9-26-77

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY Casual No. 346 A

DATE: September 26, 1977

CONFIDENTIAL

SUBJECT: Application for Full Registration of Dimilin W-25 for Cotton Ball Weevil Control 7F-1898

~~Draft Copy~~

Application for Full Registration of Dimilin [tm] W-25 for Mosquito Larvae Control 6F-1773

Final copy ~~in progress~~.

Application for Full Registration of Dimilin W-25 for Soybean Insect Pest Control 6F-1832

000962

FROM: Salvatore F. Biscardi, Pharmacologist
Toxicology Branch, Registration Division (WH-567)

TO: Product Manager, Mr. Charles Mitchell, No. 17
Chemistry Branch

PETITIONER: Thompson-Hayward Chemical Company
Kansas City, Kansas

Considerations for Final Recommendations:

In conformance with the directives of the Deputy Administrator for Pesticides Programs dated February, 1977, the following background elements are offered for consideration for the final recommendations.

EPA FORM 1320-6 (REV. 3-76)

24 1

SUBSTANCE IDENTIFICATION

1. Chemical Name:

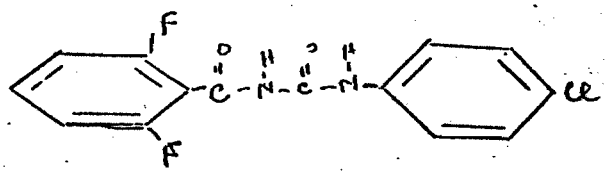
Chemical Abstracts Name: [[N-(4-chlorophenyl) amino] (carbonyl)] 2,6-difluorobenzamide.

2. Synonyms: Trade name - Dimilin

Common name - Diflubenzuron (proposed)
TH-6040, PH-6040, ENT-29054, OMS 1804

3. Structure:

Structural Formula:



Molecular Weight
310.7

4. Purity of Technical Material:

Confidential Statement of Chemical Composition:

ACTIVE INGREDIENTS	
[[N-(4-chlorophenyl) amino] (carbonyl)] 2,6-difluorobenzamide.	95%
INERT INGREDIENTS	
[redacted]	
	100%

OTHER PHYSICAL/CHEMICAL DATA:

Melting Point: Technical Material 210 - 230°C
Pure Product 230 - 232°C

Boiling Point: Not Applicable

Vapor Pressure: Volatility of the crystaline material at room temperature is nil.

Density/Specific Gravity: 1.208

Hydrolysis Rate: Decomposition at pH 5 after 3 weeks was 4%. At pH 7 decomposition was 8% after 3 weeks. At pH 9, 26% was degraded in 3 weeks. Studies conducted for 9 weeks indicated that 11% decomposition occurred at pH 5, 31% at pH 7, and 65% of the TH-6040 had degraded at pH 9.

Solubility in Various Solvents:

Water	0.2 ppm
Acetone	0.65 g/100 ml
Methyl pyrolidine	28 g/100 ml
DMSO	12 g/100 ml
DMF	12 g/100 ml
Cyclohexanone	<2 g/100 ml
Dioxane	<2 g/100 ml
Ethanol	<2 g/100 ml
Isophorone	<2 g/100 ml
Methylene chloride	<2 g/100 ml
Xylene	<2 g/100 ml

Dissociation Constant: Not Applicable

Stability: Diflubenzuron is stable at pH 2 to 8 but unstable at pH 12. The half-life in the soil is 1.5 weeks with particle sizes less than 5 microns.

Physical State: Crystaline solid

Color: White

Miscibility in Various Solvents (Formulated Product):

The formulated product is readily suspended in water, cotton seed oil, Vegedix, Sun 7-N, and other oils used to aid in spray application.

Storage Stability (formulated Product):

Dimilin W-25 was stable at room temperature and 54°C for one year.

Analytical Method for the Principal Component and Impurities:

Determination of impurities in the technical material is accomplished by [redacted]

Confidential Statement of Chemical Composition (formulated):

ACTIVE INGREDIENTS	
[[N-(4-chlorophenyl) amino] (carbonyl)] 2,6-difluorobenzamide (95%)	26.23%

REFERENCED PETITIONS:

There are no approved permanent tolerances since this is a new compound. Petitions submitted for requests for tolerances are:

1. 7F 1898 -- Application for Full Registration of Dimilin W-25 for Cotton Ball Weevil Control.

2. 6F 1773 -- Application for Full Registration of Dimilin tm W-25 for Mosquito Larvae Control.

3. 6F 1832 -- Application for Full Registration of Dimilin W-25 for Soybean Insect Pest Control.

4. 6G 1744 -- Application for Experimental Permit of Dimilin tm W-25 for Cotton Ball Weevil Control.

The following attachment presents the list of additional Experimental Use Permits issued either to Thompson-Hayward or to the U.S.D.A.

000962

Section 1 - Vol. I of VII

Acute Oral Toxicity Studies with DU 112307 In Mice and Rats

Report No. 56645/14/73

Test Compound: DU 112307 (Batch No. 309181)

Test Specie: Swiss mice
SPF Wistar Strain Rat

Number of Animals: Five males & five females/test level

Route of Administration: Intragastic intubation

Dose and Duration of Exposure: The doses were 4640, 2150, 1000, 464, 215 and 100 mg/kg bodyweight. Animals observed 14 days.

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

17 b

29

000962

Methodology: DU 112307 was administered as a suspension in 1% aqueous gum tragacanth by intragastric intubation. Five males and five females were used per test level. The volume of test material was 10 ml/kg body weight except for the highest dose which volume was 20 ml/kg.

Animals were observed for signs of intoxication during the subsequent 14 day period.

Results: No overt effects were observed for the subsequent 14 days in male and female Swiss mice and SPF Wistar rats when DU 112307, suspended in 1% gum tragacanth, was administered in dose of 100, 215, 464, 1000, 2153, and 4640 mg/kg.

Comment: The test material DU 112307 must be completely identified.

Note: Doses of 4000, 3000, 2000 mg/kg in a mutogenic study (IBT) produced hypoactivity in mice when DU 112307 was administered i. p..

Conclusion: DU 112307 produced no signs of toxicity in Swiss mice and SPF Wistar rats when red at 100, 215, 464, 1000, 2150 and 4640 mg/kg intragastrically. The acute LD-50 is greater than 4,640 mg/kg in both species.

Validation: Invalid- no experimental test data presented.

IR 7

30

BB-1056
TXR-962

INFORMATION WHICH MAY REVEAL THE IDENTITY OF AND INERT INGREDIENT IS NOT INCLUDED

000962

Section 2 - Vol. I of VII

Acute Oral Toxicity Studies with DU 112307 [redacted]
(Batch #203071)

A. Acute Oral Toxicity in Mice and Rats

B. Acute Dermal Toxicity in Rabbits

C. Local Toxicity to the Rabbit Eye

A. Acute oral toxicity in Swiss Mice

Text Compound: DU 112307 [redacted]

Test Species: Swiss mice

Number of Animals: Five males and five females per dose level.

Route of Administration: Intragastric intubation

Dose: 10,000; 4,640; 2,150; 1,000; 464; 215; 100; 46.4; 21.5; 10 mg/kg.

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

1 of 30

8

Methodology: DU 112307 [redacted] was adminsitered as a suspension in gum tragacanth by stomach tube to 5 males and 5 females per test level. The dose levels were 10,000; 4,640; 2,150; 1,000; 464; 215; 100; 46.4; 21.5; and 10 mg/kg p.o. Each volume was administered at 10 ml/kg except 10,000 mg/kg dose level which was given at 30 ml/kg. Toxicity was observed for 14 days post administration of test material.

Results: No mortalities were recorded. There were no signs of toxicity.

Conclusions: The LD-50 of DU 112307 [redacted] is greater than 10,000 mg/kg p.o. when administered to male and female Swiss mice.

Validation: Invalid-no experimental data presented.

22 A2

9

Section 2 - Vol. I of VII

Acute Oral Toxicity in Rats

Test Compound: DU 112307 [redacted]

Test Specie: SPF Wistar Rat

Number of Animals: Five males and five females per dose level

A. Route of Administration: Intragastric intubation.

Doses: 20,000; 9.280; 4.300; 2,000; 928; 430; 200; 92.8; and 43 mg/kg.

