UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

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OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

82-2

SUBJECT: Dicofol: Review of A 4-Week Dermal Toxicity Study in Rabbits

TO: D. Edwards, PM (12)
Registration Division (H7505C)

FROM: Whang Phang, Ph.D. 11/16/89
Pharmacologist
HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 11/16/89
and
Marcia van Gemert, Ph.D. 11/17/89
Branch Chief
HFAS/Tox. Branch II/HED (H7509C)

The registrant, Rohm & Haas Co., submitted a 4-week dermal toxicity study on dicofol. This reviewer has evaluated the study, and the data evaluation report for this study is attached. The conclusion is as follows:

Groups of rabbits (6/sex/dose) received water, vehicle control, and dicofol at doses of 4.1, 10.2, and 61.1 mg a.i./kg/day by dermal application for 4 week (6 hrs/day). The test material caused dermal irritation at all dose levels. The vehicle control also caused similar dermal irritation. The degree of dermal irritation and incidences of acanthosis and hyperkeratosis in the treated skin were similar in vehicle controls (formulation ingredients) and in the high dose animals; therefore, the dermal irritation could be caused by the vehicle control.

The test article also decreased body weight in mid and high dose males and high dose females.

Based upon these results, the NOEL for systemic toxicity was 4.1 mg a.i./kg/day; the LOEL, 10.2 mg a.i./kg/day.

The study is classified as <u>minimum</u>.

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Reviewer: Whang Phang, Ph.D. 11/16/89
HFAS/Tox. Branch II/HED (H7509C)

Secondary Reviewer: K. Clark Swentzel, Section Head
HFAS/Tox. Branch II/HED (H7509C) 11/16/89

DATA EVALUATION REPORT

Chemical: Dicofol; Kelthane® MF Miticide; 4-chloro-alpha-(4-chlorophenyl)-alpha-(trichloromethyl)benzenemethanol

Study Type: 4-Week dermal toxicity study (rabbits)

Caswell No.: 93 **HED Proj. No.:** 9-1431
MRID No.: 410770-01 **EPA ID No.:** 707-203
EPA Record No.: 245029

Sponsor: Rohm and Haas Co.

Testing Laboratory: Rohm and Haas Co.
Toxicology Department
727 Norristown Rd.
Spring House, PA 19477

Citation: Bonin, R, Hazelton, GA, and Kulwich, BA, Dicofol (Kelthane® MF miticide): 4-Week Dermal Toxicity Study in rabbits. Rohm & Haas; Report No.: 86R-047; Dec. 1, 1986.

Conclusion

Groups of rabbits (6/sex/dose) received water, vehicle con-control, and dicofol at doses of 4.1, 10.2, and 61.1 mg a.i./kg/day by dermal application for 4 week (6 hrs/day). The test material caused dermal irritation at all dose levels. The vehicle control also caused similar dermal irritation. The degree of dermal irritation and incidences of acanthosis and hyperkeratosis in the treated skin were similar in vehicle controls (formulation ingredients) and in the high dose animals; therefore, the dermal irritation could be caused by the vehicle control.

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The study is classified as minimum.

Materials and Methods

Test article: Kelthane® MF miticide (lot AG5020A; TD 85-254);

brown liquid (43.6% a.i.). The composition of this formulation is presented in the report. Since this information is proprietary, it is not included in this data evaluation report.

Animals: New Zealand White rabbits were obtained from Hazelton Research Animals, Denver, PA. The animals were acclimatized in the laboratory conditions for 5 weeks. At the initiation of the study, the rabbits were 19 weeks old and weighed from 2.6 to 3.2 kg for males and 2.8 to 3.3 kg for females.

Study Design

Sixty rabbits (30/sex) were randomly assigned to different dose groups as follows:

Groups	Dose (a.i.) mg/kg (Kelthane® MF)	Number of rabbits Male	Female
1	0 (water control)	6	6
2	0 (vehicle control)	6	6
3	4.1 (low dose)	6	6
4	10.2 (mid dose)	6	6
5	61.1 (high dose)	6	6

One week before dosing, the hair on the back of each test rabbit was clipped, and then it was clipped as needed one day prior to the application of the test material. A week before the initiation of the test, each rabbit was fitted with a plastic collar which was kept in place with metal snaps for 24 hrs/day. The plastic collar was placed on each test animal throughout the dosing period.

