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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Dicofol: Review of an acute neurotoxicity study in rats

Caswell No. 93 DP Barcode: D187705
EPA ID No. 010501 Submission No. S434734

TO: Judith Loranger/Linda Propst, PM Team 73
Re-registration Division (H7508C)

FROM: Whang Phang, Ph.D. *Whang Phang 6/28/93*
Pharmacologist
Tox. Branch II/HED (H7509C)

THROUGH: James Rowe, Ph.D. *James N. Rowe 7/28/93*
Section Head
and
Marcia van Gemert, Ph.D. *M van Gemert 7/28/93*
Branch Chief
Tox. Branch II/HED (H7509C)

The registrant, Rohm and Haas, submitted an acute neurotoxicity study in rats. This study has been reviewed, and the Data Evaluation Report is attached. The citation of the study and the conclusion of the review are presented below:

Citation: Foss, J. A. (1992) Acute neurotoxicity study of dicofol (Kelthane[®] Technical B Miticide) administered orally via gavage to Crl:CD[®]BR VAF/Plus[®] Rats. Unpublished study conducted by Argus Research Lab., Inc.; Argus Study No. 018-017. October 2, 1992. Submitted to EPA by Rohm & Hass. EPA MRID No. 426333-03.

Conclusion: Groups of Crl:CD[®]BR VAF/Plus[®] rats (10/sex/group) were administered (by gavage) dicofol once at doses of 0, 15, 75, and 350 mg/kg. Functional observational battery and motor activity evaluations were conducted in the test animals.

The results of this study indicated that dicofol at the doses tested did not cause any deaths; however, it produced

- 1. A statistically significant increase in the incidence of urine- or feces stained fur in 350 mg/kg male and female rats.

2. An increase in the incidence of ataxia in 350 mg/kg male and female rats,
3. Changes in air righting response as indicated by an increased incidence of uncoordinated landing in 350 mg/kg females rats,
4. An increase in the incidence of female rats in 350 mg/kg group showing signs of being asleep,
5. A decrease in body weights in both male and female rats of 75 and 350 mg/kg groups at approximately days 2 to 5 of the study,
6. A decrease in food consumption in both male and female rats in 75 and 350 mg/kg groups during days 1-3, and
7. A decrease in motor activity as measured by a decrease of the number of movements/5 minutes and by an increase in time spent in a movement in both male and female rats of 350 mg/kg groups.
8. It should be noted that some of the effects (such as air righting responses) seen in 75 mg/kg male and female rats were scattered and less clear, and often they did not show a statistically significant difference from those of the vehicle controls.

Dicofol, under the conditions of this study, did not cause any histopathological changes in the central or peripheral nervous systems. In general, the results appeared to indicate that dicofol was not a "specific or selective neurotoxicant". Based upon the decreases in body weights and reduced food consumptions, the LEL for systemic toxicity was 75 mg/kg; NOEL, 15 mg/kg.

The study meets the data requirements for a neurotoxicity screening study (Guideline No. 81-8); it is classified as **minimum**.

Reviewer: Whang Phang, Ph.D.
Tox. Branch II/HED (H7509C)

Whang Phang 6/28/93

Secondary Reviewer: James Rowe, Ph.D.
Section Head, Tox Branch II/HED (H7509C)
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DATA EVALUATION REPORT

Study: Acute neurotoxicity in rats (by gavage)

Chemical: Dicofol (Kelthane^R)

Caswell No.	93	PC No.	010501
DP Code:	D187705	CASE:	818577
Submission No.	S434734	MRID No.	426333-03

Performing Laboratory: Argus Research Laboratories, Inc.
905 Sheehy Dr.
Horsham, PA, 19044

Sponsor: Rohm and Haas Co.

Citation: Foss, J. A. (1992) Acute neurotoxicity study of dicofol (Kelthane^R Technical B Miticide) administered orally via gavage to Crl:CD^RBR VAF/Plus^R Rats. Unpublished study conducted by Argus Research Lab., Inc.; Argus Study No. 018-017. October 2, 1992. Submitted to EPA by Rohm & Hass. EPA MRID No. 426333-03.

