011268

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT - 6 1994

#093

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicofol: Review of a subchronic neurotoxicity in rats

Caswell No. 93 **DP Barcode:** D196309
PC code **EPA ID No.** 010501 **Submission No.** S452169
MRID No. 429714-01

TO: Judith Loranger/Linda Propst, PM Team 73
Re-registration Division (7508W)

FROM: Whang Phang, Ph.D. 9/30/94
Pharmacologist
Tox. Branch II/HED (7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 10/3/94
Section Head
and
Marcia van Gemert, Ph.D. Marcia van Gemert 10/4/94
Branch Chief
Tox. Branch II/HED (7509C)

The registrant, Rohm and Haas, submitted a subchronic neurotoxicity study in rats. This study has been evaluated, and the DER is attached. The citation and the conclusion are presented below:

Foss, J. A. (1993) Subchronic neurotoxicity study of dicofol (Kelthane® Technical B Miticide) administered orally via diet to Crl:CD®BR VAF/Plus® Rats. Unpublished study conducted by Argus Research Lab., Inc.; Argus Study No. 018-018. Rohm and Haas Report No. 92RC-004. October 14, 1993. Submitted to EPA by Rohm & Hass. EPA MRID No. 429714-01.

In a 90-day neurotoxicity study, groups of Crl:CD®BR VAF/Plus® rats (10/sex/group) received dicofol (95% a.i.) at dietary concentrations of 0, 5, 100 and 500 ppm, (0, 0.3, 5.6, and 27.8 mg/kg for males and 0, 0.3, 6.5, and 31.3 mg/kg for females). Dicofol produced the following effects:

1. a decreases in body weights and body weight changes in 100 and 500 ppm male rats and in 500 ppm female rats,

2. a decrease in food consumption in 500 ppm males and females,

3. a decrease in the incidence of rears in the open field, a slight and persistent decrease in the forelimb and hindlimb grip strength, and a slight increase in the landing foot splay in 500 ppm males and females,

4. a decrease in total number of movements and total time spent in movement in 100 and 500 ppm males and 100 ppm females,

5. an increase in the absolute and relative liver weights (liver/brain) were increased in 100 and 500 ppm female rats, and the absolute and relative kidney weights (liver/brain) were also increased in 500 ppm females, and

6. a significant increase in absolute brain weights in 500 ppm males.

Based on the decreased motor activity and the increased liver weights, the LOEL was 100 ppm; NOEL, 5 ppm.

The study meets the data requirements for a 90-day neurotoxicity screening study (Guideline No. 82-7). It is classified as **minimum.**

Reviewer: Whang Phang, Ph.D. 9/30/94
Tox. Branch II/HED (7509C)

Secondary Reviewer: William Sette, Ph.D. 9/30/94
SACB/HED (7509C)
James Rowe, Ph.D. 10/3/94
Section Head, Tox Branch II/HED (7509C)

DATA EVALUATION REPORT

Study: Subchronic neurotoxicity study in rats (feeding)

Chemical: Dicofol (Kelthane®)

Caswell No.	93	**PC No.**	010501
DP Code:	D196309	**CASE:**	818577
Submission No.	S452169	**MRID No.**	429714-01

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

Sponsor: Rohm and Haas Co.

Citation: Foss, J. A. (1993) Subchronic neurotoxicity study of dicofol (Kelthane® Technical B Miticide) administered orally via diet to Crl:CD®BR VAF/Plus® Rats. Unpublished study conducted by Argus Research Lab., Inc.; Argus Study No. 018-018. Rohm and Haas Report No. 92RC-004. October 14, 1993. Submitted to EPA by Rohm & Hass. EPA MRID No. 429714-01.

Conclusion: In a 90-day neurotoxicity study, groups of Crl:CD®BR VAF/Plus® rats (10/sex/group) received dicofol (95% a.i.) at dietary concentrations of 0, 5, 100 and 500 ppm, (0, 0.3, 5.6, and 27.8 mg/kg for males and 0, 0.3, 6.5, and 31.3 mg/kg for females). Dicofol produced the following effects:

1. a decreases in body weights and body weight changes in 100 and 500 ppm male rats and in 500 ppm female rats,

2. a decrease in food consumption in 500 ppm males and females.

3. a decrease in the incidence of rears in the open field, a slight and persistent decrease in the forelimb and hindlimb grip strength, and a slight increase in the landing foot splay in 500 ppm males and females,

4. a decrease in total number of movements and total time spent in movement in 100 and 500 ppm males and 100 ppm females,

5. an increase in the absolute and relative liver weights (liver/brain) were increased in 100 and 500 ppm female rats, and the absolute and relative kidney weights (liver/brain) were also increased in 500 ppm females, and

6. a significant increase in absolute brain weights in 500 ppm males.

