### Acknowledgment

The authors are indebted to J. R. Busvine, A. J. Forgash, G. C. LaBrecque, and C. N. Smith for providing cultures of resistant houseflies on repeated occasions, and to P. V. Stone of the Wisconsin Alumni Research Foundation for maintaining a constant available supply of susceptible houseflies. Malaoxon [*O,O*-dimethyl *S*-(1,2-biscarboethoxy)ethyl phosphorothiolate] was kindly provided by R. W. Young, American Cyanamid Co., Stamford, Conn.

The authors further acknowledge the assistance of W. C. Dauterman in preparing the radiolabeled malathion and methyl parathion and of Janet L. Mengle in performing the bioassays. A discussion with K. van Asperen and F. J. Oppenoorth led to the experiments on the decapitation of houseflies. The initial stages of this study were made with the counsel and assistance of R. D. O'Brien.

### Literature Cited

(1) Aldridge, W. N., Davison, A. N., *Biochem. J.* **51,** 62 (1952).
(2) Asperen, K. van, *Entomol. Exptl. et Appl.* **1,** 130 (1958).
(3) Asperen, K. van, *Nature* **181,** 355 (1958).
(4) Asperen, K. van, Dekhuijzen, H. M., *Biochim. et Biophys. Acta* **28,** 603 (1958).
(5) Asperen, K. van, Oppenoorth, F. J., *Entomol. Exptl. et Appl.* **2,** 48 (1959).
(6) *Ibid.*, **3,** 68 (1960).
(7) Augustinsson, K-B., Casida, J. E., *Biochem. Pharmacol.* **3,** 60 (1959).
(8) Augustinsson, K-B., Heimburger, G., *Acta Chem. Scand.* **9,** 310 (1955).
(9) Brown, A. W. A., *Advances in Pest Control Research* **2,** 351 (1958).
(10) Brown, A. W. A., *Ann. Rev. Entomol.* **5,** 301 (1960).
(11) Busvine, J. R., *Entomol. Exptl. et Appl.* **2,** 58 (1959).
(12) Busvine, J. R., *Trans. Roy. Soc. Trop. Med. Hyg.* **51,** 11 (1957).
(13) Casida, J. E., *Acta Chem. Scand.* **12,** 1691 (1958).
(14) Casida, J. E., Augustinsson, K-B., *Biochim. et Biophys. Acta* **36,** 411 (1959).
(15) Casida, J. E., Augustinsson, K-B., Jonsson, G., *J. Econ. Entomol.* **53,** 205 (1960).
(16) Chadwick, L. E., *Rend. ist. super. sanitá, Suppl.*, 219 (1954).
(17) Eldefrawi, M. E., Miskus, R., Hoskins, W. M., *Science* **129,** 898 (1959).
(18) Eldefrawi, M. E., Miskus, R., Sutcher, V., *J. Econ. Entomol.* **53,** 231 (1960).
(19) Fay, R. W., Kilpatrick, J. W., Morris, G. C., *Ibid.*, **51,** 452 (1958).
(20) Forgash, A. J., Hansens, E. J., *Ibid.*, **52,** 733 (1959).
(21) Hodgson, E., Casida, J. E., *Biochim. et Biophys. Acta*, **42,** 184 (1960).
(22) Keiding, J., *Science* **123,** 1173 (1956).
(23) Kilpatrick, J. W., Schoof, H. F., *J. Econ. Entomol.* **51,** 18 (1958).
(24) Krueger, H. R., O'Brien, R. D., *Ibid.*, **52,** 1063 (1959).
(25) Krueger, H. R., O'Brien, R. D., Dauterman, W. C., *Ibid.*, **53,** 25 (1960).
(26) LaBrecque, G. C., Wilson, H. G., *Ibid.*, **53,** 320 (1960).
(27) LaBrecque, G. C., Wilson, H. G., Bowman, M. C., Gahan, J. B., *Florida Entomologist* **42,** 69 (1959).
(28) LaBrecque, G. C., Wilson, H. G., Gahan, J. B., *J. Econ. Entomol.* **51,** 616 (1958).
(29) LaBrecque, G. C., Wilson, H. G., Smith, C. N., *Ibid.*, **52,** 178 (1959).
(30) Lord, K. A., Solly, S. R. B., *Chem. and Ind. (London)* **1956,** 1352.
(31) March, R. B., *Misc. Publ., Entomol. Soc. Am.* **1,** 13 (1959).
(32) Meltzer, J., *Mededel. Landbouwhogeschool en Opzoekingsstats. Staat Gent* **21,** 459 (1956).
(33) Mengle, D. C., Casida, J. E., *J. Econ. Entomol.* **51,** 750 (1958).
(34) Mengle, D. C., O'Brien, R. D., *Biochem. J.* **75,** 201 (1960).
(35) Nguy, V. D., Busvine, J. R., *Bull. World Health Organization*, in press.
(36) Oppenoorth, F. J., *Entomol. Exptl. et Appl.* **2,** 216 (1959).
(37) *Ibid.*, p. 304.
(38) Oppenoorth, F. J., *Nature* **181,** 425 (1958).
(39) Pal, R., *Indian J. Malariol.* **12,** 539 (1958).
(40) Plapp, F. W., Casida, J. E., *J. Econ. Entomol.* **51,** 800 (1958).
(41) Reiff, M., *Rev. suisse zool.* **63,** 317 (1956).
(42) Roan, C. C., Maeda, S., *J. Econ. Entomol.* **46,** 775 (1953).
(43) Sacca, G., *Riv. parassitol.* **18,** 289 (1957).
(44) Schmidt, C. H., LaBrecque, G. C., *J. Econ. Entomol.* **52,** 345 (1959).
(45) Schoof, H. F., Kilpatrick, J. W., *Ibid.*, **51,** 546 (1958).
(46) Soap and Chem. Specialties Blue-Book, 219, 1959.
(47) Technology Branch, Communicable Disease Center, *Summ. Invest.* **12,** 169 (1958).
(48) Wilson, H. G., LaBrecque, G. C., Gahan, J. B., Smith, C. N., *J. Econ. Entomol.* **52,** 308 (1959).