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

Methodology: DU 112307 [redacted] was administered in a 1% aqueous gum tragacanth intragastically to groups 5 males and 5 females per dose level. Dose levels were 20,000; 9,280; 4,300; 2,000; 928; 430; 200; 92.8; and 43 mg/kg.

Results: No mortalities were reported or signs of toxicity 14 days subsequent to dosage.

Conclusion: The LD-50 of DU 112307 [redacted] is greater than 20,000 mg/kg when administered p.o. to SPF wistar male and female rats.

Validation: Invalid-no experimental test data presented.

Section 2 - Vol. I of VII

Acute Dermal Toxicity in Rabbits

B. Test Compound: DU 112327 [redacted]

Test Specie: New Zealand White Rabbits

Number of Animals: Sixteen rabbits

Route of Administration: Dermal application

Dose: 0.6567 g/ml of a solution of DU 112307 [redacted] suspended in 1% aqueous gum-tragacanth-applied at 15 and 30 ml/kg body weight.

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

27
5 12

Methodology: Two group of rabbits 4 males and 4 females per group were dosed as above after clipping the skin of hair. Half of each group was braded through the stratum corneum. Trunks were wrapped in sticking plaster, covered with aluminum foil Animals were individually housed. Food and water was "ad lib". Twenty-four hours after dosage, the coverings were removed and the skin washed with soap and water.

Results: No skin irritation was reported or mortalities up to 14 days post treatment.

Validation: Invalid-no experimental test data presented.

13

Section 2 - Vol. I of VII

Primary Irritation of Eye Mucosa

C. Test Compound: DU 112307 [redacted].

Test Specie: Rabbits -

Number of animals: Nine-three groups of three

Route of Administration: Eye-instillation

Doses: One-tenth milliliter DU 112307 [redacted] in concentration of 0.5 g/ml in 1% aqueous gum-tragacanth

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

000962

Methodology: The test material at the above stated dose was instilled into the left conjunctival sac of each animal. The other eye was used as control. The first group of rabbit eyes remained unwashed. The record group had their eyes rinsed with lukewarm water (20 mT) two second after instillation. The third group had their eyes rinsed 4 seconds after instillation. Reactions were observed at 24, 48, 72 hours and at 4 and 7 post treatment.

Results: No irritation was observed either in the eyes or adjoining tissues.

Validation: Invalid-no experimental test data was submitted.

26 15
55 8

000962

Section 3 - Vol. I of VII

Acute Dermal Toxicity In Rabbits with DU 112307 w.p. 25%

Report # 56645/17/73

Test Compound: DU 112307 w.p. 25%
Batch # 311301

Test Specie: New Zealand White Rabbits

Number of animals: Thirty-two rabbits four groups of 4 males and 4 females

Route of Administration: Dermal

Doses: (a) 4640 mg/kg (a.i.) DU 112307 w.p. 25% suspension in water.

(b) 2150 mg/kg (a.i.) DU 112307 w.p. 25% suspension in 0.5% aqueous gum-tragacanth.

Solutions: (a) and (b) were applied at the rate of 20 ml/kg body weight.

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

Methodology: The skin of the rabbits was clipped of hair around the trunk. Half of the animals in each group was abraded to penetrate the stratum corneum. Sticking plaster, covered by aluminum foil wrapped the trunks. The test material was introducted under the plaster. Animals were housed individually. Food and water was "ad libitum". Twenty-four hours, later, the bandages were removed and skin washed with soap and lukewarm water. Toxicity was recorded for the subsequent 14 days.

Results: No mortality or overt signs of toxicity were observed.

Conclusion: The dermal LD-50 of the test materials exceeded the highest dose of 4,640 mg/kg (a.i.) for solution (a) and 2150 mg/kg (a.i.) for solution (b).

Validation: Invalid. No experimental test data submitted.

17

000962

Section 4 - Vol. I of VII

Acute Toxicity Studies with DU 112307 (25%) w.p.) in Mice and Rats

Report No. 56645/15/73

No. 56645 (3/77

Test Compound: DU 112307 25% w.p. (Batch No. 311231)

Test Specie: Mice - Swiss strain
Rats - SPF Wistar strain

Number of Animals: Five males and five females per dose level

Route of Administration: Per os

Doses: 10,000; 4640; 2150; 1000; 464; and 215 mg/kg body weight

Testing Laboratory: Philips-Duphar, B.V., Weesp, The Netherlands

11

18

000962

Methodology: DU 112307 was administred as a suspension in water to mice and rats at dose levels 1000, 4640, 2150, 1000, 464 and 215 mg/kg (a.i.)

Injection volumes were prepared at 10 ml/kg except for the 10,000 mg/kg dose which was given at 30 ml/kg. Intragastric intubations were given to five males and five females per dose level. Toxicity was recorded up to 14 days post treatment.

Results: The LD-50 of DU 112307 25% w p. is greater than 10,000 mg/kg (a.i.) when administered to mice and rats intragastically.

Note: This experiment was initially reviewed in 1976 and considered invalid because of absence of ~~analytical~~ animal test data.

Analytical test data was subsequently received in June 1977. The study was re-examined.

Validation: Core - Guideline

Supporting data from:

Section 11 - #962

Additional Data Applicable to PP Nos. 6F1773, 6F1832, 7F1898, June 10, 1977.

12

19

000962

Section 5 - Vol. I of II

Acute Percutaneous Toxicity to Rabbits of Du 112307 (Technical)

Report No. 2171/0175/73

Test Compound: DU 112307 - 50% paste in aqueous gum tragacanth (0.5%)

Test Specie: New Zealand White Rabbits

Number of Animals: Eight rabbits - 4 males and 4 females

Route of Administration: Dermal

Dose: 4 ml/kg body weight of above test material

Testing Laboratory: Huntingdon Research Center

Testing Labortory: Huntingdon Research Center
Huntingdon, England

Methodology: Hair was clipped from trunk of rabbits. DU 112307 in a 50% aqueous gum tragacanth (0.5%) paste was spread over the skin 5% of total body surface. The skin was covered with aluminum foil and waterproof plaster around trunk. At 24 hours, coverings were removed and skin washed with dilute soap solution.

Animals were observed for 14 days for signs of toxicity. Lesions were scored and graded. Rabbits were sacrificed to exam tissues microscopically to determine any target organ.

Results: There were no mortalities. There were signs of slight lethargy only in 3 rabbits during first week of observations.

Slight irritation was observed in ~~five~~ three rabbits soon after treatment. All reactions ameliorated during the first week.

Post mortems were normal.

Conclusions: LD-50 to rabbits of DU 112307 50% paste in gum tragacanth is greater than 4 ml/kg body weight.

Validation: Core - Guidelines

000962

Section 6 - Vol. I of VII

Acute Inhalation Toxicity to the Rat of DU 112307 Technical Grade Powder

Report No. PDR 74/73849.

HRC Report No. 4920/72/355

Test Compound: Technical DU 112307

(Batch # 309181)

Test Specie: Sprague - Dawley albino rats

Number of Animals: Thirty rats, ten per dose level, ten controls.

Route of Administration: Inhalation

Dose: 5.0 mg/liter, 35 mg/liter, 0 mg/liter controls

Testing Laboratory; Huntingdon Research Center
Huntingdon, England

Sponsor: B.V. Philips-Duphar, Weesp, Holland

22

15

Methodology: Two groups of ten rats each were exposed to DU 112307 at nominal concentrations at 5.0 mg and 35 mg/liter air respectively. The dust was generated by a Timbrell or Wright dust generator. Exposure lasted 6 hours. Dust particle sizes were mostly of respirable size. Gravimetric analysis showed the 5.0 mg/liter nominal concentration to be equal to 0.64 mg/liter and the 35 mg/liter nominal concentration to be equal to 2.88 mg/liter estimated concentration.

Results: No mortalities were recorded. Macroscopic examination of tissues showed no abnormalities throughout.

Conclusion: The LC-50 of DU 112307 Technical is greater than 35 mg/liter nominal concentration or 2.88 mg/liter.