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Dicofol, under the conditions of this study, did not cause any histopathological changes in the central or peripheral nervous systems. In general, the results appeared to indicate that dicofol was not a "specific or selective neurotoxicant". Based upon the decreases in body weights and reduced food consumptions, the LEL for systemic toxicity was 75 mg/kg; NOEL, 15 mg/kg.

The study meets the data requirements for a neurotoxicity screening study (Guideline No. 81-8); it is classified as minimum.

Methods and Materials

Test article: Dicofol (Kelthane^R technical B miticide) which contained 95.5% active ingredient (Lot No. 464). The test article was a brown solid. Corn oil was used as a vehicle.

Animals: Approximately 50 day old Crl:CD^RBR VAF/Plus^R rats were obtained from Charles River Lab. Inc., Portage, Michigan. The male rats weighed from 200 to 230 gm, and females weighed from 133 to 181 gm on the day of arrival at the testing laboratory.

Study Design

The test animals were acclimated to the test laboratory for approximately 1 week. Forty males and 40 females were selected for the study based on good health, and randomly assigned to the following test groups:

<u>Group</u>	<u>Dose (mg/kg)</u>	<u>No. of males</u>	<u>No. of females</u>
I	0	10	10

II	15	10	10
III	75	10	10
IV	375	10	10

The test animals were housed individually. Food (Certified Rodent Chow[®] #5002 Ralston Purina) and water were available ad lib.

Compound administration: The test solutions were prepared by mixing an appropriate amount of dicofol and corn oil at approximately 80°C (with heating) the day before the administration. The prepared test solution was stored at room temperature and protected from light. Samples were taken for analysis of targeted dose levels, homogeneity, and stability.

Based upon the body weights, the test animals were administered by gavage the test solution at doses of 0, 15, 75, or 375 mg/kg in a volume of 5 ml/kg body weight.

Observations: All test animals were observed twice daily, and the body weights and food consumptions were measured weekly prior to dosing and daily after dosing. The observations were conducted for 14 to 19 days post-dosing.

Neurotoxicity evaluations: The neurotoxicity evaluation consisted of functional observational battery (FOB) and the motor activity test. These evaluations were conducted prior to the administration of the test compound, 8 hrs after dosing, one day after dosing, 7 days after dosing, and 14 days after dosing.

A. The FOB assessed the following parameters:

1. Lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to the light, piloerection, respiration, urination, and defecation.
2. Sensorimotor response to visual, auditory, tactile and painful stimuli.
3. Reaction to handling and behavior in the open field.
4. Gait pattern in the open field, severity of gait abnormalities, air righting reaction, visual placing response and landing foot splay.
5. Forelimb and hindlimb grip strength.
6. Abnormal clinical signs including convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance, and deposits around the eyes, nose, or mouth.

The report stated that all observations were made by the same individual who was unaware of each rat's dosage group.

- B. Motor activity test: Motor activity of each test rat was "monitored by a passive infrared sensor mounted outside a wire-bottomed stainless-steel cage. Each test session was 1.5 hrs ... the number of movements and the time spent in movement were tabulated at each five-minute interval".

Positive control data on several substances (acrylamide, IDPN, carbaryl, DDT, and triadimefon) were submitted to show that the FOB and motor activity tests were sensitive (Appendix J of the report).

- C. Necropsy: The test animals received a combination of heparin and an anesthetic and were perfused in situ with neutral buffered 10% formalin. Then a gross examination was conducted. Testes along with epididymides, ovaries, adrenal glands, liver, kidneys and urinary bladder were removed and fixed in neutral buffered 10% formalin for future histological examination. Brain, spinal cord, and hind limb peripheral nerves were removed and fixed in neutral buffered 10% formalin.

Six rats/sex/dose groups were randomly selected for neurological examination with respect to the possible lesions in the nervous tissues. The central nervous tissues were embedded in paraffin, and peripheral nerves were embedded in plastics.

Statistical analysis: The details of the statistical analysis were excerpted from the report and presented in Appendix A. A statement of GLP, a statement of no claim of confidentiality, a flagging statement which indicated the study neither met or exceeded any of the applicable criteria, and two quality assurance statements were signed, dated, and submitted in the report.

