### 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.

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**INSECTICIDE RESIDUES**

# Meat and Milk Residues from Livestock Sprays


**H. V. CLABORN, R. C. BUSHLAND, H. D. MANN, and M. C. IVEY**

**Entomology Research Division**

**and**

**R. D. RADELEFF**

**Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Kerrville, Tex.**



**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

**Table I. Residues in Fat of Cattle Following a Single Spray Treatment with Various Insecticides**

| Insecticide | Dosage, % | Time Interval,[a] Weeks | Maximum Residue, P.P.M. | Duration of Detectable Residues, Weeks | Reference |
|---|---|---|---|---|---|
| DDT | 0.5 | 2 | 11.2 | >27 | (*4*) |
| TDE | 0.5 | 2 | 11.0 | >27 | (*4*) |
| Methoxychlor | 0.5 | 2 | 2.8 | <10 | (*4*) |
| Lindane | 0.03 | 2 | <2.5 | ... | (*4*) |
| | 0.075 dip | 1 | 21.8 | 8 | |
| | 0.075 | 1 | 17.3 | 8 | |
| Co-Ral | 0.5 | 1 | 0.40[b] | 2 | |

[a] Time after spraying.
[b] These results were corroborated by Chemagro Corp. using method described by Anderson (*1*).

**Table II. Residues in Fat of Cattle Following Multiple Spray Treatments with Various Insecticides**

| Insecticide | Dosage, % | Number of Sprays | Interval, Weeks | Time of Maximum Residue,[a] Weeks | Maximum Residue, P.P.M. | Duration of Detectable Residues, Weeks | Reference |
|---|---|---|---|---|---|---|---|
| Gamma chlordan | 0.5 | 6 | 2 | 2 | 24.0 | <16 | (*4*) |
| Chlordan | 0.5 | 6 | 2 | 2 | 20.7 | >16 | (*4*) |
| DDT | 0.5 | 6 | 3 | 3 | 35.2 | >36 | (*4*) |
| Dieldrin | 0.05 | 4 | 3 | 3 | 24 | >28 | (*4*) |
| Heptachlor | 0.5 | 6 | 2 | 2 | 19.3 | >16 | (*4*) |
| Lindane | 0.03 | 6 | 3 | 3 | <2.5 | ... | (*4*) |
| Methoxychlor | 0.5 | 6 | 3 | 3 | 2.4 | <12 | (*4*) |
| TDE | 0.5 | 6 | 3 | 3 | 28.4 | >36 | (*4*) |
| Strobane | 2.0 | 6 | 2 | 2 | 28.5 | >14 | (*4*) |
| | 0.5 | 12 | 2 | 2 | 6.6 | >6 | (*4*) |
| Toxaphene | 0.5 | 12 | 2 | 2 | 16.0 | 6 | (*4*) |
| Malathion | 0.5 | 16 | 1 | 1 | <0.5 | ... | (*5*) |

[a] Time after last spray treatment.

treatment. Also, a group of control animals were used in each experiment.

The spraying and dipping operations were carried out with emulsions or suspensions of an appropriate chemical. Suspensions were prepared from wettable powders supplied by the manufacturers, and emulsions from both laboratory and commercially supplied concentrates. In spraying, each animal was wet as thoroughly as possible. In dipping, the entire animal was submerged. Residues were determined from single spray treatments and from multiple treatments that simulated the continuous use of the insecticides during a season of insect control.

**Single Treatments.** Residue studies from single spray applications were made on DDT, TDE, methoxychlor, lindane, Co-Ral [*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate], and Delnav [2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)].

DDT AND TDE. They were determined by the method described by Schechter, Pogorelskin, and Haller (*19*), methoxychlor by the method described by Claborn and Beckman (*5*), lindane by the method of Davidow and Woodard (*10*) or by the method of Schechter and Hornstein (*18*). Co-Ral was determined by the method of Claborn *et al.* (*6*) and by radiochemical analysis using the phosphorus-32–labeled insecticide. Delnav was determined by radiochemical analysis using phosphorus-32–labeled Delnav.

A summary of these results is shown in Table I. References to previously published material are given in the tables. Other experiments which could not be tabulated are briefly described below.

LINDANE. Twelve hogs were sprayed with 0.06% lindane, six with an emulsion, and six with a suspension. Two untreated animals were kept for controls. One hog from each group was slaughtered at 1, 2, 4, and 6 weeks after spraying, and fat samples were taken for analysis. In the group sprayed with the suspension, the lindane residue in the fat was 0.47 p.p.m. after 1 week, 0.14 p.p.m. after 4 weeks, and none could be detected after 6 weeks. In the emulsion-sprayed group, 0.66 p.p.m. was found after 1 week, but none after 4 weeks.