Validation: Core - Guidelines

000962

Section 7 - Vol. I of VII

Acute Inhalation Toxicity to the Rat of DU 112307 Insecticide Powder

Report No. 4920/72/355

Test Compounds: PH 60-40 Concentrate 50%
(No. 203071 - P 7087) and
[redacted]

Test Specie: Albino rats - CFHB Strain

Number of Animals: Fourty rats, ten per test group

Route of Administration: Inhalation

Doses: 50 mg/liter [redacted]
5.0 mg/liter DU 112307
50 mg/liter DU 112307
Controls.

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: M.V. Philips - Duphar, Weesp, Holland

Methodology: Two groups of 10 rats were exposed to DU 112307 dust for 6 continuous hours while a third group of 10 rats were exposed [redacted] The two doses of DU 112307 were 5.0 and 50 mg/liter of air respectively. The dusts were generated by "Wright" and Timbrell dust generators. Particle sizes were determined to be within respirable ranges. Gravimetric analysis showed [redacted] to have as extimated concentration of 5.83 mg/liter air. DU 112307 at nominal concentrations of 5.9 mg/liter to have an estimated concentration of 0.21 mg/liter air and DU 112307, nominal concentration of 50 mg/liter of air have an estimated concentration of 2.71 mg/liters air.

Results: No mortalities or signs of toxicity were observed during the subsequent 7 day post treatment period as might be reflected by either body weight change or through microscopic examination of the lungs.

Conclusion: The LC-50 of DU 112307 is greater than the nominal concentration of 50 mg/liter or of 2.71 mg/liter estimated concentration.

Validation: Core - Guidelines - tentative

3c 25

would appear to be a

000962

Comment: There ~~is~~ discrepancy whether PH 60-40 concentrat 50% is equal to DU 112307 technical, as the report states.

Test material must be completely identified.

Section 8 - Vol. I and VII

000962

Acute Inhalation Toxicity To the Rabbit

Of DU112307 - Technical Grade Powder

Report PDR 198/74988

Test Compound: PO 112307 - Technical Grade Powder (Batch # 405093)

Test Specie: Albino rabbits - Duncan Hartley strain

Number of animals: Eight rabbits - 4 test, 4 controls

Route of Administration: Inhalation

Dose and duration of exposure: Nominal concentration of 30 mg/liter air

One six-hour test period

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: M.V. Philips - Duphar, Weesp, Holland

27

320

Methodology:

The test material in dust form was generated by a Timbrell dust generator into a 100-liter exposure chamber holding 4 albino rabbits, 2 males, 2 females of the Duncan Hartley strain. The duration of exposure was 6 continuous hours. Animals were examined daily for the subsequent 14 days for signs of toxicity. Animals had food and water and were weighed 5 x post-exposure period. Methemoglobinemia content was estimated by a modified Hainline, A. Jur method. Animals were autopsied and organs examined microscopically. Gravimetric analysis showed the estimated concentration to be 3.75 mg/liter air.

Results:

Both males in the treated group lost weight progressively from day 7 to day 14. Female weights ~~gradually~~ generally increased as did all control animals. Nothing appreciable was seen in methemoglobinemia in respect to control values. Macroscopic pathology revealed no abnormalties despite progressive decrease in weight in males.

Conclusion: DU112307 technical grade produces progressive weight loss in male rabbits at the actual inhalation dose at 3.75 mg/liter of air. Clarification is needed as to cause and whether this reflects toxicity.

Validation: Supplementary. The experiment does not accomplish the intended purpose.

28

000962

Section 9 - Vol. II and VII

Irritant Effects Of DU112307 (Technical) On Rabbit Eye

Report number 2170/176D/7

Test Compound: DU1123307 (Technical)
Batch # 309181

Test Specie: New Zealand White Strain

Number of Animals: Eight rabbits

Route of Administration: Instillation into eye

Dose: Forty mg instilled into eye

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N.V. Philips - Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

22 29

Methodology: The methodology employed is described in Federal Register 37 No. 83, April 1972 # 19112

Results: After rinsing the eyes in one group of five animals for five minutes the ocular examination showed no indication of irritancy or corrosiveness according to the method of numerical scoring as outlined in the method.

A slight dulling of the cornea was observed in one rabbit only one hour after instillation. Mild conjunctival reactions were noted in all five animals.

The same results of mild conjunctival irritation were noted when eyes were rinsed after 24 hours.

Conclusion: DU112307 when administered, 40 mg. to the rabbit eye can be considered a "marginal irritant."

Comment: For solids, the dose of test material should be 50 mg. and not 40 mg. as per the above method. Because the dose level used was not the prescribed amount, the categorization of the results were not appropriate.

Validation: Supplementary.

Section 10 - Vol. II of VII

Irritant Effects Of DU112307 (W.P. 25%) On Rabbit Eye Mucosa

Report No. 56645/18/73

Test Compound: DU112307 (W.P. 25%)
Batch No. 311301

Test Specie: New Zealand White Strain

Number of Rabbits: Eight Rabbits

Route of Administration: Instillation into eye

Dose: 1/10 ml DU112307 w.p. 25% in a concentration of 0.5 gm/ml in 0.9% NaCl solution

Testing Laboratory: B.V. Philips - Duphar, Weesp, Holland

Methodology: The testing procedure was according to FDA procedures published in the Federal Register 37 No. 83, April 1972.

Results: The report states that the test material had no irritation effect on the eye and associated tissues in the subsequent seven days post treatment.

Conclusion: DU1123307 w.p. 25% (0.5% gm/ml) was not irritating to the rabbit eye when tested according to the method of FDA published in the Federal Register 37 No. 83, April 1972 according to Dr. A. V. Eldik.

Validation: Invalid -- no animal test data submitted.

000963

Section 11 - Vol. II of VII

Primary Skin Irritation Study

Test Compound: TH-6040 Technical

Test Specie: Albino rabbit

Number of Animals: Six rabbits

Route of Administration: Dermal

Dose: According to FHSLA protocol

Testing Laboratory: Harris Laboratories

Methodology: Protocol as described in "Regulations Under the Federal Hazardous Substances Labeling Act," Federal Register, August 12, 1961, Sec. 191.11

Results: No erythema, eschar or edema formation was noted at 24 or 72 hours post treatment.

Conclusion: When TH-6040 (Technical) was applied to the skin of rabbits as outlined in "Regulations Under The Federal Hazardous Substances Labeling Act," August 12, 1961, Sec. 191.11, no erythema, eschar or edema formation on abraded or intact skin was noted in any of the six rabbits either at 24 or 72 hours. TH-6040 (Technical) is not a primary skin irritant to the rabbit, when tested according to the above method.

Comment: Complete identification of test material is required.

Validation: Core - Guidelines.

000963

Section 12 - Vol. II of VII

Primary Skin Irritation Study

Test Compound: TH6040 W-25 Wettable Powder

Test Specie: Albino Rabbits

Number of Animals: Six Rabbits

Route of Administration: Dermal

Dose: According to FHSLA Protocol

Testing Laboratory: Harris Laboratories
Lincoln, Nebraska

Methodology: Protocol as described in "Regulations Under the Federal Hazardous Substances Labeling Act" Federal Register, August 12, 1961, Sec. 191.11

Results: No erythema, eschar or edema formation was noted at 24 or 72 hours post treatment.

Conclusion: When TH-6040 wettable powder was applied to the skin of rabbit as in the method outlined in "Regulations Under The Federal Hazardous Substances Act," August 12, 1961, Sec. 191.11, no erythema, eschar or edema formation was noted on abraded or intact skin in any of the six rabbits either at 24 or 72 hours. TH-6040 W-25 wettable powder is not a primary skin irritant to the rabbit when tested according to the above method.

Comment: Complete identification of test material is required.

Validation: Core - Guidelines

000963

Section 13 - Vol. II of VII

Acute Toxicity Studies With DU112307 (Technical) In Mice After Intra-Peritoneal Administration

Report No. 56645/1/74

Test Compound: DU112307 technical product (Batch # 201252)

Test Specie: Swiss strain mice

Number of Animals: Ten mice, five males and five females/dose level, total 80 mice.