Results

- a. Test article analysis: The analytical results of homogeneity and stability of the test material indicated that the prepared solution of corn oil and the test substance was adequately mixed, and approximated the targeted doses (109% to 112% of the targeted doses) at the top, middle, and bottom of the mixture. The prepared solution, which was kept in the refrigerator for 3 days, was shown to be stable. The report also stated that the doses were stable over the time period of the study, but no data were included in the report to

substantiate this conclusion.

b. Mortality of the test animals: No death was seen in any dose groups during the study.

c. Functional observation battery (FOB): At the 8 hr examination period, 1/10, 3/10, and 8/10 male rats of the 15, 75, and 350 mg/kg groups, respectively, showed urine-stained or feces-stained fur. At a similar examination period, 7 female rats of the 350 mg/kg groups also showed signs of urine-stained or feces-stained fur, but this observation was not found in lower dose females.

At the day 2 examination period, 10/10 male rats in 350 mg/kg group still showed signs of urine- or feces-stained fur, and 3/10 males also had ataxia. In females, 1/10 and 7/10 animals in 75 and 350 mg/kg groups still showed signs of urine- or feces-stained fur; 2/10 females in 350 mg/kg group had ataxia. The air righting response of treated females was different from those of the vehicle controls as indicated by 1/10, 3/10, 3/10, and 5/10 females of 0, 15, 75, and 350 mg/kg groups, respectively, showing signs of uncoordinated landing and by 2/10 females of 350 mg/kg group landing on their backs.

At the day 8 examination period, 1/10 male in 350 mg/kg group had urine- or feces-stained fur, and other parameters were similar to those of the vehicle controls. In females, 1/10 and 4/10 animals of 75 and 350 mg/kg groups, respectively, showed sleepy signs, and 3/10 animals in 350 mg/kg group had urine- or feces-stained fur. All other parameters examined in the treated animals were comparable to those of the vehicle controls.

At the day 15 examination period, a statistically significant increase in the incidence of female rats (5/10) showing signs of immobility but awake in 350 mg/kg group was noted. The incidence of all other parameters in male or females rats were comparable to that of the vehicle controls.

A summary of clinical observations for both male and female rats was excerpted from the report and presented in Table 1. This table presents each finding as total number of observations/number of rats with the observation. This set of data appeared to be derived separately relative to the FOB because the data on stained fur and several other findings presented in Table 1 appear to be different from those reported under the heading of FOB; the author of the report should have offered an explanation as to exactly how the data were derived and why were they different from FOB data.

d. Body weights: In male rats, the mean body weights of 350 mg/kg group was decreased from day 2 to the end of the study,

and the decrease showed a statistical significance ($p < 0.01$) during days 2 to 9 of the study (Table 2 & Figure 1A). The percentage of the mean body weight reduction in 350 mg/kg males ranged approximately 6 to 10% of the controls. In female rats, a statistically significant decrease ($p < 0.01$) in mean body weights was found in 350 mg/kg group ($\approx 10\%$) from days 2 to 5 and in 75 mg/kg group ($\approx 7\%$) ($p < 0.05$) in days 2 and 3 (Table 3 & Figure 1B). At the end of study, the mean body weights were essentially comparable between the treated and control groups of male and female rats.

The calculated mean body weight changes indicated that the largest mean body weight change (decrease) occurred between day 1 and day 2 in both males and females of 75 and 350 mg/kg groups, and the decreases were statistically significant (Table 4). In 350 mg/kg males, the mean body weight change (gain) from day 1 to day 15 was less than that seen in the controls; in 350 mg/kg females, the body weight gain during the duration of the study was comparable to that of the controls.

e. Food consumption: - The mean food consumption values of 350 mg/kg males were substantially reduced ($> 50\%$) during the periods of days 1-2 and 2-3 and of 75 mg/kg males ($\approx 27\%$) during days 1-2 relative to those of the vehicle controls, and subsequently the food consumption values either approached or were significantly greater than those of the vehicle control male rats (Table 5). Similar patterns of changes in food consumption were also found in 350 mg/kg female rats as those of 350 mg/kg males. A significant drop in food consumption was also seen in 75 mg/kg females during days 1-2 and 2-3, and subsequently the food consumption of this group of females was comparable to that of the controls (Table 6). The food consumption of 15 mg/kg males and females was similar to that of the vehicle controls.

f. Motor activity: The effects of dicofol on motor activity were determined by the number of movements/5 minutes and time spent in movement. Both of these parameters were measured by a passive infrared sensor, and the operational sensitivity of this sensor was tested with neurotoxic chemicals such as acrylamide and triadimefon (Bayleton[®]) (positive controls) (Figure 2, A & B). It should be noted that the positive control studies were conducted approximately a year prior to the current study.