Four sheep and four goats were dipped in a 0.025% suspension of lindane. Fat samples were taken from all animals before dipping, and from alternate pairs at 2-week intervals after treatment. The lindane residue averaged 4.22 p.p.m. after 2 weeks, decreased to 0.26 p.p.m. at 8 weeks, and could not be detected at 12 weeks.

CO-RAL. Two calves were sprayed with phosphorus-32–labeled Co-Ral. One animal, sprayed with 25 mg. per kg. and slaughtered after 1 week, had an apparent Co-Ral residue of 0.12 p.p.m. in the fat. The other animal, sprayed with 58 mg. per kg. and slaughtered 2 weeks later, had only 0.04 p.p.m. in the fat. Higher residues were indicated in the kidney and liver, but apparently these residues were not Co-Ral, but some phosphorus-containing metabolites.

DELNAV. A calf was sprayed with 16.2 mg. per kg. of phosphorus-32–labeled technical Delnav. The animal was slaughtered 7 days after treatment and tissues were taken for analysis. Radioactivity counts indicated the following residues in the tissues: omental fat, 1.51 p.p.m.; renal fat, 1.07 p.p.m.; muscle, 0 p.p.m.; liver, 0.20 p.p.m.; and kidney, 0.07 p.p.m.

**Multiple Treatments.** Studies of residues resulting from multiple spray treatments were made on DDT, TDE, methoxychlor, lindane, chlordan (1,2,4,-5,6,7,8,8 - octachloro - 2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene), gamma chlordan, heptachlor, dieldrin, Strobane (a mixture of chlorinated terpenes with about 66% chlorine), toxaphene, and malathion {*S* - [1,2, - bis(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate}.

The analytical methods for the first four insecticides were the same as indicated above, and malathion by the method described by Norris *et al.* (*13*), but the residues from all the other compounds were estimated from total chlorine analysis. The insecticides were extracted from the fat with hot nitromethane, the organically bound chlorine was reduced with sodium, and the chlorides were titrated amperometrically with silver nitrate.

The results from these studies are summarized in Table II. Again, other experiments which could not be tabulated are briefly described below.

EXCESSIVE TREATMENTS WITH DDT. Earlier experiments had shown that the storage of DDT in the fat reached a maximum after six applications. An experiment was then run to determine the residues that would result from spray treatments over an extended period of time (*17*). Fat samples were taken from six 6-month-old calves with unknown history and analyzed for DDT. Before treatment, the DDT content ranged from 5 to 75 p.p.m. and averaged 45 p.p.m. The calves were sprayed five times with 0.5% DDT emulsion at 3-week intervals and then, after 5 days, were given 28 treatments at 2-week intervals. The DDT content of the fat samples taken 2 weeks after the last treatment ranged from 76 to 89 p.p.m. and averaged 84 p.p.m. The one animal that had 75 p.p.m. at the start had only 81 p.p.m. after 33 additional sprayings.

Another group of six calves, fat samples from which were negative for DDT

before treatment, were sprayed 28 times at 2-week intervals. Fat samples taken from five of the animals 2 weeks after the last treatment contained from 80 to 103 p.p.m. of DDT, and averaged 88 p.p.m., 4 p.p.m. higher than for the first group.

COWS AND CALVES SPRAYED WITH DDT. Eight Hereford cows with young calves were used for this experiment. All the cows and four of the calves were sprayed five times at 28-day intervals with 0.5% DDT, half of these animals with an emulsion and the other half with a suspension. The other four calves were not sprayed. Each calf lived upon the milk supplied by its mother. All the animals were slaughtered 28 days after the last spraying. An average of 52 p.p.m. of DDT was found in the fat from the calves that were getting DDT by both absorption and ingestion, and 25 p.p.m. from those that were getting it only from the milk. More DDT was stored in the fat of the calves that were not sprayed than in the fat of their mothers, which averaged only 15 p.p.m.

Four calves in good condition were sprayed with 0.5% emulsion of chlordan 12 times at 2-week intervals. Four other calves in very poor condition were given the same treatment. Fat samples were taken 2, 8, and 16 weeks after the last spraying. Two weeks after the last spraying the poor cattle had 28 p.p.m. of chlordan in the fat, and the fat cattle had 21 p.p.m. Fourteen weeks later the residues were 3.9 and 4.5 p.p.m., respectively.

### Milk Contamination

The dairy cows used were Jerseys in full lactation. They were sprayed all over, 2 quarts for each cow. They were milked by machine and the samples taken from the full yield. Care was taken to avoid mechanical contamination of the milk. The butterfat of each sample was determined and the results were calculated and adjusted to a uniform butterfat content of 4%. Milk samples were taken from each cow before spraying and at intervals after treatment, and untreated animals were included in each experiment.

