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*Received for review June 6, 1957. Accepted October 26, 1957. Division of Analytical Chemistry, 131st Meeting, ACS Miami, Fla., April 1957.*

**INSECTICIDE RESIDUES**

# Residues in Milk from Dairy Cattle Treated with Methoxychlor for Fly Control

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**Samples of milk from methoxychlor-treated cows on four New Jersey farms were analyzed to determine the insecticide residue present at various intervals after application. Results indicate that methoxychlor is present in minute, but detectable, amounts in the milk of treated cows and that the concentration diminishes rapidly with successive samplings after spraying or dusting.**

PRELIMINARY INVESTIGATIONS of milk samples from cows treated with methoxychlor spray formulations in 1955 indicated detectable amounts of methoxychlor residue. Therefore, residue analyses were conducted on milk samples taken in conjunction with tests made by Granett and Hansens in 1956 (*4, 5*), in which they set out to establish more firmly the finding that control of biting flies can result in a significant increase in milk production. Previous work on effect of methoxychlor residues in milk and on rate of excretion has been done (*7*). No attempt was made in this study to reproduce conditions of treatment used by other investigators.

### Procedure

Four farms in Salem County, N. J., were used in the experiment. On farms I and II, water emulsion sprays were applied once a week and, on farm III, twice a week. At each location, one third of the herd (group A) was treated with a 10% methoxychlor formulation diluted 1 to 19 parts of water; and one third (group B) with a formulation of 5% methoxychlor plus 50% butoxy polypropylene glycol (Crag Fly Repellent) diluted 1 to 9 parts of water. The methoxychlor was applied at the rate of approximately 1 quart of 0.5% solution per animal. Sprays were applied from a knapsack sprayer operated at 20 to 40 pounds pressure and with a nozzle 1 to 2 feet from the animal. The other third (group C) was untreated.

On farm IV, the cows were similarly grouped and 7.6 grams of 50% methoxychlor wettable powder per animal was applied as a dust to group A for 4 weeks. The above wettable powder of methoxychlor plus 10% butoxy polypropylene glycol was applied to group B and group C was left untreated. After a 2-week interval, groups A and B were treated with sprays of the wettable powders at a level of 1 quart of 0.5% methoxychlor per cow (8 pounds per 100 gallons of water). There were from four to six cows in each group on all four farms.