On each dosing day, the test article was prepared fresh by mixing appropriate amounts of Kelthane® MF miticide with distilled water to concentrations of 1.0, 2.5, and 15% W/V. Vehicle control (formulation blank) was also diluted with distilled water "so that the concentrations of the non-active ingredients were equal to that in the high dose group" (page 12 of the report). The dosing solutions were sampled on first, tenth, and nineteenth day of treatment, and the the samples were analyzed.

The test material was applied onto a 96 cm^2 area of the shaved skin on the back of each rabbit at a constant volume of 1.0 ml/kg. The animals were treated 5 days/week for a total of 20 to 21 days. After treatment, the application sites remained unoccluded during the exposure time (6 hrs/day). At the end of the treatment period, the test material was removed by wiping the application sites with paper tissues which were moistened with a 2% Ivory soap solution and wet paper tissues. The animals were individually housed.

Each animal was observed twice daily during the treatment period and once daily during the weekend for any clinical signs of

toxicity. Physical examinations were conducted on each animal one week before the initiation of the study and on Mondays and Fridays of each week during treatment. During each physical examination, gait, posture, behavior, and external structure were also evaluated.

Skin irritation was evaluated prior to the daily treatment. The severity of irritation was graded according to the Draize's system (Attachment 1).

The body weight of each test animal was measured one week prior to the initiation of the test and weekly during the test.

Blood samples were collected from fasted animals via the jugular vein one week prior to the first treatment and 24 hrs after the last treatment. The following hematology and clinical chemistry parameters were examined:

Hematology

hematocrit	erythrocytes counts & morphology*
hemoglobin	white blood cell (WBC) counts (total and differential)*
platelet counts	mean corpuscular hemoglobin (MCH)
mean corpuscular volume (MCV)	
Mean corpuscular hemoglobin concentration (MCHC)	

* Erythrocyte counts and morphology and differential WBC counts were conducted only on animals of high and the two control groups.

Clinical Chemistry

glucose (gluc)	serum urea nitrogen (BUN)
serum glutamic pyruvic transaminase (SGPT)	serum glutamic oxalacetic transaminase (SGOT)
alkaline phosphatase (Alk)	total protein (T. Prot.)
cholesterol (Chol)	albumin (Alb)
globulin (Glob)	A/G ratio
creatinine	total bilirubin (T. Bili)
triglycerides (Trig)	calcium (CA)
phosphorus	gama glutamyl transferase (GGT)

Animals were sacrificed after 20 or 21 days of treatment. The test animals were grossly examined. Liver, brain, kidneys, and gonads were weighed. Thyroids and parathyroids were weighed post fixation. The following organs were fixed in 10% neutral buffered formalin:

adrenal	mammary gland
bone with bone marrow	skeletal muscle
brain	peripheral nerve
epididymides	pancreas
esophagus	pituitary
eye	prostate/vesicular gland
gall bladder	salivary gland
gonads	seminal vesicles
gross lesions	skin (treated & untreated)
spinal cord	heart
spleen	intestine (colon, cecum,
kidneys	duodenum, rectum, ileum, &
stomach	jejunum)
trachea	thymus
thyroid/parathyroid	liver
urinary bladder	uterus
vagina	lungs
lymph node (mesenteric)	

Histologic examinations were carried out on gonads, adrenal, thyroids, liver, kidney, and skin from treated and untreated areas, and gross lesions from all dose groups.

Data analyses were performed using two-way analysis of variance, analysis of covariance, and t-test. Mann-Whitney U test or Cochran-Mantel-Haenszel test was also used.

Compliance: Signed statements of data confidentiality, and quality assurance for GLP's were included in the report.

Results

Clinical signs: No clinical signs which could be related to the toxicity of the test agent were seen. No compound-related death was found.