Route of Administration: Intraperitoneally

Doses: 2150; 1000; 464; 205; 100; 46.4; and 21.5 mg/kg

Testing Laboratory: B.V. Philips-Duphar, Weesp, Holland

Methodology: DU 112307 Technical was administered i.p. as a 25% PVP suspension in 0.9% saline solution to five male and five female mice per dose level. The doses were 2150, 1000, 464, 215, 100, 46.5 and 21.5 mg/kg mouse body weight. Controls were treated with vehicle. Animals were observed for signs of toxicity for 14 days subsequent to treatment.

Results: When DU 112307 (Technical) was administered i.p. to mice at doses up to 2,150 mg/kg, no mortality or signs of overt toxicity were noted in any of the mice during the fourteen days post treatment observation period.

Conclusion: There is no toxicity to mice when DU 112307 Technical is administered i.p. up to a dose of 2,150 mg/kg.

Validation: Invalid. No animal test data is available.

Comment: When TH6040 was administered i.p. in the mutagenicity study (IBT) at 2,000 mg/kg, hypoactivity was noted.

000963

Section 14 - Vol. II of VII
Section 15 - Vol. II of VII

Dietary Administration Of DU112307 to Male and Female Rats for 3 Months

Report # 56645/13A/73
Report # 56645/13B/73

Test Compound: DU112307 (Batch No. 201252)

Test Animals: Wistar Rats

Number of Animals: 60 males and 60 females

Route of Administration: Dietary

Doses: 0, 3.125, 12.5, 50 and 200 ppm

Testing Laboratory: Philips-Duphar, Weesp, Holland

Methodology: Sixty males and sixty female rats were divided into five groups., An additional 10 animals, 5 males and 5 females were placed in controls and again in the high dose level for serum for clinical chemistry analysis. The experiment lasted 3 months. The dose levels were 0, 3.125, 12.5, 50 and 200 ppm. Parameters monitored were clinical signs, body weight and food consumption, haematology, clinical chemistry, organ weights and gross and microscopic pathology.

Results: At seven weeks there was a gradual dose-related decrease in P.C.V. and hemoglobin in all female treated groups becoming statistically significant at the highest dose level.

At thirteen weeks, there was a dose-related decrease in R.B.C., P.C.V. and Hb in males becoming statistically significant at the highest dose level.

At seven weeks dealing with group mean values again, there was an increase in urea in males at the highest dose level and an increase in alkaline phosphatase over control values. Male rats at 13 weeks also showed an increase in S.G.O.T. and S.G.P.T. at the highest level. At 13 weeks female rats also showed a dose-related increase in S.G.P.T. and S.G.O.T.

Male rats showed a dose-related increase in absolute testes weight becoming statistically significant at the highest dose level. This same effect could be observed in adrenals in all treated groups.

Summary: The above-mentioned results show that certain hematolcgical and biochemical factors reflecting toxicity, while becoming statistically significant at higher dose levels, ~~over~~ more severe the less show trends at all dose levels including the lowest dose level of 3.125 ppm.

The establishment of liver pathology at the 50 and 200 ppm dose level cannot be established at a non-effect dose level when even one animal out of nine shows the same pathology at the lowest dose level of 3.125 ppm.

The establishment of a no-effect dose level based on the histopathological findings of liver effects when no other tissues were examined at ~~any~~ each of the other dose levels is inappropriate.

Validation: Invalid.

000963

Section 16 - Vol. II of VII

DU112307
Toxicity in Repeated Dietary
Administration to Beagle Dogs
(Repeated Administration for 13 weeks)

Report No. 169/74157

Test Compound: DU112307 (Batch # P7227)

Test Species: Pure-bred Beagle dogs

Number of Animals: 5 groups at 3 males and 3 females/group.

Route of Administration: Dietary

Doses and duration: 0, 10, 20, 40, 160 ppm for 13 weeks.

Testing Laboratory: Huntingdon Research Center
Hungtindon, England

Methodology: Thirty pure bred beagles, fifteen males and fifteen females were equally divided into 5 groups with 3 males and 3 females per group. The dose levels were 0, 10, 20, 40, 160 ppm for 13 weeks. Four hundred ~~grams~~ grams of dog food was offered each day to each dog. Amounts not eaten were recorded. A supplement of milk was included and water was "ad lib." Clinical signs, body weights, food and water consumption were periodically recorded. Ophthalmoscopic examination was conducted at the beginning of testing at 6 and 12 weeks into the study. Laboratory investigations included haematology, biochemistry, urinalysis and histopathology.

Results: The actual consumption of test compound in the 0, 10, 20, 40, 160 ppm dose levels was 0, 0.42 mg/kg/day, 0.84/mg/kg/day, 1.64 mg/kg/day and 6.24 mg/kg/dog/day body weight. During a test period of 13 weeks, there were no mortalities and no clinical signs were observed. Body weight gains appeared satisfactory with the exception of one control dog. This dog had a condition of hydrocephalus.

Opthalmoscopy revealed no abnormalities in the eyes of all dogs.

At two weeks into the study, haematology and biochemistry appear to be within normal limits.

At four weeks one dog at 160 ppm dose level had a decrease in RBC. Increased SAP values were noted for dog #315 (40 ppm) and 319 and 322 (160 ppm). Three dogs, 319 , 320 and 322 (100 ppm) showed elevated SGPT values. At six weeks the RBC counts were significantly lower at 160 ppm. Methaemoglobinemia was greater in the high test group than controls but only those two dose levels were tested for methemoglobinemia. The report states that abnormal haemoglobin pigments were observed at 160 ppm. It is unknown whether these abnormal blood pigments exist at the lower dose levels. These abnormal pigments are in addition to sulphaemoglobin. An increase in SAP values at 40 and 160 ppm and a dose related increase in SGPT starting at the lowest dose level of 10 ppm. At 12 weeks, haematological values appear to be within normal limits. Methemoglobineamia is present to an appreciable degree only in one dog at 160 ppm dose level. While plasma free haemoglobin seemed to increase in some groups, there were no dose related effects. At the end of the experiment, no histological abnormalties were seen which could be related to test compound administration.

Validation: Core-Guidelines

000963

Section 17 - Vol. III of VII

PH 60-40 Excretion Of Radioactivity And Metabolic Patterns In Rats Following Oral Administration

Report No. 56654/20/74

Test Compound: PH60-40

Testing Laboratory: B.V. Philips - Duphar

Purpose: To determine metabolic patterns following oral administration of PH60-40

Methodology: PH60-40 was labelled in various parts of the molecule with C^{14} and H^{3}. Urine, bile, feces and air were monitored analytically to detect their presence.

Results: The poor recoveries of the labelled moieties in these excretion fluids failed to indicate a metabolic pattern.

Comment: A more sensitive technique is required to assess excretion patterns in rats. Elucidation is needed concerning differences in excretion patterns between male and female rats or whether the results are artifacts.

Validation: Invalid - the experiment did not achieve its intended purpose.

000963

Section 16 - Vol. III of VII

Diflubenzuron (PH 60-40)
Balance Studies In the Rat

Report No. 56654/22/75

Test Compound: PH 60-40

Purpose: 1. To obtain consistent recoveries of labelled material.

2. To identify the metabolites.

Methodology: As in experiment Section 17 - # 56654/20/74

Results: It was established that about 50% of the dose of test material was absorbed from the intestines. About 75-80% of the urinary metabolites were established, as far as the benzoyl moity or the parent compound was concerned. About one-half of this amount was identified as 2,6 difluorobenzoic and not much could be stated about the 4-chlorophenylurea moiety.

Validation: Supplementary data.

Section 19 - Vol III of VII

Treatment of Chickens With [^{14}C] TH-6040

Treatment of Female Poland China-Duroc Cross Pig With [^{14}C] TH-6040

These experiments are within the competancy of the Chemistry Branch with the exception of the pathology reports on the chicken and pigs.

Results: Pathology report # 427186 on the chickens states that 4 mature chickens had been fed each a single dose of TH 6040 at 5 mg/kg. The histologic examination diagnosed fatty metamorphosis of the liver. The birds were necropsied 12 days after dosage.