*Received for review June 8, 1960. Accepted September 8, 1960. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Study supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by grants from the U. S. Atomic Energy Commission [Contract No. AT(11-1)-64, Project No. 14] and the U. S. Public Health Service, National Institutes of Health (Contract RG-5304).*

**INSECTICIDE RESIDUES IN MILK**

# Excretion of Co-Ral in the Milk of Dairy Cattle

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Co-Ral, *O* - (3 - chloro - 4 - methylumbelliferone) *O,O* - diethyl phosphorothioate, known also as Bayer 21/199, has shown considerable promise as a systemically active insecticide against the cattle grubs *Hypoderma lineatum* (DeVill.) and *H. bovis* (Deg.) (*1, 2*) and as a contact insecticide against a number of other external parasites (*5*). Recent reports indicate that it may have activity against certain helminths (*3*).

The use of Co-Ral on lactating dairy cattle could be recommended only if Co-Ral were absent from their milk. The present series of studies was initiated to determine the amount of Co-Ral which might appear in milk of cattle sprayed with the material.

### Material Used

Through the courtesy of the Bayer Chemical Co., Leverkusen, Germany, and the Chemagro Corp., Kansas City, Mo., Co-Ral labeled with phosphorus-32 was made available to the Animal Disease and Parasite Research Division. This material was chromatographically demonstrated to be of at least 99.5% purity and at the time of shipment demonstrated a specific activity of 4.6 mc. per gram.

The radioactive material was diluted with ordinary Co-Ral and made up into a 20% emulsifiable concentrate by dissolving the chemical in a mixture of 65 parts of xylene, and 10 parts of Triton X-100.

This emulsifiable concentrate was then diluted to 0.5 and 0.75% concentrations with tap water immediately before application to the cows.

Co-Ral, O-(3-chloro-4-methylumbelliferone)O,O-diethyl phosphorothioate, also known as Bayer 21/199, is an effective systemic and contact insecticide for livestock use. To determine whether it would be excreted in milk of sprayed cattle, dairy cows were sprayed with 0.5 and 0.75% concentrations. Maximum organo-soluble extractive (Co-Ral plus other organo-soluble compounds) was approximately 0.2 and 0.25 p.p.m., respectively, for the 0.5 and 0.75% concentrations, reached 5 hours after treatment. These levels declined gradually over 7 days, being only a trace at 10 days.

**Table I. Excretion of Phosphorus-32 and Organo-soluble Phosphorus-32 Compounds in Milk Following Spray Treatment of Cows with Phosphorus-32–Labeled Co-Ral**