Based on the decreased motor activity and the increased liver weights, the LOEL was 100 ppm; NOEL, 5 ppm.

The study meets the data requirements for a 90-day neurotoxicity screening study (Guideline No. 82-7). It is classified as **minimum.**

Methods and Materials

Test article: Dicofol (Kelthane® technical B miticide) contained 95.5% a.i. (Lot No. 464, TD No. 92-003)). The test article was a brown solid.

Animals: Approximately 35 day old Crl:CD®BR VAF/Plus® rats were obtained from Charles River Lab. Inc., Portage, Michigan. The male rats weighed 84 to 110 gm, and females weighed 79 to 106 gm on the day of arrival at the testing laboratory.

Study Design

Based on the information presented in the report, the test animals were acclimated to the conditions of the testing laboratory for approximately 2 weeks. Forty healthy males and 40 healthy females were selected for the study, and randomly assigned to the following test groups:

Group	Diet. Conc.(ppm)	No. of males	No. of females
I	0	10	10
II	5	10	10
III	100	10	10
IV	500	10	10

The dietary concentrations of dicofol for this study were selected based on the previous dietary studies with dicofol. According to the report, in a previous 90-day feeding study, 500 ppm did not produce any deaths, but 1500 ppm caused deaths in more than one-half of the rats in this group. The 100 ppm dose produced histological changes in the liver.

The test animals were housed individually. Food (Certified Rodent Chow® #5002, Purina Mills) and water were available ad

lib. Later 6 rats/sex/dose group were randomly selected for neuropathological examination.

Compound administration: The test diet was prepared weekly by initially dissolving dicofol in acetone and then mixed with an appropriate amount of diet to achieve the desired concentrations (5, 100, and 500 ppm). Samples (125 gm each) were taken from the top, middle, and bottom to be analyzed for homogeneity and substance concentration. During the first week of test diet preparation, a duplicate set of samples (125 gm each) were collected from each concentration for the analysis of stability and substance concentration. The diet analyses were performed by Enviro-Bio-Tech, Ltd..

Observations: All test animals were observed twice daily, and the body weights and food consumption were measured weekly prior to dosing and daily during dosing.

Neurotoxicity evaluations: The neurotoxicity evaluation consisted of a functional observational battery (FOB) and the motor activity tests. These evaluations were conducted prior to the administration of the test compound, during weeks 4, 8, and 13 of the treatment period. Motor activity was evaluated with an automated apparatus on the same days when the FOB examinations were performed.

A. The FOB assessed the following parameters:

1. Autonomic functions: Lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to the light, piloerection, respiration, urination, and defecation.

2. Reactivity & sensitivity: Sensorimotor response to visual, auditory, tactile and painful stimuli.

3. Excitability: Reaction to handling and behavior in the open field.

4. Gait & sensorimotor coordination: 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". The test rats were evaluated "in sets of 10 males and 10 females, and each sex was assigned to a different block of 10 cages and sensors... Rats remained in the same assigned cage in all test sessions."

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 H 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, weighed, 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 along with the ganglia and spinal nerve roots, were embedded in paraffin, and peripheral nerves were embedded in plastics. "Sections were stained with hematoxylin and eosin, luxol fast blue/cresyl violet or toluidine blue, and Bielschowsky's technique"

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 of the test diet indicated that each test diet preparation was adequately mixed and approximated the targeted doses (from 87% to 98% of the intended doses) at the top,

middle, and bottom of the mixture. The results of the stability test showed that the test diet was approximately 96% of the targeted concentrations after 7 days of storage at room temperature. The average percent of the target at each concentration was reported to be 95.5%, 99.0%, and 99.4% for 5.0, 100, and 500 ppm respectively.

B. Mortality of the test animals: No compound-related death was seen in any dose groups during the study.

C. Clinical observations: Increased incidence of compound-related clinical signs were not seen in any treated groups.

D. Body weights: In male rats, the body weights were decreased in 100 and 500 ppm groups, and the decrease in 500 ppm was statistically significant ($p \leq 0.01$) starting from day 15 to the end of the study (Table 1 & Figure 1A). The body weight decrease in 100 ppm did not showed statistical significance until day 92 measuring interval. The body weights of 5 ppm and vehicle control males were comparable.

The body weight changes in 100 and 500 ppm males were reduced relative to those of the control. The reduction showed statistical significance ($p \leq 0.01$) in 500 ppm at several measuring intervals and at days 57-64 in 100 ppm group (Table 2).

In female rats, the body weight was significantly decreased in 500 ppm groups relative to the control ($p \leq 0.05$ and $p \leq 0.01$) (Table 3 & Figure 1B). There was also a significant reduction in body weight changes from days 1 to 92 of 500 ppm female rats ($p \leq 0.01$)(Table 4).

