# Extent of Residues in Milk Resulting from Use of Guthion-Treated Forage

W. W. LOEFFLER, Jr.
Chemagro Corp., Kansas City 20, Mo.
G. W. TRIMBERGER, F. H. FOX, R. L. RIDGEWAY, D. J. LISK, and G. G. GYRISCO
Cornell University, Ithaca, N. Y.

The use of Guthion for insect control on forage crops, particularly alfalfa, necessitated an investigation of the possible excretion of the insecticide and/or its metabolites in the milk of dairy cattle. Feeding levels, equivalent to 4.2 to 33.3 p.p.m. in fresh forage, did not result in detectable residues of the insecticide in milk. However, residues of one or more metabolites of the benzazimide portion of Guthion were detected, directly proportional to the amount of Guthion ingested. The preliminary study indicated the necessity for further work to determine the nature and extent of these residues in milk.

GUTHION, *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate, is an insecticide which exhibits a broad spectrum of insect control including Coleoptera, Diptera, Homoptera, Hemiptera, Lepidoptera, and various mite species. It is registered for use on a wide variety of crops, including fruit, nuts, vegetables, grains, forage, and field crops, and on ornamentals, shade, and forest trees.

The use of Guthion as an insecticide on forage crops, particularly alfalfa, prompted an investigation to determine the amount of residue that might be present in milk as a result of using forage crops containing residues of the pesticide.

## Experimental

**Treatment and Sampling.** Two tests were conducted. The first, at Cornell University, Ithaca, N. Y., included seven Jersey cows weighing from 295 to 475 kg.; the second, at the Chemagro Research Facility in Kansas City, involved seven Holstein cows weighing from 364 to 454 kg.

Feeding rates for the Cornell test were 0.3 and 0.6 mg. per kg. and for the Chemagro test, 0.6, 1.2, and 2.4 mg. per kg. These rates were calculated to be equivalent to 4.2, 8.3, 16.7, and 33.3 p.p.m. in fresh forage, based on the assumption that a 454-kg. animal would consume 9 kg. of dry hay or its equivalent of 32.4 kg. of green forage per day. The latter figure is based on a green forage–dry hay conversion factor of 3.6 recommended by the Food and Drug Administration, U. S. Department of Health, Education and Welfare. If a factor of 5 had been used instead, as recommended by some workers, the rates would be equivalent to 3, 6, 12, and 24 p.p.m. A factor of 3.6 was used in our work.

Half of each daily dose of Guthion was mixed with 1 pound of grain and given before the regular grain ration both morning and evening. The amounts of Guthion fed were adjusted to the actual weights of the animals. Untreated hay was permitted *ad libitum*.

Milk samples (including milk from all four quarters) were collected on various days during 14-day treatment and 10- to 14-day posttreatment periods. The samples were placed in tin cans, sealed, and frozen immediately. The samples remained frozen until analyzed.

**Table I. Typical Recovery of Guthion and Its Oxygen Analog Added to 200 Grams of Whole Milk**

| Compound | Added, P.P.M. | Found, P.P.M. | Recovery, % |
|---|---|---|---|
| Guthion | 0 | 0.019[a] | .. |
| | 0.10 | 0.094 | 94 |
| | 0.15 | 0.135 | 90 |
| | 0.20 | 0.170 | 85 |
| Guthion oxygen analog | 0 | 0.026[b] | .. |
| | 0.10 | 0.083 | 83 |
| | 0.15 | 0.107 | 71 |
| | 0.20 | 0.174 | 87 |

[a] Mean of 24 samples with standard deviation of 0.008 p.p.m., benzene-chloroform eluate.
[b] Mean of 22 samples with standard deviation of 0.013 p.p.m., chloroform-acetone eluate.