Milk samples were taken from each group before treatment and at intervals

**Table I. Methoxychlor Residue Found in Milk from Cows**

| | Hours after Spraying or Dusting | P.P.M. Group A | Group B | Group C |
|---|---|---|---|---|
| 6–19–56 | | | Farm I | |
| 1st spray | Before | 0.04 | 0.06 | 0.05 |
| | 12 | 0.18[a] | 0.20[a] | 0.08[a] |
| | | | | 0.03 |
| | 24 | 0.10[a] | 0.11[a] | 0.06 |
| | 48 | 0.12[a] | 0.09[a] | 0.06 |
| | 72 | 0.07[a] | 0.08[a] | 0.03 |
| | 96 | 0.06 | 0.07 | 0.07 |
| | 120 | 0.05[a] | 0.05[a] | |
| | | 0.02 | 0.05 | 0.04 |
| 7–17–56 | | | | |
| 5th spray | Before | 0.05 | 0.05 | 0.02 |
| | 12 | 0.20[a] | 0.13[a] | 0.03 |
| | 24 | 0.13[a] | 0.12[a] | 0.06 |
| 8–14–56 | | | | |
| 9th spray | Before | 0.09 | 0.05 | 0.05 |
| | 12 | 0.19[a] | 0.15[a] | 0.12 |
| | 48 | 0.11[a] | 0.07[a] | 0.05 |
| | 120 | 0.07 | 0.10 | 0.07 |
| 6–18–56 | | | Farm II | |
| 1st spray | Before | 0.26 | 0.08 | 0.12 |
| | 12 | 0.11[a] | 0.14[a] | 0.12 |
| | | | 0.13[a] | |
| | 24 | 0.11[a] | 0.13[a] | 0.03 |
| | 48 | 0.12[a] | 0.12[a] | 0.03 |
| | 72 | 0.10[a] | 0.09[a] | 0.06 |
| | 96 | 0.06 | 0.05[a] | 0.04 |
| | 120 | 0.03 | 0.03 | 0.06 |
| | | | 0.09[a] | |
| 7–16–56 | | | | |
| 5th spray | Before | 0.05 | 0.05 | 0.06 |
| | 12 | 0.21[a] | 0.13[a] | 0.05 |
| | | | 0.14[a] | |
| | 24 | 0.11[a] | 0.12[a] | 0.06 |
| 8–13–56 | | | | |
| 9th spray | Before | 0.10 | 0.05 | 0.08 |
| | 12 | 0.10[a] | 0.11[a] | 0.37[ab] |
| | 48 | 0.12[a] | 0.15[a] | 0.06 |
| | 120 | 0.06 | 0.06 | 0.02 |
| 6–18–56 P.M. | | | Farm III | |
| 1st spray | Before | 0.05 | 0.12 | 0.16 |
| | 12 | 0.32[a] | 0.24[a] | 0.09 |
| | 24 | 0.15[a] | 0.12[a] | 0.10[a] |
| | | | | 0.05 |
| | 48 | 0.15[a] | 0.15[a] | 0.10[a] |
| | | | | 0.06 |
| | 72 | 0.08[a] | 0.10[a] | 0.07 |
| | | | 0.06[a] | |
| 6–22–56 P.M. | | | | |
| 2nd spray | 96 | 0.17[a] | 0.21[a] | 0.01 |
| | | | 0.14[a] | |
| | 120 | 0.25[a] | 0.12[a] | 0.07 |
| | | 0.25[a] | | |
| 7–16–56 | | | | |
| 9th spray | Before | 0.09[a] | 0.14[a] | 0.06 |
| | 12 | 0.25[a] | 0.22[a] | 0.05 |
| | 24 | 0.27[a] | 0.20[a] | 0.06 |
| 8–13–56 P.M. | | | | |
| 17th spray | Before | 0.09[a] | 0.03[a] | 0.04 |
| | 12 | 0.15[a] | 0.06[a] | 0.03 |
| | | 0.17[a] | 0.11[a] | |
| | 24 | 0.12[a] | 0.12[a] | 0.07 |
| | 48 | 0.18[a] | 0.08[a] | 0.09 |
| 8–15–56 A.M. | | | | |
| 18th spray | 72 | 0.08[a] | 0.06[a] | 0.06 |
| | 96 | 0.17[a] | 0.08[a] | 0.03 |
| | 120 | 0.08[a] | 0.06[a] | 0.04 |
| 6–18–56 | | | Farm IV | |
| 1st dusting | Before | 0.04 | 0.09 | 0.07 |
| | | 0.07 | | |
| | 12 | 0.14[a] | 0.13[a] | 0.07 |
| | 24 | 0.06[a] | 0.05[a] | |
| | 48 | 0.09[a] | 0.09[a] | 0.08 |
| | 72 | 0.07[a] | 0.10[a] | 0.04 |
| | 96 | 0.08[a] | 0.08[a] | 0.04 |
| | 120 | 0.08[a] | 0.08[a] | 0.12 |
| | | | | 0.03 |
| 7–10–56 | | | | |
| 4th dusting | Before | 0.10[c] | 0.08 | 0.09 |
| | | 0.10 | | |
| | 12 | 0.06[a] | 0.07[a] | 0.10 |
| | | | 0.10[a] | |
| | 24 | 0.06[a] | 0.16[a] | 0.08 |
| | 48 | 0.13[a] | 0.04[a] | 0.10 |
| | 72 | 0.14[a] | 0.10[a] | 0.09 |
| | 96 | 0.12[a] | 0.12[a] | 0.05 |
| | 120 | 0.11[a] | 0.12[a] | 0.03 |
| 7–26–56 | | | | |
| 1st spray | Before | 0.10 | 0.10 | 0.12 |
| | 12 | 0.11[a] | 0.16[a] | 0.08 |
| | 24 | 0.10[a] | 0.14[a] | 0.13 |
| | 48 | 0.17[a] | 0.12[a] | 0.03 |
| | 72 | 0.06 | 0.12 | 0.03 |
| | 96 | 0.05 | 0.09 | 0.05 |
| | 120 | 0.08[a] | 0.03 | 0.08 |
| 8–14–56 | | | | |
| 4th spray | Before | 0.07 | 0.05[a] | 0.06 |
| | | | 0.05 | 0.05 |
| | 12 | 0.09[a] | 0.19[a] | 0.05 |
| | 24 | 0.20[a] | 0.14[a] | |
| | 48 | 0.09[a] | 0.14[a] | 0.03 |
| | 72 | 0.17[a] | 0.11[a] | 0.11 |
| | 96 | 0.09[a] | 0.14[a] | |
| | 120 | 0.08 | 0.09 | 0.09 |

[a] These samples gave pink color; unmarked samples did not.
[b] Contaminated.
[c] Slight pink color.

up to 120 hours after treatment and kept frozen until analyzed. The method of analysis was based on that of Fairing and Warrington (*3*) with extraction and cleanup techniques as modified in an unpublished method (*2*). The methoxychlor was extracted from the milk with *n*-hexane, and the extract was purified using a series of separations with nitromethane. The isolated methoxychlor was dehydrohalogenated with alcoholic potassium hydroxide. Petroleum ether was used to separate the derivative from the reaction mixture after which it was passed through a chromatographic column to remove fats. The dehydrohalogenated methoxychlor was then treated with 85% sulfuric acid to produce a red complex which was read on a Beckman DU spectrophotometer.