E. Food consumption: The food consumption was significantly ($p \leq 0.01$) reduced in 500 ppm male and female at essentially all the measuring intervals (Tables 5 & 6). In 100 ppm male and female rats, the values for food consumption were comparable to the controls except during days 85-92 measuring interval, when there was a significance decrease in males ($p \leq 0.05$). Dicofol did not produce any effect on food consumption in 5 ppm males; in 5 ppm females, the food consumption was significantly increased during the exposure period.

F. Compound intake: Based on the body weight and food consumption measurements, the test animals received dicofol at the following mean daily doses: 0, 0.3, 5.6, and 27.8 mg/kg body weight (bwt) for males and 0, 0.3, 6.5, and 31.3 mg/kg bwt for females.

G. Functional observation battery (FOB): In the treated males, a decrease in the incidence of rears in the open field was seen in all treated males, and that in the 500 ppm group was statistically significant at the measuring periods of 8 and 13 weeks (Tables 7A, 7B & 7C). A consistent decrease in grip strength of the forelimb and hindlimb was also seen in 500 ppm group at weeks 4, 8, and 13 (Tables 7A, 7B, & 7C). The decrease in the forelimb grip strength was statistically significant only at week 4 (Tables 7A). An increase in the average landing foot splay was seen in 500 ppm males at 4, 8, & 13 weeks measuring periods (Tables 7A, 7B, & 7C), although the increase in landing foot splay was not statistically significant.

In female rats, a decrease in the incidence of rears in the open field was seen in all dicofol treated females at weeks 4, 8, & 13 measuring intervals, and the decrease was statistically significant only at week 8 of the study in 5 and 500 ppm females (Tables 8A, 8B, & 8C). There was a slight decrease in forelimb and hindlimb grip strength at weeks 4 and 8, but the decrease was not statistically significant. An increase in landing food splay was seen in 500 ppm female rats at all 3 measuring intervals, and that at week 13 was statistically significant ($p \leq 0.05$) (Table 8C).

H. Motor activity: The motor activity measurements as determined by the number of movements and time (seconds) spent in movement are excerpted from the report (Table B8 & pages 86-93 for males; Table C8 & pages 195-202 for females) and are presented in Table 9.

In male rats, the total number of movements and total time spent in movement were decreased in 100 and 500 ppm males. The decreases in these two parameters were statistically significant in all measuring interval in 500 ppm males ($p \leq 0.05$). In 100 ppm males, the decrease in total number or movements and time spent in movement was statistically significant ($p \leq 0.05$) at week 8 or week 13, respectively (Table 9 & Figures 2B to 2D).

In female rats, the total number of movements and time spent in the movement was decreased in 100 ppm group whereas those in 5 and 500 ppm were comparable to those of the controls (Table 9 & Figures 3B to 3D).

Table 9+. Motor activity presented as total number of movements and total time spent in movement (seconds)

	0 ppm	5 ppm	100 ppm	500 ppm
Males				
Week 4				
No. of movements	757 ± 184	936 ± 205	649 ± 154	467 ± 176*
Time spent in movement	1309 ± 364	1665 ± 505	1101 ± 359	675 ± 217**
Week 8				
No. of movements	846 ± 271	706 ± 177	573 ± 276*	535 ± 156*
Time spent in movement	1509 ± 431	1337 ± 373	1043 ± 628	864 ± 273**
Week 13				
No. of movements	954 ± 209	873 ± 223	697 ± 222	603 ± 192*
Time spent in movement	1631 ± 466	1466 ± 428	1055 ± 376*	928 ± 347*
Females				
Week 4				
No. of movements	819 ± 228	833 ± 256	568 ± 206	840 ± 338
Time spent in movement	1324 ± 389	1351 ± 560	766 ± 340*	1250 ± 507
Week 8				
No. of movements	776 ± 265	664 ± 276	465 ± 149*	901 ± 235
Time spent in movement	1325 ± 403	1153 ± 552	744 ± 270*	1509 ± 598
Week 13				
No. of movements	881 ± 290	821 ± 230	591 ± 212	792 ± 243
Time spent in movement	1240 ± 421	1143 ± 439	725 ± 342*	1123 ± 400

+: The values presented in this table are the totals of the means of 18 blocks of measurements. Each test animal under went 18 blocks of observavation, and each block consisted of a 5 minute period. These values were excerpted from the report (MRID No. 429714-01).