**Analytical Method.** Samples were analyzed by a fluorometric method which was later modified to improve sensitivity (*1*). The extraction procedure employed was identical to the one described in the final version of the method (*1*). After evaporation of the combined acetonitrile extracts, the samples were chromatographed on a 30-gram column of Florisil using 300 ml. of 10% benzene in chloroform followed by 400 ml. of 5% acetone in chloroform. Guthion was eluted with the benzene-chloroform and the oxygen analog and/or benzazimide metabolites with the acetone-chloroform. The two eluates were analyzed separately by transferring two aliquots of each corresponding to $^1/_5$ of the original sample into a pair of test tubes, one of which contained 5 μg. of Guthion as an internal standard. Following evaporation of the solvent the samples were hydrolyzed for 20 minutes at room temperature using 2 ml. of 0.5*M* potassium hydroxide in isopropyl alcohol. At the conclusion of the hydrolysis period, 2 ml. of 0.5*N* hydrochloric acid and 4 ml. of

**Table II. Guthion Metabolite Residues[a] in Milk during and after Feeding of Insecticide to Dairy Cattle**

| | Level Fed | | | | | |
|---|---|---|---|---|---|---|
| | 4.2 P.P.M. | | | 8.3 P.P.M. | | |
| | Animal No. | | | | | |
| Days on Feed | 5 | 6 | 9 | 1 | 3 | 8 |
| 0 | <0.02 | <0.02 | <0.02 | <0.02 | 0.02 | 0.02 |
| 1 | 0.04 | <0.02 | 0.03 | 0.07 | 0.06 | 0.07 |
| 2 | 0.05 | 0.05 | <0.02 | 0.04 | 0.11 | 0.04 |
| 3 | 0.06 | 0.05 | 0.04 | 0.08 | 0.09 | 0.08 |
| 5 | 0.04 | 0.04 | 0.03 | 0.03 | 0.09 | 0.10 |
| 7 | <0.02 | 0.05 | <0.02 | 0.03 | 0.07 | 0.12 |
| 10 | 0.03 | 0.04 | 0.04 | 0.08 | 0.05 | 0.03 |
| 13 | 0.05 | <0.02 | 0.03 | 0.04 | 0.06 | 0.06 |
| Av. values[b] | | 0.035 | | | 0.061 | |
| Days Posttreatment | | | | | | |
| 3 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| 7 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| 14 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |

[a] Net residue values expressed as p.p.m. mercaptomethylbenzazimide.
[b] In calculating averages, values recorded as <0.02 p.p.m. assumed to be 0.02 p.p.m.

**Table III. Guthion Metabolite Residues[a] in Milk during and after Feeding of Insecticide to Dairy Cattle**

| | Level Fed | | | | | |
|---|---|---|---|---|---|---|
| | 8.3 P.P.M. | | 16.7 P.P.M. | | 33.3 P.P.M. | |
| | Animal No. | | | | | |
| Days on Feed | 528 | 540 | 928 | 981 | 531 | 532 |
| 0 | <0.02 | <0.02 | 0.03 | 0.08 | 0.15 | 0.17 |
| 1 | 0.04 | <0.02 | 0.07 | 0.08 | 0.18 | 0.26 |
| 2 | 0.03 | 0.05 | 0.10 | 0.12 | 0.30 | 0.24 |
| 3 | 0.05 | 0.04 | 0.20 | 0.29 | 0.13 | 0.19 |
| 4 | 0.03 | 0.05 | 0.13 | 0.14 | 0.12 | 0.21 |
| 5 | 0.03 | 0.03 | <0.02 | 0.10 | 0.19 | 0.28 |
| 6 | 0.05 | 0.03 | <0.02 | <0.02 | 0.14 | 0.15 |
| 7 | <0.02 | <0.02 | 0.05 | 0.03 | 0.07 | 0.07 |
| 10 | 0.03 | <0.02 | 0.07 | 0.09 | 0.05 | 0.06 |
| 14 | <0.02 | 0.06 | <0.02 | 0.02 | 0.21 | 0.14 |
| Av. value[b] | 0.030 | | 0.076 | | 0.165 | |
| Days Posttreatment | | | | | | |
| 3 | <0.02 | ... | <0.02 | <0.02 | <0.02 | <0.02 |
| 7 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| 10 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |

[a] Net residue values expressed as p.p.m. mercaptomethylbenzazimide.
[b] In calculating averages, values recorded as <0.02 p.p.m. assumed to be 0.02 p.p.m.

buffer solution were added. The buffer consisted of 15 parts of 0.2*M* disodium phosphate and 85 parts of 0.1*M* citric acid in isopropyl alcohol–water (1 to 1). Fluorescence measurements were made as previously described (*1*), except that the activating and fluorescence wavelengths were 330 and 425 mμ, respectively, and the instrument was not standardized with a quinine sulfate solution.