Skin irritation: Table 1 presents the mean scores for skin irritation. The individual animal data revealed that, in both sexes of the low dose group, there were 1 or 2 rabbits which showed signs of "very slight" erythema at sporadic intervals of the treatment period. At the mid dose, more than two males or females showed signs of very slight erythema at all treatment days and some animals in this dose group also had slight edema. All males and females of the high dose group showed signs of well defined erythema and slight edema, and skin irritation persisted throughout the treatment period. The animals in the vehicle control group also showed signs of skin irritation throughout the treatment period.

Body weights: The data on the group mean body weights are excerpted from the report and presented in Table 2. There

was a decrease in mean body weight gain in all males which received the test material relative to the water controls as indicated by the body weight changes during the treatment period (days 0 to 27). The reduced body weight gains were statistically significant at mid and high dose levels ($p<0.05$), and it was a compound-related effect. The difference in body weight gain in low dose males was small and not statistically significant.

In female rabbits, the high dose animals showed significantly lower body weights relative to the water and vehicle controls ($P<0.05$) on days 6, and 13. In addition, there was also statistically significant drop ($p<0.05$) (63%) in body weight gain in high dose females relative to that of the water controls (Table 2).

Hematology: No treated effects were seen in any of the parameters examined.

Clinical chemistry: There were decreases in globulin and gamma glutamyl transferase seen in some test material treated female rabbits, but these changes did not showed any dose-response effect and were small.

Gross pathology: Other than the changes in the treated skin no other gross changes were found. In the treated skin of 4/6 vehicle control males, reddening, thickening, and/or desication were seen. Similar effects were seen in all vehicle control females, 3/6 high dose males, 2/6 high dose females, In the mid dose group, thickening or reddening was also seen in a female rabbit. Other gross changes were comparable among groups, and they were not compound-related.

Organ weights: There were some changes in organ weights of the treated rabbits relative to the vehicle control, but these changes were small and were not dose-related (Table 3).

Histopathology: Histopathological changes were seen mainly in the treated skin, and the summary of these changes were excerpted from the report and presented in Table 4. The incidence of histopathologic changes in other organs was comparable among the various treatment groups and the controls.

In the treated skin, notable findings were increases in the incidence of acanthosis and hyperkeratosis in essentially all vehicle controls and high dose males and females. In addition, acanthosis was seen in all dicofol treated animals (Table 4). Increases in the incidence of chronic and/or subacute inflammation were also seen in all dose groups (Table 4); these incidences were more marked in vehicle controls and the high dose animals. Acanthosis was characterized by increased numbers of cell layers and thickening of the epidermis; subacute inflammation, a mixed cell infiltration with significant numbers of heterophils; chronic inflammation, primarily

diffused lymphohistocytic cell infiltration.

Some vehicle control animals also showed signs of chronic inflammation in the untreated skin, and similar findings were seen in 2/6 males and 3/6 females of the high dose groups (Table 3). The untreated skin of these animals had probably accidentally come into contact with either vehicle controls or the test material.

Other histopathologic changes in liver, kidneys, thyroids, adrenal, and gonads were comparable among the two control groups and the various treatment groups.

Discussion

The data showed that the test material, when applied dermally, caused dermal irritation at all dose levels. The vehicle control also caused similar dermal irritation. The degree of dermal irritation and incidences of acanthosis and hyperkeratosis were similar in vehicle controls (formulation ingredients) and in the high dose animals; therefore, the dermal irritation could be caused by the vehicle control.

The test article also decreased body weight in mid and high dose males and high dose females.

Based upon these results, the NOEL for systemic toxicity was 4.1 mg a.i./kg/day; the LOEL, 10.2 mg a.i./kg/day.

The EPA guidelines for subchronic dermal toxicity study recommend that the area of dermal application site be at least 10% of the body surface of a test animal. The experimental design of this study employed only 96 cm^2 (5%) of the body surface. This small application area could have contributed to accidental contact of the test material with the untreated skin as indicated by incidence of skin irritation in the untreated area. Although this was a shortcoming of this study, it did not interfere with the interpretation of the results. The study is classified as minimum.