Residue studies were made on milk from cows sprayed with DDT, dieldrin, methoxychlor, Dilan—1 part of 1,1-bis(*p*-chlorophenyl) - 2 - nitropropane plus 2 parts of 1,1 - bis(*p* - chlorophenyl)-2 - nitrobutane — malathion, Strobane, toxaphene, Co-Ral- and Perthane [1,1-dichloro - 2,2 - bis(*p* - ethylphenyl)-ethane].

The method used for the determination of DDT is described by Schechter *et al.* (*19*); the total chlorine method for dieldrin, by Carter (*3*); the method for methoxychlor, by Prickett *et al.* (*14*); for Dilan, by Jones and Riddick (*11*, *12*), with modification by Cundiff (*8*); for malathion, by Norris *et al.* (*13*); and for Perthane, by Weiss (*20*). Co-Ral was determined by a radiochemical method using phosphorus-32–labeled Co-Ral, and toxaphene and Strobane by the total chlorine method described under Meat Contamination.

**Table III. Residues in Milk from Cows Sprayed with Various Insecticides**

| Insecticide | Concentration of Spray, % | Time of Maximum Residue,[c] Days | Maximum Residue, P.P.M. | Duration of Detectable Residue, Days | Reference |
|---|---|---|---|---|---|
| DDT | 0.5[a] | 2 | 2.8 | >21 | (*6*) |
| Dieldrin | 0.5[a] | 3 | 7.0 | >21 | (*6*) |
| Methoxychlor | 0.5[a] | 1 | 0.70 | <21 | (*6*) |
| | 0.5[b] | 2 | 0.44 | <21 | (*6*) |
| Dilan | 0.5[b] | 2 | 0.75 | >14 | (*6*) |
| Malathion | 0.5[a] | 5[d] | 0.08 | 3 | (*5*) |
| | 1.0[a] | 5[d] | 0.20 | 3 | (*5*) |
| | 0.5[b] | 5[d] | 0.27 | 3 | (*5*) |
| | 1.0[b] | 5[d] | 0.36 | 3 | (*5*) |
| Strobane | 0.5[a] | 1 | 0.74 | 14 | (*4*) |
| | 0.5[b] | 2 | 0.61 | 14 | (*4*) |
| Toxaphene | 0.5[a] | 2 | 0.61 | 14 | (*4*) |
| | 0.5[b] | 2 | 0.74 | 14 | (*4*) |
| Co-Ral | 0.5[a] | 5[d] | 0.20 | 14 | (*16*) |
| | 0.75[a] | 5[d] | 0.25 | 14 | (*16*) |
| Perthane | 0.5[a] | 3 | 0.17 | <21 | (*4*) |

[a] Emulsion.
[b] Suspension.
[c] Time after spraying.
[d] Hours.

The results of these studies are summarized in Table III. Other experiments, which for various reasons could not be tabulated, are described separately.

An experiment with methoxychlor was conducted on a herd basis, since there had been some evidence with other insecticides that lower residues result when larger groups of animals are sprayed with commercial equipment. Two herds were sprayed with a 0.5% suspension of methoxychlor. The samples were analyzed by the method described by Claborn and Beckman (*5*). Pooled milk samples from one herd contained 0.18, 0.13, 0.11, and 0.05 p.p.m. of methoxychlor after 1, 2, 3, and 5 days, and less than 0.05 p.p.m, after 7, 14, and 21 days. Similar data were obtained on another herd.

In other experiments, two dairy cows were sprayed twice daily with 1 ounce of a 2% oil solution of toxaphene and two cows were sprayed in the same manner with Strobane. Samples of milk were taken at frequent intervals during the 21-day spray period. The two insecticides caused about the same contamination. On the third day after the first spraying, the contamination reached 0.3 p.p.m. and, with few exceptions, remained at this level for the remainder of the 21 days.

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**INSECTICIDE RESIDUES**

# Determination of Heptachlor and Heptachlor Epoxide in Soil

**R. T. MURPHY and W. F. BARTHEL**

**Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture, Gulfport, Miss.**

An improved method is described which makes possible the determination of 0.01 p.p.m. of heptachlor and heptachlor epoxide in soil with satisfactory accuracy. Soil from plots treated with 0.25, 0.50, 1, and 2 pounds per acre of heptachlor was analyzed 3 and $5^1/_2$ months after treatment. A second group of samples was taken after 8 months from an area which was treated with two different granular formulations. A third group of samples was taken in a treated area where biological control was incomplete, in an attempt to correlate chemical and biological data. The initial loss of insecticide from the soil appears to be rapid, and is followed by a much slower loss and conversion of a portion of heptachlor to heptachlor epoxide.

