted to 22.02 per cent of that in the dose. This activity was distributed throughout the body, with the greatest amounts in the carcass and lesser quantities in the excised organs, but with no strong evidence of selective storage sites.

More than 50 per cent of the administered tetramine* was eliminated from the body in 48 hours. Elimination was mainly in the feces, to a lesser extent in the urine, and to a much smaller degree in the expired air.

Rates of fecal and urinary elimination. Excretion of radioactive material was evident from the start and continued throughout the experiment (Table 3). Rates of elimination via both the feces and urine were highest during the first 48 hours and decreased gradually thereafter to the lowest level by the end of the experiment. At this time, only 0.24 per cent of the radioactive dose remained in the gastrointestinal tract, indicating that the tracer was practically eliminated from the tract in 96 hours.

Total radioactivity eliminated in the feces was more than three times that eliminated in the urine. This presents additional evidence that (1) only small amounts of radioactivity were absorbed into the blood and were available for distribution in the tissues and elimination in the urine, and (2) the feces was the principal route of the chemical's elimination from the animal's body.

Nature of radioactivity. As shown earlier (Radwan, 1967), the tracer separated into tetramine* (lower spot) at an R_F value of 0.40, and an impurity related to tetramine* (upper spot) at the R_F value of 0.75 (Fig. 1). The impurity accounted for approximately 20 per cent of the tracer's activity. Extracts of feces and gastrointestinal contents contained tetramine* and the impurity in the same 80:20 ratio indicating that the unabsorbed tracer was not subject to metabolic change. On the other hand, extracts of the tissues and urine contained spots corresponding to that of the impurity and almost no tetramine*. Apparently, these spots did not arise from selective absorption of the tracer's impurity. Rather, they seem to be the resultant metabolites of absorbed tetramine*. This view is supported by: (1) post-treatment reactions of all test animals indicating absorption of toxic tetramine in the blood, and (2) liberation of $C^{14}O_2$ by treated animals. Absorbed tetramine*, therefore, was probably degraded by cleavage of one or more carbons to $C^{14}O_2$ and a metabolite with the same R_F as that of the tracer's impurity.

Activity in the metabolite was very slight because most of the tracer was eliminated in the feces. The pooled metabolite from all experiments, therefore, was not sufficient

TABLE 3. Rates of fecal and urinary elimination of radioactivity in white mice after a single oral dose of 15 micrograms tetramine- C^{14} .

Hours after dosing	Eliminated radioactivity			
	Feces		Urine	
	Counts/min ¹	% of dose ²	Counts/min ¹	% of dose ²
0 - 24	2,598 ± 54	7.36	4,288 ± 136	12.15
24 - 48	14,094 ± 138	39.93	915 ± 62	2.59
48 - 72	3,961 ± 78	11.22	888 ± 57	2.52
72 - 96	422 ± 34	1.20	68 ± 18	.19
Total	21,075	59.71	6,159	17.45

¹ Averages of four mice and variation expressed as standard error of the mean.

² 35,300 counts per minute per dose.

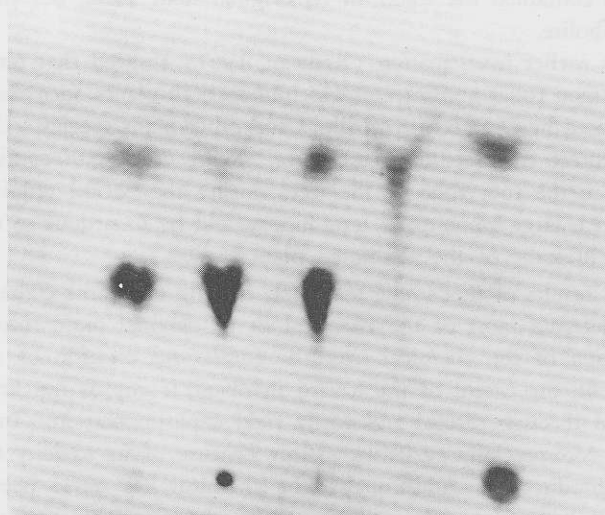


Figure 1. Autoradiograms of paper chromatograms developed ascendingly in formamide-saturated chloroform. Extracts shown are from mice orally treated with a single dose of 15 micrograms tetramine- C^{14} and sacrificed 48 hours after dosing. Spotting from left to right: feces extract, extract of gastrointestinal tract contents, tetramine*, tissues extract, and urine extract.

for a bioassay test on white mice; and it was not possible to determine the toxicity of the compound.

Other animals. Limited experiments similar to those outlined above were carried out with four snowshoe hares (*Lepus americanus washingtonii*). Results were essentially the same as those with mice. The tracer was found in the blood, heart, lungs, kidneys, liver, muscle, and expired air, and was also excreted in the urine. No feces were excreted during the experiments, but feces collected from the colon showed small amounts of activity. The tracer was not changed from its original form in the gastrointestinal tract or in the feces, but was converted into the same metabolite found in mice in the tissues and urine.

The fate of tetramine in other species of mammals is still unknown. However, a number of these species, after ingesting tetramine, repeatedly showed reaction to sublethal and lethal dosages of the chemical similar to that of hares and mice.³ It is reasonable, therefore, to assume that the fate of tetramine in other mammals would probably be similar to that in hares and mice.

Summary and Conclusions

Absorption, distribution, excretion, and metabolism of C^{14} -labeled tetramine were investigated in white mice following oral administration of the chemical, and results were confirmed in limited similar experiments with snowshoe hares. Tetramine- C^{14} was absorbed from the gastrointestinal tract rapidly, but in limited amounts. Absorbed activity was distributed in all tissues and organs examined. More than 50 per cent of the administered radioactivity was eliminated in 48 hours. Elimination was mainly in the feces, to a lesser extent in the urine, and to a much smaller degree in the ex-

³ See footnote 2.

pired air. Feces contained the tracer in its original toxic form, but tissues and urine contained a metabolite.

Results of an earlier investigation (Radwan, 1967) showed that tetramine did not penetrate leaves upon foliar application, was immobile in plants once absorbed by roots, and was decomposed by Douglas-fir seedlings and other plant species. Use of tetramine to adequately protect tree seedlings, therefore, would necessitate continuous presence of the chemical in the root zone of the trees during the period requiring protection. Application of such large amounts of tetramine would be hazardous to man and would produce toxicity in some of the associated plant species.

According to results of this study, mammals feeding on plants containing tetramine would tend to defecate most of the chemical in its original toxic form. This would lead to distribution of the chemical over the forest and creation of additional toxic plants. At present, the extent of the hazard involved, if any, is not known and could only be evaluated through careful and costly field experimentation. In the meantime, tetramine is not commercially available, mainly because of its toxicity and the hazards involved in its production. Additionally, the chemical has never been shown to be more effective in protecting tree seedlings than other available and much less toxic chemicals. Available evidence, therefore, does not seem to justify any further consideration of tetramine for protection of seedlings from animals.

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