| | | Total $P^{32}$ in Whole Milk, P.P.M.[a] | | Organo-soluble $P^{32}$ Labeled Compounds, P.P.M.[a] (4% Butterfat Basis) | |
|---|---|---|---|---|---|
| Date | Time | 0.5% spray | 0.75% spray | 0.5% spray | 0.75% spray |
| 8/30 (5 hr.) | P.M. | 0.544 | 0.522 | 0.196 | 0.245 |
| 8/31 | A.M. | 0.783 | 0.833 | 0.126 | 0.158 |
| | P.M. | 1.219 | 1.427 | 0.121 | 0.154 |
| 9/1 | A.M. | 1.177 | 1.666 | 0.064 | 0.082 |
| | P.M. | 1.311 | 2.024 | 0.069 | 0.082 |
| 9/2 | A.M. | 1.039 | 1.858 | 0.037 | 0.063 |
| | P.M. | 1.086 | 1.963 | 0.034 | 0.064 |
| 9/3 | A.M. | 1.061 | 1.769 | 0.034 | 0.036 |
| | P.M. | 0.975 | 1.819 | 0.033 | 0.040 |
| 9/4 | A.M. | 0.855 | 1.555 | 0.019 | 0.024 |
| | P.M. | 0.778 | 1.453 | ... | ... |
| 9/5 | A.M. | 0.719 | 1.303 | ... | ... |
| | P.M. | 0.678 | 1.372 | ... | ... |
| 9/6 | A.M. | 0.664 | 1.294 | 0.012 | 0.014 |
| | P.M. | 0.625 | 1.470 | ... | ... |
| 9/9 | A.M. | 0.375 | 0.661 | ... | ... |
| 9/10 | A.M. | 0.336 | 0.504 | 0.001 | 0.003 |
| 9/13 | A.M. | 0.244 | 0.444 | ... | ... |
| 9/16 | A.M. | 0.244 | 0.299 | ... | ... |
| 9/20 | A.M. | 0.114 | 0.230 | ... | ... |
| 9/23 | A.M. | 0.069 | 0.119 | ... | ... |

[a] Co-Ral equivalents.

### Experimental Animals

Two Jersey cows, approximately 6 years of age, were selected, as near alike in weight, size, hair coat, and milk production as possible. Both cows were in excellent health and were maintained on good quality feeds and average dairy conditions.

### Methods

Each cow was sprayed with 2 liters of emulsion, using suitable precautions for protection of personnel. Material in excess of that held in the hair was collected and its quantity determined radiometrically.

The cow receiving the 0.5% spray retained 16.03 mg. per kg., while the one receiving 0.75% retained 22.9 mg. per kg.

Each cow was milked by machine behind a protective shield of plastic sheeting. The operator was specially trained to handle the necessary procedures in a minimum of time.

Milk samples were collected at each milking, beginning 5 hours after spraying through the first week, then from the morning milk only at intervals through the 25th day. Weight of the milk produced was recorded and the percentage of butterfat was determined at each sampling time.

Total phosphorus-32 was determined on the whole milk samples. Organo-soluble compounds containing phosphorus-32 were determined following extraction.

**Extraction.** Two hundred milliliters of the milk sample was diluted with an equal volume of ethyl alcohol and extracted three times with a 75 to 25 *n*-hexane–ethyl ether mixture, using 200 ml. of the ether mixture for the first extraction and 100 ml. each for the other two extractions. The ether extracts were combined and washed with 125 ml. of water. The extracts were dried over anhydrous sodium sulfate and filtered. The ether was removed as completely as possible and the fatty residue was dissolved in 200 ml. of *n*-hexane and extracted in a separatory funnel with four 50-ml. portions of acetonitrile. Each portion of acetonitrile was drained through another separatory funnel containing 50 ml. of *n*-hexane. The acetonitrile was distilled off to a volume of 10 ml. The residue was transferred to a 125-ml. glass-stoppered Erlenmeyer flask with a side stem calibrated at 5 ml. using 50 ml. of *n*-hexane to make the transfer. The solvent was evaporated on a steam bath, or hot plate at low heat, to a volume of less than 5 ml. The volume was then made up to exactly 5 ml. with benzene and the flask stoppered tightly to prevent evaporation of the solvent. Aliquots were taken from this 5-ml. volume for radiometric assay. Recoveries of radioactive Co-Ral were essentially 100% by this method.

### Results

Milk yield, butterfat percentage, total phosphorus-32, extractable labeled material, and percentage extracted are given in Table I. The greatest excretion of organo-soluble material was observed at the 5-hour sampling, being approximately 0.2 p.p.m. for the 0.5% spray and 0.25 p.p.m. for the 0.75% spray. The quantity declined steadily throughout the next 14 days. A greater percentage of the total radioactivity was extracted at the 5-hour period than at any later time. The extractable portion declined rapidly in the first 48 hours, then more slowly during the remaining time.

The total organo-soluble material was not sufficient for our paper chromatographic identification; therefore, it can only be presumed that a portion of the extractive was Co-Ral.

An experiment has been described (*4*) in which a Holstein cow was sprayed with a 0.75% concentration of Co-Ral and the total radioactivity in milk was similar to that obtained in our study. The authors report a very small fraction of this total as Co-Ral, the highest value being equivalent to that obtained by us 48 hours after treatment. Those results are not necessarily contradictory of our findings, since the formulation, spraying, and animals differed considerably between the two tests.

Total phosphorus-32 in the milk increased rapidly during the first 48 hours, then declined gradually during the following 3 weeks.