Results: Pathology report # 387765 on Rhode Island Red X Barred Rock Cross chickens states that histologic examination showed "almost all the liver cell. nuclei were undergoing karyorrhexis". The diagnosis was "Karyorrhexis, liver cells, marked".

Note: Karyorrhexis constitutes the fragmentation of nuclei into chromatin particles which scatter in the cytoplasm.

Results: Pathology report # 387824 on a pig fed TH 6040 at 5 mg/kg in a single dose 10 days prior to necropsy-- diagnosis, "Diffuse changes of a mild fatty metamorphosis."

Section 20 - Vol III of VII

A Preliminary Report On The Metabolic Fate of Dimilin - ^{14}C

In A Lactating Cow

This review is within the competency and jurisdiction of the Chemistry Branch.

Comment: TB takes note however that radioactive carbon residues in tissues of a lactating cow 7 days after oral treatment with Dimilin ^{14}C at 10 mg/kg shows liver to have 2.9 ppm Dimilin equivalents.

Section 21 - Vol. 111 of V11

000963

TH-6040

MILK AND TISSUE RESIDUE STUDY IN DAIRY COWS

This review is within the competency and jurisdiction of the Chemistry Branch.

Section 22 - Vol. III of VII

Isolation, Purification and Identification of the TH 6040 and Its Metabolites From The Liver Of The Cow Exposed to ^{14}C TH 6040

This review is within the competency and jurisdiction of the Chemistry Branch. However, TB notes the following results which may impinge upon the evaluation of safety.

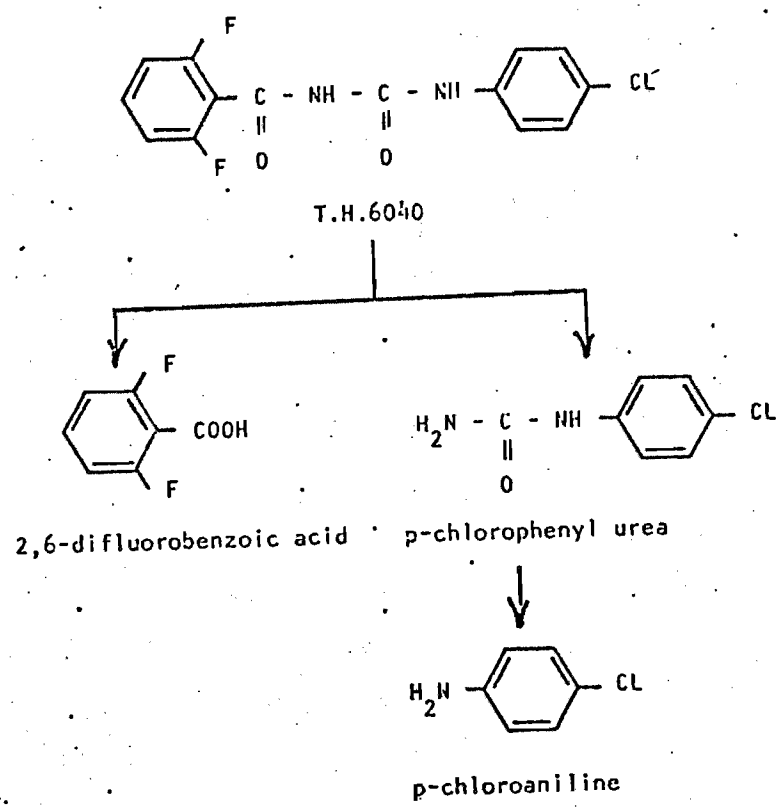
Results: The following figure 1 presents the schematic outline of the degradation of TH 6040 in bovine liver when the cow was fed TH 6040 at 250 ppm in the diet for seven consecutive days.

Figure 1: Proposed Metabolism Scheme for T.H. 6040 in Bovine Liver

Cannon Laboratories, Inc.

000963

FIGURE 1: PROPOSED METABOLISM SCHEME FOR T.H.6040 IN BOVINE LIVER



64

000963

Section 23 - Vol. III of VII

T.H. 6040 Egg And Tissue
Residue Study in Poultry

This review is within the competency and jurisdiction of the Chemistry Branch.

000963

Section 24 - Vol. III of VII

Isolation, Purification And Identification Of T.H. 6040 And Its Metabolites From Tissues And Eggs of Poultry Exposed To ^{14}C-T.H. 6040

This review is within the competency of the Chemistry Branch.

000963

Section 25 - Vol. III of VII

The Administration Of TH-6040 At A Dose Level of 300 PPM, To a Steer For 14 Consecutive Days

Lab. No. 5E-8153

Test Compound: Dimilin-W-25 (TH-6040)

Test Specie: One Steer

Route of Administration: Capsule

Duration of Study: 14 days

Dose: 300 ppm

Test Laboratory: Cannon Laboratories, Inc.
Reading, Pennsylvania

Sponsor: Thompson-Hayward Chemical Co.

Methodology: A steer (no weight given) was administered Dimilin W-25 (TH-6040) at 300 ppm for 14 consecutive days. The report states that the steer was dosed by capsule at 9:00 A.M. each day. After 14 consecutive doses, the animal was sacrificed and a detailed gross observation was made to the liver, kidney, brain, heart, lungs, stomach and GI tract. The report states that those organs were preserved in 10% buffered formalin and submitted for histopathological evaluation.

Results: There was a slight diminution in food and water consumption early in the experiment but soon returned to normal. Gross pathology examination showed all organs to be normal. The histopathology report states that the tissues examined were heart, kidney, bile duct and liver. Foci of chrome inflammation were found in heart and liver.

Comment: Clarification is needed:

(1) The report states that liver, kidney, brain, heart, lungs stomach, GI tract were submitted for histopathological examination yet, the tissues examined microscopically were only heart, kidney, bile duct and liver.

(2) The protocol states that the steer was dosed at 300 ppm yet the protocol also states that the test material was given in a capsule.

OP

(3) The protocol states that one steer was treated but results were on on two steers, one treated and one control.

(4) No data is available on food, water consumption on the control steer.

(5) Dimilin W-25 is equated to TH-6040. Is Dimilin W-25 a 25% wettable powder?

Validation: Invalid, unless clarification is forthcoming concerning questions raised under comments.

69

000963

Section 26 - Vol. III of VII

Effect of Repeated Applications Of DU 112307 To the Skin of Rabbits For Three Weeks

Report # PDR 146/73845

Test Material: DU112307 (Batch No. P7131)

Test Specie: New Zealand White Rabbits

Number of Animals: 60 rabbits, 30 males, 30 females

Route of Administration: Dermal

Dose: 1.5 ml/kg/day of DU112307-23% to group 3, group 4

1.5 ml/kg/day of DU112307-70% of group 5, group 6.

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: M.V. Philips-Duphar, Weesp, Holland

000963

Methodology: Sixty New Zealand rabbits, were randomly divided into 6 groups, 5 males and 5 females/group. All rabbits were acclimatized, weighed, earmarked. Rabbits were housed individually in metal cages with wire mesh floors, and had free access to water and standard rabbit diet medicated with 0.1% formosulphathiazole. Hair was clipped from trunks exposing an area about 5% of total body surface. Rabbits in groups 2, 4 and 6 were abraded while rabbits in groups 1, 3, 5 were non-abraded. Each rabbit was supplied with an "Elizabethan" collar to avoid oral ingestion of test materials. Test material was applied at 1.5 ml/kg five days per week for three weeks. Controls received vehicle, gum tragacanth 0.5% alone. Along with recording ~~gain~~ reactions, clinical signs, food consumption, body weight, hematology, blood chemistry, and ophthalmological data were recorded. Some organ weights were taken and tissues were preserved in buffered formaldehyde.

Results: Sporadic transient erythema was observed in all groups showing nothing remarkable that might be attributed to DU112307. DU112307 at 70% did show a more marked erythema (grade 2) but still a transient effect.

There were no signs of toxicity as might be observed clinically in any of the rabbits.

7130

Weight gains were depressed in rabbits treated with DU112307 at 70% especially in the females.

Hematologically, there was seen a tendency to higher reticulocyte counts in both sexes with both the 23% solution as well as the 70% solution.