RECENT WORK in this laboratory demonstrated a need for a method to determine quantitatively heptachlor and heptachlor epoxide residues as low as 0.01 p.p.m. in soil. Since this is below the limits of present published methods (*2*, *4*, *10*), a study was undertaken to find a more sensitive procedure.

There were two possible approaches to the problem. The first would involve finding a reagent capable of yielding a more intense color than does the Polen-Silverman reagent (*7*) for both heptachlor and its epoxide. Davidow's (*1*) and Radomski's (*8*) work was in this direction, but did not attain the necessary sensitivity. The second approach would make use of a much larger soil sample with suitable elimination of the interfering substances. The latter method was selected for this study.

**Table I. Insecticide Residues[a] of Four Different Dosages of Heptachlor after 3 and $5^1/_2$ Months; Series I**

| Lb./ | 3 Months[b] | | | 3 Months[c] | | | $5^1/_2$ Months[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| Acre | Hept. | Epox. | Total | Hept. | Epox. | Total | Hept. | Epox. | Total |
| $^1/_4$ | <0.1 | 0.2 | 0.2 | <0.01 | 0.16 | 0.16 | <0.01 | 0.17 | 0.17 |
| | <0.1 | 0.2 | 0.2 | <0.01 | 0.12 | 0.12 | 0.01 | 0.17 | 0.17 |
| Av. | <0.1 | 0.2 | 0.2 | <0.01 | 0.14 | 0.14 | 0.01 | 0.17 | 0.17 |
| $^1/_2$ | <0.1 | 0.2 | 0.2 | <0.01 | 0.24 | 0.24 | 0.01 | 0.26 | 0.26 |
| | 0.1 | 0.2 | 0.2 | <0.01 | 0.12 | 0.12 | 0.01 | 0.27 | 0.28 |
| Av. | 0.1 | 0.2 | 0.2 | <0.01 | 0.18 | 0.18 | 0.01 | 0.26 | 0.27 |
| 1 | 0.1 | 0.6 | 0.7 | 0.04 | 0.62 | 0.66 | 0.04 | 0.67 | 0.71 |
| | 0.1 | 0.4 | 0.5 | 0.05 | 0.33 | 0.38 | 0.03 | 0.33 | 0.37 |
| Av. | 0.1 | 0.5 | 0.6 | 0.05 | 0.48 | 0.52 | 0.03 | 0.50 | 0.54 |
| 2 | 0.2 | 0.8 | 1.0 | 0.20 | 0.78 | 0.98 | 0.07 | 0.86 | 0.75 |
| | 0.3 | 0.9 | 1.2 | 0.28 | 0.86 | 1.14 | 0.08 | 1.06 | 1.14 |
| Av. | 0.3 | 0.8 | 1.1 | 0.24 | 0.82 | 1.06 | 0.08 | 0.96 | 1.04 |

[a] All values represent p.p.m. in the top inch of soil.
[b] Data from use of 50-gram soil samples without acid wash.
[c] Data from use of 250-gram soil samples with acid wash.

## Experimental

**Series I.** Ten plots measuring 200 × 400 feet were set up in a large pecan grove. Two of these were not treated and were used as check plots throughout the experiment. The others were treated in duplicate with 0.25, 0.50, and 1 pound of heptachlor per acre, using 10 pounds of 2.5, 5, and 10% heptachlor granules, respectively, and with 2 pounds of actual heptachlor per acre, using 20 pounds of granules which contained 10% of heptachlor. The granules were applied by a Buffalo turbine mounted on the back of a jeep. The plots were treated on May 25, 1959, and sampled on August 29 and on November 2, 1959, 3 and $5^1/_2$ months, respectively, after treatment. Results are given in Table I.

**Series II.** An airfield of approximately 500 acres was divided into two treatment areas of similar size. Five 1-acre plots were selected throughout each treatment area, from which the soil samples were taken. Before treatment, blank samples were taken from these plots for use on the recovery curve. On one treatment area, a granular formulation containing 10% oil and 10% heptachlor was applied at 10 pounds per acre. On the other treatment area, granules which were formulated with no oil were applied at the same dosage. After 8 months, the five plots of each area were sampled and the residues were determined.

**Series III.** Samples were also taken from plots of another location which was treated with 10% oil-containing granules at the same time and dosage as in Series II. At the time of sampling, active ant mounds were found in the treated area. These mounds appeared to be in rows parallel to the line of flight taken by the aircraft while applying the insecticide. Samples were taken along