### Conclusions

Co-Ral, labeled with phosphorus-32, applied dermally to lactating dairy cows, results in excretion of phosphorus-32 in the milk in both organo-soluble and organo-insoluble forms. Significant quantities of both forms appear in milk within 5 hours after the treatment. The maximum quantity of organo-soluble material was found 5 hours post-treatment, the amount declining rapidly. Organo-insoluble phosphorus-32 reached maximum levels 48 hours post-treatment, then declined slowly over a 3-week period.

Total organo-soluble substances, calculated but not identified as Co-Ral, did not exceed 0.2 p.p.m. for a 0.5% spray nor 0.25 p.p.m. for a 0.75% spray.

### Literature Cited


(1) Brundrett, H. M., McGregor, W. S., Bushland, R. C., *Agr. Chem.* **12,** 36–37, 123, 125 (1957).

(2) Graham, O. H., *J. Econ. Entomol.* **51,** 359–60 (1958).

(3) Herlich, Harry, Porter, D. A., *Vet. Med.* **53,** 343–8, 360 (1958).

(4) Krueger, H. R., Casida, J. E., Niedermeier, R. P., J. AGR. FOOD CHEM. **7,** 182 (1959).

(5) Lindquist, A. W., "Systemic Insecticides for Control of Cattle Grubs and Other Livestock Insects," Annual Proceedings, 61st U. S. Livestock Sanitary Association Meeting, 1957.



*Received for review August 24, 1959. Accepted August 23, 1960.*


**INSECTICIDE RESIDUES**

# Meat and Milk Residues from Livestock Sprays


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**Before an insecticide can be recommended for use on livestock, studies must be made to determine whether it will contaminate meat and dairy products. Results of residue studies show that all the chlorinated hydrocarbon insecticides, Co-Ral, and malathion were excreted in the milk after spray treatments. Studies were also made on DDT, TDE, methoxychlor, chlordan, gamma chlordan, heptachlor, dieldrin, lindane, Strobane, toxaphene, malathion, and Co-Ral in the fat of beef cattle following spray treatments.**


CHLORINATED hydrocarbons and some of the phosphorus insecticides are fat-soluble and when sprayed on animals may be absorbed through the skin and stored in fatty tissues or they may also be excreted in milk. Therefore, before an insecticide can be recommended for use on livestock, it must be determined whether the dosages used will contaminate meat and milk. The U. S. Department of Agriculture has supported studies on residues in meat and milk at Kerrville, Tex., for the last 12 years. These studies have shown that a number of effective insecticides cannot be recommended for use on livestock because of their residues.

Several of these studies have already been reported in the literature (*4, 6, 7, 17*). The purpose of this paper is to summarize them and, where possible, compare the residues in both meat and milk resulting from the use of various insecticides under analogous conditions.

If the insecticides are stored in the animal's body, they will be found in fatty tissues and excreted in the butterfat of milk because of their solubility in fats and insolubility in water. The small concentrations found in other tissues can usually be attributed to the fat content of the tissue (*17*). In a sample of milk the amount of insecticide is proportional to the amount of butterfat.

In some instances, it is not enough to analyze for the insecticide used, because contamination may be caused by the storage of toxic metabolites in tissues. Davidow and Radomski (*9*) have shown that heptachlor (*3a*,4,5,6,7,8,8 - heptachloro - 3*a*,4,7,7*a* - tetrahydro - 4,7-methanoindene) is converted within the animal's body to heptachlor epoxide, which is stored in fatty tissues. Bann *et al.* (*2*) presented evidence showing that aldrin (1,2,3,4,10,10 - hexachloro - 1,4,4*a*, 5,8,8*a* - hexahydro - 1,4 - *endo* - *exo*-5,8 - dimethanonaphthalene) is converted by metabolic processes to dieldrin (1,2,3,4,10,10 - hexachloro - 6,7 - epoxy-1,4,4*a*,5,6,7,8,8*a* - octahydro - 1,4-*endo* - *exo* - 5,8 - dimethanonaphthalene), which is stored in fat.

Although it has complicated chemical analysis, the storage of insecticides in the fat has simplified residue studies. With the biopsy technique developed by Radeleff (*15*), it has been possible to take samples of abdominal fat from the same animals at various intervals after spray treatments. This has reduced the number of experimental animals needed and has permitted following the level of insecticides in the fat of individual animals.

The analytical methods used were found to give satisfactory recoveries before any test samples were analyzed.

Various solvents were used for extractions, but no extraction procedure was considered satisfactory that did not extract fat from the tissues or butterfat from milk.

The spray concentrations used were those determined to be the most practical for insect control. Both single and multiple treatments representing frequent and prolonged or seasonal applications were included.

### Experimental

**Meat Contamination.** Hereford calves were used for most of the meat contamination studies. Except where otherwise specified, they were in good condition and fed on a fattening diet during the experiments. Fat samples were taken from all animals before