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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicofol (010501) - Preliminary report on poultry metabolism study [Accession No. 257600, RCB No. 964].

FROM: Susan V. Hummel, Chemist
Special Registration Section II
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Susan V. Hummel

THRU: Charles L. Trichilo, Branch Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: Bruce Kapner, PM#70
Special Review Branch
Registration Division (TS-767)

and

Edward Allen, PM#12
Insecticide-Rodenticide Branch
Registration Division (TS-767)

Rohm and Haas has submitted a preliminary report on a poultry metabolism study, required by the registration standard.

Laying hens were orally dosed with 1.7mg/day of ^{14}C-dicofol for seven days. The hens were sacrificed approximately 24 hours after the last dose. Fat, liver, and whole egg samples were taken. The samples were homogenized, subsampled for radioanalysis, and kept frozen until analysis.

The number of hens used in the study, and the breed, age, and weight of the hens was not reported. The preparation of the dose and the method of administration of the dose was not described, nor was any evidence that the hen has receoved the complete dose presented. These data are needed. The dose in mg/animal was given. However, the weight and feed consumption are needed to calculate the dose in mg/kg and ppm in the feed.

Tissue samples were mixed with sodium sulfate and extracted three times with dichloromethane. The extracts were combined and evaporated to near dryness and reconstituted with dichloro-

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methane/cyclohexane 50:50. An aliquot was cleaned up by Gel Permeation Chromatography (GPC) to remove high molecular weight impurities. The extracts were evaporated to dryness and reconstituted with methanol. Samples were separated by HPLC on an Altech C18 column using acetonitrile/water 85:15 as mobile phase, and analyzed by UV at 230 nm. Then one minute fractions were collected. The fractions were analyzed by Liquid Scintillation Counting (LSC). The minimum quantifiable level (MQL) was reported to be 0.036 ppm in fat, 0.011 ppm in liver, and 0.011 ppm in whole egg. Some sample calculations were included.

Extraction Efficiencies and recoveries from the GPC cleanup step, as reported, are tabulated below.

Tissue	Extraction Efficiency (%)	Recovery (%)
fat	104%	102%
egg	88%	78%
liver	52%	77%

More information is needed on the test material and the standards. What is the composition of the ^{14}C-dicofol? Is it p,p'-dicofol only or is it a mixture of p,p'- and o,p'- dicofol? Where is the compound labeled? What is the radiochemical purity? The specific activity radiochemical purity, and the correct value for dpm for each standard solution is needed.

Poultry muscle, in addition to liver, and fat must be analyzed. Analysis of urine and feces would be helpful, although this is not required. Sample collection and storage procedures should be completely described, including the length of sample storage. The preparation of the spiked fat sample should be completely described. What is meant by "analyzed with the fat", and "analyzed with the liver and whole egg" in Table 2.

The recoveries reported for the extraction step, indicate that an acid, base, or enzyme hydrolysis step is needed to release more residues. The GPC recoveries indicate that the GPC parameters need to be changed, so that less residue is lost.

No p,p'-DDE or p,p'-Cl-DDT was reported in any of the fat, liver, or egg samples. We tentatively conclude that DDE and Cl-DDT are not metabolites of dicofol in poultry, pending submission of the final report.

Dicofol, per se, was reported as follows.

Tissue	ppm dicofol	% total activity
fat	9.36	82.8
liver	0.26	13.0
egg	0.49	35.5

We calculated different values for % total activity. Our values are 82.9%, 39.3%, and 85.9% in fat, liver, and egg, respectively. The registrant should explain the calculations or verify that our calculations are correct.

We note that the residue reported as dicofol is not necessarily dicofol. It is residue eluted in the same HPLC fraction as dicofol, reported as dicofol equivalents. Part (14-60%) of the extractable residue elutes before the dicofol fraction. All HPLC fractions with measurable activity need to be further characterized. TLC with an appropriate identification technique may be useful.

The activity should be characterized by fractions (e.g. water soluble, organosoluble, released by hydrolysis, etc.). The results should be expressed in counts, ppm, and % total activity in the sample.

The calculations presented are not clear. A complete set of calculations using the raw data should be presented. Table 2 indicates that the control fat sample was spiked with 8.6×10^{27} dpm ER-8. The correct value should be given.

Conclusions

We tentatively conclude that DDE and Cl-DDT are not metabolites of dicofol in poultry, pending submission of the final report.

The metabolism study has a number of deficiencies, which need to be resolved.

1. Submit the number of hens used in the study, and the breed, age, and weight of these hens. Completely describe the preparation of the dose and the method of administration of the dose. Present evidence that the hens have received the complete dose. The dose in mg/animal was given. However, the weight and feed consumption are needed to calculate the dose in mg/kg and ppm in the feed.

2. More information is needed on the test material and the standards. What is the composition of the ^{14}C-dicofol? Is it p,p'-dicofol only or is it a mixture of p,p'- and o,p'- dicofol? Where is the compound labeled? What is the radiochemical purity? The specific activity radiochemical purity, and the

correct value for dpm for each standard solution is needed.

3. Poultry muscle, in addition to liver, and fat must be analyzed. Analysis of urine and feces would be helpful, although this is not required. Sample collection and storage procedures should be completely described, including the length of sample storage. The preparation of the spiked fat sample should be completely described. What is meant by "analyzed with the fat", and "analyzed with the liver and whole egg" in Table 2.

4. The recoveries reported for the extraction step, indicate that an acid, base, or enzyme hydrolysis step is needed to release more residues. The GPC recoveries indicate that the GPC parameters need to be changed, so that less residue will be lost.

5. We calculated different values for % total activity found in the dicofol fraction. Our values are 82.9%, 39.3%, and 85.9% in fat, liver, and egg, respectively. The registrant should explain the calculations or verify that our calculations are correct.

6. The residue reported as dicofol is not necessarily dicofol. It is residue eluted in the same HPLC fraction as dicofol, reported as dicofol equivalents. All HPLC fractions with measurable activity need to be further characterized. TLC with an appropriate identification technique may be useful.

7. The activity should be characterized by fractions (e.g. water soluble, organosoluble, released by hydrolysis, etc.). The results should be expressed in counts, ppm, and % total activity in the sample.

8. The calculations presented are not clear. A complete set of calculations using the raw data should be presented. Table 2 indicates that the control fat sample was spiked with 8.6×10^{27} dpm ER-8. The correct value should be given.

Recommendations

We recommend that the Rohm and Haas be advised of our conclusions, and advised to resolve the deficiencies in the study.

cc:R.F., circu, S. Hummel, B. Kapner (SRB), K. Barbehenn (SIS), EAB, EEB, TOX, dicofol S.F., Reg. Std. File, Special Review file (Hummel), PM#12, PMSD/ISB
RDI:EZ:5/13/85:RDS:5/13/85
TS-769:RCB:SVH:svh:RM:810:CM#2:5/13/85