### Results

The results of the residue analyses are given in Table I. Even with the extensive cleanup procedure, some charring of milk fat and sugars or other impurities still occurred when the final pink color was developed with sulfuric acid; the blank samples gave positive readings at 550 mμ, even when no color was present. The uncolored blanks are recorded in the tables as the equivalents of their readings in parts per million of methoxychlor. Even though these readings do not represent its actual presence, they should be considered in evaluating results, as charring is assumed to have been also present in the pink-colored samples containing a methoxychlor residue. The readings due to charring averaged about 0.06 p.p.m. of methoxychlor equivalent in the blank samples. Recovery studies (Table II) indicate that the positive error might not be so great in samples which contained methoxychlor. Based on recovery values, a sample yielding a reading of 0.1 p.p.m. might reflect a positive error as high as 0.05 p.p.m. There is apparently a cancellation of the positive error due to

**Table II. Recovery of Methoxychlor (P.P.M.)**

| Added | Recovered | Added | Recovered |
|---|---|---|---|
| 0.050 | 0.050 | 0.100 | 0.095 |
| | 0.047 | | 0.099 |
| | 0.065 | | |
| 0.100 | 0.099 | 0.150 | 0.125 |
| | 0.080 | | 0.140 |
| | 0.081 | | 0.125 |

charring by the negative error due to incomplete recoveries. With samples containing over 0.1 p.p.m., the results reflect the true amount present with very slight negative errors as the values increase. None of the values are corrected for variation in percentage of butter fat.

Methoxychlor is present in minute but detectable amounts in the milk of treated cows, and the concentration diminishes rapidly with successive samplings after spraying or dusting.


### Acknowledgment

This investigation was conducted partially under research grants sponsored by Union Carbide Chemicals Corp. and E. I. du Pont de Nemours & Co., Inc., at Rutgers University. Edward Gant and Fredi Jakob assisted in the collection and analysis of samples.


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*Received for review May 8, 1957. Accepted December 5, 1957.*


**SEED DISINFECTION**

# Mechanism of Liquid Seed Treatment

## Vapor Action and Adhesion, Radioactive Studies of Initial Liquid Distribution, and Investigations with Radioactive Panogen Formulations


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The mechanism of liquid seed treatment (Panogen process) was studied with physical and chemical methods. The mercurial distribution on treated seed is governed by the mixing process in the treater and by the vapor action of the fungicide. These processes were studied by means of volatile and nonvolatile tracers, and the distribution was characterized by statistical methods. High moisture content of the seed and poor mixing favor resorption which gives poorer initial distribution. A vapor pressure of $10^{-5}$ to $10^{-4}$ mm. of mercury gives sufficient vapor action to produce the final reasonably uniform distribution. In dust treatment, higher vapor pressures are required to give equivalent vapor action, as the effective surface of the dust is less than the seed surface treated with liquid. Panogen mercurials penetrate the fruit coat rapidly but diffusion stops at the endosperm. Liquid seed treatment may be improved further by reduction of the liquid volume.


Fungicidal and fungistatic agents have been used for seed disinfection since the beginning of this century. Seed has been treated with solutions of formaldehyde or various dry or liquid copper and inorganic mercury preparations. The highly efficient organomercurial fungicides came into use at the end of World War I. Seed was soaked in a dilute aqueous solution of the mercurial and was dried in a subsequent operation. The necessity of drying was a great disadvantage, and the development work in this field during the twenties and thirties was therefore concerned mainly with this question.

The dry method, in which the seed was mixed with mercurial dust in special seed treaters, was introduced in the middle twenties. A few years later Gassner (*11*) developed the short wet treatment in which smaller amounts of liquid seed disinfectants were applied to the seed in a revolving drum. This method was superior in comparison with the dry method, especially as regards handling hazards. However, still comparatively large liquid volumes were used, and the moisture content of the treated seed was increased to such a high level that the seed had to be sown within a couple of days after the treatment to prevent spontaneous heating of the stored grain.

Slurry treatment, a modification of short wet treatment—using slightly lower liquid dosages— was first performed with dusts in aqueous suspension but is now also used with true solutions.

Zade (*27*), working along similar lines as Gassner but independently of him, was able to develop a liquid seed disinfectant to be used in a much lower dosage—0.1 of the dosage in short wet treatment—so that the moisture content of the seed remained essentially unchanged. This disinfectant, Panogen, was put on the market in Sweden in 1938, where it received wide acceptance. In 1948, the Panogen process was introduced into the United States and Canada, and it has received much attention during the last few years.

Panogen has been subjected to worldwide testing for 20 years, with good results, experimentally and commercially. However, uniform distribution of the very small amounts of the liquid disinfectant used is still a problem as it was with the short wet treatment where much larger liquid volumes were involved.

The distribution problem has been discussed in the literature (*15, 25*) and, as late as in 1953, De Ong (*10*) remarked regarding Panogen that "even with the special applicator used for large scale work, there seems to be difficulty in securing a uniform distribution." The poor distribution of the dye used in Panogen to distinguish treated seed from nontreated seed is the main reason behind this and similar statements in the literature or in the field.

Ideally, just the necessary dose of the fungicide should be distributed over every site from which diseases may develop during germination, and the disinfectant should exhibit a specific vapor action in which the molecules of the fungicide are exclusively resorbed by fungi. As this scheme is not possible, uniform distribution over all sites from which plant