I. Gross examination: No compound-related gross lesions were seen in any test animals.

J. Organ weights: In female rats, there were significant increases in absolute and relative (liver/brain) liver weights ($p \leq 0.05$) (Table 10A) in 100 and 500 ppm groups. The absolute and relative (kidney/brain) kidney weights were also increased in all treated females, and the increase was statistically significant ($p \leq 0.05$) at 5 and 500 ppm. Since the increase in kidney weights in 100 ppm was not statistically and a dose related increase was not present, there was insufficient evidence to show that the increase in kidney weights in 5 and 100 ppm females was a treatment-related effect.

In 500 ppm male rats, there was a statistically significant decrease in absolute brain weights ($p \leq 0.05$). There were increases in the organ to body weight ratios in 500 ppm males and females, but these increases were mainly a consequence of significant decreases in the body weights of these rats.

K. Neurohistopathology: Histological examination of the central nervous tissues, spinal cords with ganglia, and the peripheral nerves showed that in 500 ppm female rats, one (1/6) had neuronal vacuolation (multifocal, minimal) in the dorsal root ganglia and another (1/6) had peroneal/sural nerve fiber degeneration (focal, minimal). Since the effect was minimal and the incidence was small, the evidence was insufficient to show that the effects were treatment-related.

L. Positive Controls: The positive controls were conducted within one year of the testing of dicofol using Crl:CD®BR VAF/Plus® rats which are similar to those used in dicofol study. The data from the positive controls are intended for validating the testing laboratory's ability to perform the functional observational battery and motor activity tests and for certification of technicians conducting the tests.

Acrylamide in saline solution (40 mg/kg bwt) with was administered (ip) one dose/day for "a maximum of nine dosages".

IDPN in saline solution (200 mg/kg bwt) was administered (ip) one dose/day for three days.

DDT in corn oil (75 mg/kg bwt) was administered once by gavage.

Carbaryl in corn oil (75 mg/kg bwt) was administered once by gavage.

Triadimefon in corn oil (200 mg/kg bwt) was administered once by gavage.

d-Amphetamine in 0.5% methylcellulose (4.0 mg/kg bwt) was administered (ip) once.

The results of the motor activity and of functional observation battery for each positive control demonstrated that the laboratory was equipped to conduct the neurotoxicity study. The report has a shortcoming in that some of data on FOB and figures of motor activity did not clearly identify whether they were for male or female test rats.

Discussion

In a 90-day neurotoxicity study, groups of Crl:CD^{R}BR VAF/Plus^{R} rats (10/sex/group) received dicofol (95% a.i.) at dietary concentrations of 0, 5, 100 and 500 ppm, (0, 0.3, 5.6, and 27.8 mg/kg bwt for males and 0, 0.3, 6.5, and 31.3 mg/kg bwt for females). Dicofol did not cause any death during the study. Body weights and body weight changes were reduced in 100 and 500 ppm male rats and in 500 ppm female rats. Food consumption was decreased in 500 ppm males and females.

The FOB observation data showed that there were an increased incidence of rears in the open field , a slight and persistent decrease in the forelimb and hindlimb grip strength, and a slight increase in the landing foot splay in 500 ppm males and females. The decrease in the forelimb and hindlimb grip strength and the increase in landing foot splay were slight and at most of the measuring intervals did not attained a statistical significance relative to the controls; however, when these findings were evaluated together, they appeared to be treatment-related.

The motor activity was decreased in 500 ppm males and females and in 100 ppm males as indicated by a decreases in total number of movements and total time spent in movement.

The absolute and relative liver weights (liver/brain) were increased in 100 and 500 ppm female rats, and the absolute and relative kidney weights (liver/brain) were also increased in 500 ppm females. There was a significant increase in absolute brain weights in 500 ppm males.

The positive control DDT at 75 mg/kg (the only dose tested) caused an increase in the incidence of rears in the open field, a decrease in body weights, and essentially no effect on motor activity. Since the positive control DDT is structurally similar to dicofol, it is important to carefully evaluate any effects produced by this control. However, under the conditions of the studies, it is inappropriate to compare the results of DDT to those of dicofol because DDT was administered as a single dose by gavage.

Based on the decreases in motor activity and increases in liver weights, the LOEL was 100 ppm (5.6 mg/kg); NOEL, 5 ppm (0.3 mg/kg).

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

_____

Page ____ is not included in this copy.

Pages __12__ through __38____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X ___ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

_____ Internal deliberative information.

_____ Attorney-client communication.

_____ Claimed confidential by submitter upon submission to the Agency.

_____ Third party confidential business information.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

13544

010517

Chemical:	**Dicofol**
PC Code:	**010501**
HED File Code	**13000 Tox Reviews**
Memo Date:	**10/06/94**
File ID:	**TX011268**
Accession Number:	**412-02-0004**

HED Records Reference Center
10/01/2001