On day 2 of the study, there was a statistically significant decrease in the number of movements/5 minutes and a significant decrease in the time spent in a movement for approximately the first 7 blocks of the measuring session in 350 mg/kg males (Tables 7 & 8). The decrease in the number of movements in 350 mg/kg males on day 2 of study can be clearly

seen in Figure 3C. During the other measuring times, the motor activity measurements were comparable between the vehicle controls and the test article treated male rats.

In females, similar results as those seen in males were also found in 350 mg/kg group at 8 hours (hrs) after dosing on day 2 of the study (Tables 9 & 10 and Figure 4C). The measurements during the other examination times were comparable between the treated and the vehicle control rats.

g. Sacrifice: Gross examination of the male and female rats at the sacrifice did not reveal any compound-related gross lesions in any organs.

h. Organ weights: The brain weights and brain weight to body weight ratios were not affected by the test article in either treated males or females (Table 11).

i. Microscopic examination: Histopathological evaluations of the sections of the brain and the peripheral nervous system of the randomly selected high dose and control animals (6/sex) did not show any evidence of treatment-related neuropathologic changes. However, minimal evidence of axonal fragmentation within an individual nerve fiber in a longitudinal section of the sciatic nerve was seen in a control male with Bielschowsky's technique. One 350 mg/kg female rat had a minimal degree of myelin sheath swelling in only one nerve fiber within a section of the thoracic spinal cord.

Discussion

Groups of Crl:CD^RBR VAF/Plus^R rats (10/sex/group) were administered (by gavage) dicofol once at doses of 0, 15, 75, and 350 mg/kg. Functional observation battery and motor activity evaluations were conducted in the test animals. The results of several positive controls such as acrylamide, DDT, and triadimefon (Bayleton) were included in the report for validation of the sensitivity of test systems. In comparing the neurotoxicity of dicofol to that of DDT, dicofol was shown to produce limb tremors in 2 males, decreased activity, and sleepiness, whereas DDT was shown to produce whole body tremors in 2/8 males, increased rears, and some increased eye open in treated rats. Based upon the comparison of these **limited** parameters, the neurotoxicity of dicofol did not appear to be similar to that of DDT.

The results of this study indicated that dicofol at the doses tested did not cause any deaths; however, it produced the following effects:

1. An increase in the incidence of urine- or feces stained fur in 350 mg/kg male rats and in 350 mg/kg female rats,
2. An increase in the incidence of ataxia in 350 mg/kg male and female rats,
3. Changes in air righting response as indicated by an increased incidence of uncoordinated landing in 75 and 350 mg/kg females rats,
4. An increase in the incidence of female rats in 350 mg/kg group showing signs of being asleep,
5. A decrease in body weights in both male and female rats of 75 and 350 mg/kg groups at approximately days 2 to 5 of the study,
6. A decrease in food consumption in both male and female rats in 75 and 350 mg/kg groups during days 1-3, and
7. A decrease in motor activity as measured by a decrease of the number of movements/5 minutes and by an increase in time spent in a movement in both male and female rats of 350 mg/kg groups.
8. It should be noted that some of the effects (such as air righting responses) seen in 75 mg/kg male and female rats were scattered and less clear, and often they did not show a statistically significant difference from those of the vehicle controls.

Dicofol, under the conditions of this study, did not cause any histopathological changes in the central or peripheral nervous systems. In general, the results appeared to indicate that dicofol was not a "specific or selective neurotoxicant". Therefore, based upon the decreases in body weights and reduced food consumptions, the LEL was 75 mg/kg; NOEL, 15 mg/kg.

The study meets the data requirements for a neurotoxicity screening study (Guideline No. 81-8); it is classified as **minimum**.

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Pages 11 through 32 are not included in this copy.

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