Blood chemistry demonstrated very high methemoglobinemia values in all DU112307 treated groups but especially in the males. Control males showed 0.1 g % methemoglobinemia while abraded rabbits in the 23% and 70% groups showed 1.6 g % and 1.3 g % methemoglobinemia respectively. The report states that the blood from all groups had an "unusual dark colouration," probably due to methemoglobin and other unidentified blood pigments.

Eye examinations did not detect any abnormalties.

Macroscopic examinations did not reveal anything unusual or attributable to DU112307 treatment.

Organ weight analyses showed some variations but values remained within normal range.

Microscopic examination of tissues showed nothing remarkable except that "peribronchial lymphoid hyperplasia was seen in the majority of animals examined and was associated with perivascular and subplural aggregations of lymphocytes in many instances."

Conclusions: DU112307 produces methemoglobinemia in New Zealand rabbits when applied at 1.5 ml/kg at both 23% and 72% concentrations.

Comments: The report states that other blood pigments were present. These were not identified.

There is no data to show whether this methemoglobinemia is transitory or permanent in rabbits.

Histopathology was done only on groups 2, 5 and 6.

Peribronchial lymphoid hyperplasia would appear to be common to all the animals both in group 2 controls and in group 5 and 6 treated. Since white blood cell counts were well within normal range, it is questionable whether the lymphoid hyperplasia in the lungs can be reflective of a generalized inflammatory condition.

Q00963

Section 27 - Vol. III of VII

Effect of Repeated Applications Of DU112307 To the Skin of Rabbits For Three Weeks

Report # PDR 200/7485

Test Compound: DU112307 (Batch # 405093)

Test Animals: New Zealand Winter Rabbits

Number of Animals: 80 Rabbits, 40 males and 40 Females

Route of Administration: Dermal

Dose: 1.5 ml/kg/day of DU112307 in gum tragacanth. Solutions of DU112307 used were 4.64% , 10%, 21.5%

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: M. V. Philips-Duphar, Weesp, Holland

74

Methodology: Same as in Section 26 - Vol. III of VII.

Results: Sporadically, a case of mild erythema grade 1 was reported in some rabbits. These local skin irritations appeared transitory in most cases and are not related to treatment.

No signs of toxicity could be observed clinically.

Food consumption, body weight data varied sporadically and would not be related to treatment.

~~Hematology~~ other observations appeared within normal limits.

Opthalmoscopy showed no effects which could be related to toxicity.

Blood chemistry appeared sound with the exception of methemoglobinema and sulphemia globinemia. As the dose levels rise so do the levels of these parameters.

Macroscopic and microscopic pathology showed no signs of toxicity due to DU112307.

Conclusion: Methemoglobinema is induced by DU112307 dermally on the intact skin of male New Zealand rabbits down to the lowest rate

of application 1.5 ml/kg at 4.64%. Increased effects are observed in the males at the median and highest dose levels.

The females ~~can~~ show these effects beginning at 4.64% ~~to~~ concentration through to the median and highest dose level.

Comments: Clarification is needed:

The experimental procedure states that 60 New Zealand white rabbits were obtained for the experiment and 80 rabbits were reported in the experiment.

Validation: Core-Guidelines - Tentative

000963

Section 28 Vol. IV of VII

Subacute Inhalation Toxicity To The Rat Of DU 112307 W.O. 25% Insecticide Powder

PDR 148/73848
HRC Rysit 4920/72/355

Test Compound: DU 112307 (PH 60-40 w.p. 25%) # 304051

Test Specie: Albino Rat-Sprague Dawley CD1 (Grade IV)

Number of Rates: Twenty males and twenty females
Five males and five females per dose level
Five males and five females controls

Route of Administration: Inhalation

Dose and Duration of Exposure: Exposure periods 1 hour
Five days per week
Three weeks
Nominal concentrations 0.5, 5.0, and 50 mg/liter air

Testing Laboratory: Huntingdon Research Center, Huntindon, England

Sponsor: N.V. Philips-Duphar, Weesp, Holland

Petition: Thompson Hayward Chemical Company

Methodolgy: A wright dust generator and/or a Timbrell dust generator was used to aerosolize DU 112307 wp. 25% into chambers at a rate to offer nominal concentrations of 0.5, 5.0, 50 mg of test material per liter of air. The dust was generated into chambers for one hour per day, five days per week for three weeks. The chamber contained one group of ten rats, per test period, with wire mesh separating each rat. Control group was treated only with air. Actual concentrations of dust were calculated by gravimentric sampling. Mean gravimentric findings for the three dose levels were 0.61 mg/liter air, 0.351 mg/liter air and 3.29 mg/liter air for the nominal concentrations of 0.5, 5 and 50 mg/liter of air, respectively. Particles ~~scopes~~ sizes were in the respirable range. Clearance temperature was 24°c + 2°c and humdity 35% + 3%.

Results: No clinical signs were observed in animals exposed to 0.5 mg/liter test material in air while blinking, sneezing and laboured breathing were observed at the higher dose levels. Nasal secretions were noted at 50 mg/liter dose level but all animals appeared to be normal once removed from aerosolized dust.

Mean body weights were like controls. Food and water consumption appeared normal.

Urinary sediments showed RBC present at the third week from male rats in the three test groups, but not in controls. Haematological parameters varied in the three groups but variations did not reflect treatment related effects.

Macroscopic and microscopic pathology did not reveal anything unusual for this strain of rat.

Organ weights showed a dose related increase in spleen weights (except females at 0.5 mg/liter) for both sexes in the test groups with statistical significance for both sexes at the highest dose level and also for females at the median dose level. No deaths were reported throughout the study.

Comments: Two dust generating machines, the Wright and Timbrell dust generators were mentioned as the means of generating dust. No mention is made which one generator was used or whether both were used in this inhalation experiment.

Sections of tissues were taken for histopathology examination from groups 1 (controls) and group 4 (high dose level) but no statement was made as to exactly what tissues were taken routinely for study from each animal.

DU 112307 is 25% Dimilin. A statement is needed concerning the contents of the 75% remaining formulation.

Comments: With the increases observed in spleen weights in this experiment, it is deemed necessary that histopathology be employed to determine the cause of the increase weights. Not one animal spleen was microscopically examined and reported.
There is a dose related increase in SGPT in male rats also.

Conclusion: Test material must be completely identified in report. The effects on spleen weight as might be determined by histological examination need to be known before any conclusion can be reached in regards to the inhalation effects of DU 112307 w.p. on Sprague Dawley rats at the above stated concentrations and for this time schedual.

Validation: Supplementary

8d
HC

BD-1056
THR-964

000964

Section 29 - Vol. IV of VII

Subacute Inhalation Toxicity To The Rat Of
DU 112307 W.P. 25% Insecticide Powder

(Evaluation of Methemoglobinemia)

PDR 197/741013

Test Compound: DU 112307 Technical - Batched # 405093 and #309181

Test Specie: Albino Rat - Sprague Dawley CD1 (Grade IV)

Number of Rats: males and twenty females
Five males and five females per dose level
Five males and five females controls

Route of Administration: Inhalation

Dose and Duration of Exposure: Exposure periods 1 hour
Five days per week
Three weeks
Nominal concentrations 0.5, 5 and 50 mg/liter air

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N. V. Philps-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

Methodology: A Wright dust generator and/or a Timbrell dust generator was used to aerosolize DU 112307 w.p. 25% into chambers at a rate to offer nominal concentrations of 0.5, 5.0 and 50 mg of test material per liter of air. The dust was generated into chambers for one hour per day, five days per week for three weeks. The chamber contained one group of ten rats per exposure period, with wire mesh separating each rat. Control groups were treated only with air. Actual concentrations of dust were calculated by gravimetric sampling. Actual mean gravimetric finding for the three dose levels were 0.121 mg/liter air, 0.866 and 1.85 mg/liter respectively for the nominal concentrations of 0.5, 5 and 50 mg/liter. Particle sizes were within respirable range. Chamber temperatures were 24°C± 2°C and humidity 35%± 3%.