A standard curve established that a linear response is obtained for the range of 0 to 30 μg. per 8 ml. (final volume), or 0 to 0.75 p.p.m. based on a 200-gram sample. The internal standard is used to compensate for any quenching of the fluorescence resulting from the presence of the milk extract.

Recovery experiments were conducted using Guthion and its oxygen analog. Samples were spiked in the initial blending step.

The results in Table I demonstrate that the method is satisfactory for the two compounds.

On the basis that samples must have a net fluorescence equal to or greater than the untreated control in order to contain a reportable residue, the sensitivity of the method for Guthion is approximately 0.02 p.p.m. and for the oxygen analog and other benzazimide-containing moieties is 0.03 p.p.m.

### Results and Discussions

No detectable residues of Guthion were found in any of the milk samples, regardless of feeding rate. However, the results for the chloroform-acetone eluate (Tables II and III) indicate that significant residues of the oxygen analog of Guthion and/or other benzazimide-containing moieties were present at all feeding levels. These residues appeared within 1 day after the treatment was started and disappeared within 3 days after treatment was discontinued. The mean values show that there is approximately a straight-line relationship between the amount fed and the metabolite level in the milk.

For reasons discussed previously (*2*), the results in Tables II and III are expressed as residues of mercaptomethylbenzazimide, corrected for fluorescence yield. The control value of 0.026 p.p.m. of Guthion in Table I when expressed on the same basis is 0.021 p.p.m. This accounts for the stated sensitivity of 0.02 p.p.m. in Tables II and III.


### Acknowledgment

The authors thank P. Dean, H. Y. Forsythe, Jr., G. O. Poinar, Jr., R. L. Little, Jr., S. W. Lacklin, G. Levesque, R. Stark, R. J. Prokopy, and S. N. Fertig for their assistance in carrying out parts of this investigation.


### Literature Cited


(1) Adams, J. M., Anderson, C. A., J. AGR. FOOD CHEM. **14,** 53 (1966).
(2) Everett, L. J., Anderson, C. A., MacDougall, D., *Ibid.*, **14,** 47 (1966).



*Received for review March 3, 1965. Accepted August 9, 1965. Guthion is registered in United States and Canadian Patent Offices by Farbenfabriken Bayer A. G., Chemagro Corp. licensee. Work in part supported by funds provided by the Federal-State Cooperative Project—NE-36.*


**RESIDUES**

# Nature and Extent of Guthion Residues in Milk and Tissues Resulting from Treated Forage


**L. J. EVERETT, C. A. ANDERSON, and DANIEL MACDOUGALL**

**Chemagro Corp., Kansas City, Mo.**


THE usefulness of Guthion (*O,O*-dimethyl *S* - 4 - oxo - 1,2,3 - benzotriazin-3(4*H*)-ylmethyl phosphorodithioate) in controlling a variety of insects on forage crops made it necessary to determine the nature and extent of milk residues that might result from feeding forage contaminated with Guthion residues.

Previous work (*2*) has shown that feeding rates equivalent to 4.2 to 33.3 p.p.m. in fresh forage do not result in detectable residues of Guthion in milk but do result in significant residues of one or more metabolites.

To learn more regarding the nature of these metabolites additional studies were conducted using $P^{32}$- and $C^{14}$-labeled Guthion. Furthermore, since at this point it was well established that proposed label recommendations (one application of 12 ounces active per acre, 21-day preharvest interval) could result in residues in the 1-p.p.m. range in fresh alfalfa, it was considered desirable to carry out an additional low-level feeding test at this level.

### Experimental Methods

**Studies with Radiolabeled Guthion.** PHOSPHORUS-LABELED MATERIAL. Guthion-$P^{32}$ was obtained from Farbenfabriken Bayer A.G., Leverkusen, Germany. An oral dose of 5 mg. per kg. of the radioactive compound (specific activity, 1.23 mc. per gram) was administered to a 364-kg. cow. This was calculated to be equivalent to 69.5 p.p.m. in one day's feed (*2*). The animal was kept in a metabolism stall for 5 days. Milk and blood samples were collected at regular intervals. Urine and feces samples were collected at time of elimination and the time of collection was recorded. On the fifth day the animal was sacrificed and various tissue samples were collected. All samples were kept frozen until they could be analyzed.