Results: This experiment designed to detect methemoglobinemia showed that there was an increase in methemoglobinemia in Sprague Dawley rats inhaling DU 112307 25% w.p. at the estimated concentrations of 0.121 mg/liter air, 0.866 mg/liter and 1.85 mg/liter for one hour each day, five days per week for three weeks. The results were statistically significant in the 0.121 mg/liter and 0.866 mg/liter range in males and in all three dose levels in females. No effects were noted in reticulocyte counts post exposure. All other parameters appeared within normal range.

Comments: No spleen weights were recorded in this experiment not-withstanding the increase in spleen weights in previous experiment using 25% wettable powder. No histology done on tissues.

Conclusion: There is no indication in this experiment on the pathogenesis of methemoglobinemia or whether methemoglobinemia is transitory or permanent, only that it is present under the above stated conditions.

Validation: Core-Guidelines

000964

Section 30 Vol. IV of VII

Effect of DU 112307 On Pregnancy of the Rat

PDR 192/74978

Test Compound: DU 112308 Batch # 309181

Test Specie: Pathogen Free Charles River Rats

Number of Rats: Eighty female rats - 20/dose level

Route of Administration: Intragastric Intubation

Dose: Group 1 - controls

Group 2 - 1 mg/kg/day

Group 3 - 2 mg/kg/day } in 0.5% gum tragacanth

Group 4 - 4 mg/kg/day

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N.V. Philips-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

Teratology, rat

Methodology: Specific pathogen free Charles River female rats 20/test level, 20 controls were mated and then treated with DU 112307 suspended in 0.5% gum tragacanth from the 6 to 15th day of pregnancy at 1 mg/kg, 2 mg/kg and 4 mg/kg respectively for groups 2, 3 and 4. All animals had tap water "ad lib" and Spratts Laboratory Diet #1.

Animals were examined for toxicity and weighed on 1, 3, 6, 10, 14 and 17 and 20. The animals were killed by CO_2 euthanasia. Ovaries and uterine contents were examired immediately to determine number corpora lutea, viable young, resportion sites, litter weight (to obtain mean pup weight) and foetal abnormalities. The method of Wilson was used to determine soft tissue abnormalities and skeletons were examined using alizarin stain.

Results: Female rats were not affected by the administration of test material at 1, 2, and 4 mg/kd p.o. when administered from day 6 to 15th. of gestation as reflected by signs of toxicity, body weight and pregnancy rate. Litter parameters were not affected as measured by litter size, foetal loss, and litter size. Embryonic and foetal development appeared within normal limits from the data made available and in comparison to gestation history of 1,960 control animals tested in this laboratory.

Comment: Pup weights were presented as mean pup weight per day. No individual pup weight data was presented. Test material needs to be completely identified in this report.

Y C

Validation: Core - guidelines - tentative.

000964

Section 31 Vol IV of VII

Effect of DU 112307 on Pregnancy Of The
New Zealand White Rabbit

PDR 193/74937

Test Compound: DU 112307 (Batch # 309181)

Test Specie: New Zealand White Rabbit

Number of Rabbits: Thirteen per group - total 52 Rabbits

Route of Administration: Intragastric intubation

Dose: Group 1 controls

Group 2 - 1 mg/kg/day
Group 3 - 2 mg/kg/day
Group 4 - 4 mg/kg/day

} in 0.5% gum tragacanth

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N. V. Philips-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

74

87

7

Methodology: DU 112307 was administered to mature pregnant New Zealand white rabbits at 3 dose levels, 1, 2, and 4 mg/kg intragastrically during days 6 to 18 of gestation. There were 13 animals per test group. Doses were injected with luteinizing hormone to assist ovulation. Rabbits were identified by ear tags. Test volume were always 1 ml/kg. Vehicle chosen was 0.5% gum tragacanth. Diet chosen was BOCM Coney (351) and tap water.

Toxic signs were recorded daily and weights were taken on days 1, 6, 10, 14, 21 and 28. On day 29, animals were sacrificed by cervical dislocation and dissected to ascertain number of young, uterine disposition, resorption sites, and corpora lutea. All young were examined for soft tissue and skeletal abnormalities.

Results: Test material DU 112307 administered to 3 groups of female rabbits at 13 per group dosed at 1, 2 and 4 mg/kg p. o. respectively from day 6 to 18 of gestation did not produce effects substantially different from the control group of 13 rabbits. The effects did not appear substantially different from laboratory standard values of 4,292 New Zealand white rabbits assayed at Huntingdon Research Center, Huntingdon, England.

Comment: There are no individual pup weights but only mean pup weight for each dam. Test material needs complete identification in this report.

Validation: Core-Guidelines - Tentative

000964

Section 32 Vol IV of VII

Tumorigenicity of DU 112307 To Mice

PDR/75685

Test Compound: DU 112307 Technical - Batch # P7227

Test Species: DELP Swiss Mice - Carworth - Europe

Number of Mice: 52 males and 52 females per group, five groups

Route of Administration: Dietary

Duration of Study: 80 Weeks

Dietary Levels: 0 (Controls)
4 ppm
8 ppm
16 ppm
50 ppm

Note: The report states that the dietary levels were chosen by the sponsor:

Testing Laboratory: Huntingdon Research Center
Huntingdon, Cambridgeshire, England

Sponsor: B. V. Philips-Duphar, Weesp, Holland

✓ 57 44

Methodology: Mice of known litter origin were randomly distributed in the five test groups. All animals were identified by earmarks. Randomization was done according to body weights. Mice were housed four to each polypropylene cage with sifted sawdust as litter. Room temperature was 21± 20 C. Lighting was 12 hours illumination, 12 hours dark. Cages were also randomly distributed, in regards to spatial distribution. Mice had tap water "ad lib" and powdered laboratory food (Spatt's Laboratory Animal Diet No. 2). DU 112307 was incorporated into the diet to supply the above indicated dose levels. All animals were examined daily for signs of ill health, toxicity and behavioral changes. Food, water consumption were recorded. At end of test, surviving mice were killed by CO_2 asphyxiation. Gross pathology was done on all animals. All abnormalities were recorded including appearance and size of gonads, adrenals, thyroids, intra-abdominal lymph nodes and accessory reproductive organs. Adhesions, invasion between presumptive neoplasia and adjacent structures were noted.

Microscopic examination was routinely performed on adrenals, thyroids, ovaries, liver, spleen, lymph nodes, and pituitary glands and all macroscropically observed lesions suggestive of neoplasia from every animal. Blood and bone marrow smears were also made. Lung and liver tumors were classified according to Walters, 1966 (Brit. J. Cancer 20, 148-160) and lymphoreticular tumors were classified according to Dunn, 1954, (J. Natn. Cancer Inst. 14,

1281-1432). Tissues preserved but not processed in this oncogni-city study were:

heart	pancreas	seminal vescicle
lungs	kidney	skin
thymus (where present)	urinary bladder	stomach glandular and non-glandular)
salivary gland	testis	brain (medullary, cerebellar, cortical)
trachea	tongue	skeletal muscle
oesophagus	mammary gland	bone
aorta	jejunum	gall bladder
eyes	mid-colon	
sciatic nerve	prostrate	

Statistics: The report mentions.

"Where the data suggested evidence of a response to treatment, the Student's "t" test was used to determine differences between groups.

The dose levels of DU 112307 in the diet were 0, 4, 8, 16 and 50 ppm. The actual intake of DU 112307, based upon food consumption data was 0 mg/kg day for controls, 0.34 mg/kg/day, 0.67 mg/kd/day, 1.39 mg/kg/day, and 4/30 mg/kg/day respectively for males and 0 mg/kg/day, 0.42 mg/kg/day, 0.80 mg/kg/day, 1.58 mg/kg/day, and 4/87 mg/kg/day respectively for females.

Results: No clinical signs of toxicity were reported that could be related to the administration of test material. Mortalities recorded during the experimental period did not appear to be dose related. No treatment related effects were noted for food consumption, water intake, body weight, or growth rate changes. Microscopic pathology did not reveal changes unusual for this strain and age of animal.

Tumors

The histopathology data which can be evaluated for carcinogenicity are in those seven tissues which were routinely examined microscopically from all animals. These seven tissues are lymph nodes, spleen, liver, thyroids, ovaries, adrenals, and pituitary glands.

Male Mice

Of the 260 male mice on study, one pituitary adenoma was found at the 4 ppm dose level. Two thyroid papillary cystadenoma were found, one in controls and one at the 8 ppm dose level. One adrenal phaeochromocytoma was found at the 4 ppm dose level. No tumors were reported in spleens.

It is necessary to distinguish between those animals which survived the whole test period and were therefore maximully exposed to test material from those animals which died during the test period, when the data suggest a difference in tumorigenic effects.

Liver tumors

The percent incidences of single liver tumors in male mice in the group that both died during the experimental period and those that were sacrificed at termination of the experiment were 16% (8/50) at the control level 0 ppm, 14% (7/49) at 4 ppm, 18% (9/51) at 8 ppm, 22% (10/45) at 16 ppm, and 22% (11/50) at 50 ppm.

The percent incidences of single liver tumors in male mice that survived the test period and were killed at termination of the experiment (maximum time exposure) were 16% (6/38) at 0 ppm, 15% (6/39) at 4 ppm, 16% (6/38) at 8 ppm, 26.7% (8/30) at 16 ppm and 30% (9/30) at 50 ppm.

The percent of multiple liver tumors in male mice, either from the group that died during the test period or from the group that survived the 80-week period showed no dose related increase.

Liver Nodular hyperplasia

The percent incidences of liver nodular hyperplasia in male mice that both died during the experiment together with those that survived the 80-week period were 12% (6/50) at 0 ppm, 20.4% (10/49) at 4 ppm, 21.6% (11/51) at 8 ppm, 20% (9/45) at 16 ppm, and 14% (7/50) at 50 ppm.

The percent incidences of liver nodular hyperplasia in male mice that survived the test period and were then sacrificed at termination (maximum exposure) were 13% (5/38) at 0 ppm, 23% (9/39) at 4 ppm, 23.7% (9/38) at 8 ppm.

Female Mice

Of the seven routinely examined tissues in the 260 female mice, two pituitary adenoma were found in the 4 ppm dose level, two were found at the 8 ppm level and two more were found at 16

✓

93

ppm. One adrenal phaeochromocytoma was found at 50 ppm. No pituitary adenoma or phaeochromocytoma were found in control female mice. No tumors were found in thyroids and as in female mice, no tumors were found in spleen.

Liver tumors

The percent incidences of liver tumors, both animals with single and multiple liver tumors, in female mice that both died during the experimental test period and those that were sacrificed at termination were 16% (8/49) at 0 ppm, 28% (14/50) at 4 ppm, 19% (9/48) at 8 ppm, 23% (10/43) at 16 ppm and 16% (8/50) at 50 ppm.

The percent incidences of single liver tumors in female mice that were sacrificed at the end of test (maximum time exposure) were 4% (1/23) at 0 ppm, 9% (3/34) at 4 ppm, 15% (5/33) at 8 ppm, 13% (3/24) at 16 ppm and 9% (3/33) at 50 ppm.

Lymphosarcomas

The percent incidences of lymphosarcomas in female mice that died during the test period were 15% (4/26) at 0 ppm, 25% (4/16) at 4 ppm, 26.67% (4/15) at 8 ppm, 26.32% (5/19) at 16 ppm and 29% (5/17) at 50 ppm.

The percent incidences of lymphosarcomas in female mice that lived the entire test period and were sacrificed at termination (maximum time exposure) were 0% at 0 ppm, 0% at 4 ppm, 24% (4/17) at 8 ppm, 31% (4/13) at 16 ppm and 27% (6/22) at 50 ppm.

The percent incidences of lymphosarcomas of both groups, those that died during test period together with those that were sacrificed at end of test were 8.16% (4/49) at 0 ppm, 8% (4/50) at 4 ppm, 17% (8/48) at 8 ppm, 21% (9/43) at 16 ppm, and 22% (11/50) at 50 ppm.

Tumors of the lymphoreticular tissue

The percent incidences (which include lymphosarcomas, myeloid leukaemia, retriculum cell sarcoma) in all female mice that survived the test period together with those that died during test were 14% (7/49) at 0 ppm, 22% (11/50) at 4 ppm, 21% (10/48) at 8 ppm, 35% (15/43) at 16 ppm and 26% (13/50) at 50 ppm.

It is questionable whether there can be validity in histopathology data in this oncogenicity study in those instances where tissues (other than those seven routinely examined from all animals) were selected for microscopic examination only on the basis of lesions visually observable throughout the control and treated groups. However, were it to be assumed that all tumors were visibly detectable, the following data become evident.

In the testes, one interstitial cell adenoma was found at 4 ppm, 4 were found at 8 ppm, two more were found at 16 ppm and two others were found at 50 ppm. One fibrosarcoma was found in the epididymis at 4 ppm. There were no tumors found in the testes of control mice.

Lung tumors

The percent incidences of lung tumors (any grade) in the group of male mice that survived the whole test period and killed at test end were 29% (11/38) at 0 ppm, 41% (16/39) at 47%, (18/38) at 8 ppm, 47% (14/30) at 16 ppm and 23% (7/30) at 50 ppm.

The incidences of lung tumors (any grade) in the male mice that both survived the test period and those that died during test were 28% (14/50) at 0 ppm, 35% (17/49) at 4 ppm, 41% (12/51) at 8 ppm, 38% (17/45) at 16 ppm and 18% (9/50) at 50 ppm. There would appear to be no increase in lung tumors in female mice with increasing dose levels of test material.

Kidney adenomas in males were found, two at the 4 ppm dose level and one at the 16 ppm dose level. There were no kidney adenomas in the control male animals.

No kidney tumors were reported in female mice.

The remainder of the tumors are single tumors of various types in controls and in the treated groups, not unusual in this animal specie.

All Tumors - Female Mice

In the group of female mice that died during the test period, the tumor incidences (all tumors) were 42% (11/26) at the 0 ppm dose level, 56% (9/16) at the 4 ppm dose level, 60% (9/15) at the 8 ppm dose level, 68% (13/19) at the 16 ppm and 65% (11/17) at the

50 ppm. There would appear to be a trend toward a % increase in tumors as the dose level increases with the exception at the highest dose level which seems to plateau.

All Tumors - Male Mice

The groups of male mice showing an increasing trend of % tumors with dose levels are those that survived the test period and were sacrificed at test end. The percent incidences in tumors were 63% (24/38) at 0 ppm, 74% (29/39) at 4 ppm, 79% (30/38) at 8 ppm, 80% (24/30) at 16 ppm and 60% (18/30) at 50 ppm. Again there appears to be a trend towards % increases in tumors with dose level with the exception at the highest dose level.

Summary

Male Mice

There is an overall increasing trend in single liver tumors in male mice at the two highest dose levels either when examining those mice that survived the whole test period or those that survived together with those that died during test period.

In examining liver nodular hyperplasia in male mice, there is a general increase in incidences in the first three dose levels whether examining the group that survived the 80 weeks or the group composed of those that survived together with those that suc-

cumbed during test. At the highest dose level of test mat
incidences of nodular hyperplasia appear to return to contr
values.

Female Mice

Single and multiple liver tumors in female mice both those
that succumbed during test together with those that survived
showed increases at 4 ppm and 16 ppm with the 8 ppm and 50 ppm
values close to control values.

Single liver tumor incidences of females that survived the
test period generally appear to rise at 4, 8, and 16 ppm. In-
cidences decline at 50 ppm but still remain about twice control
values.

Lymphosarcomas generally rise in all groups of female mice.
Tumors of the lymphoreticular tissue rose with increasing
dose levels appreciably except for the 50 ppm dose level. At
50 ppm, however, the percent incidence was still about twice con-
trol values.

There is no basis for the evaluation of tumor incidences in
those tissues that were not routinely examined from all animals.
With the exception of the seven tissues mentioned above, histo-
pathology was performed only when visual inspection decreed the
existence of a lesion. However, if there is reason to believe that

all lesions that existed where lesions visually observable then there can be seen a trend towards increases in tumors (all types) in female mice with increasing dose levels with again the exception at 50 ppm where the tumor incidence tends to plateau but still remain well above control value. Again there is a continuing increase in tumors in male mice with increasing dose levels with the exception of the 50 ppm dose level. At 50 ppm, the tumor incidences decline to control values.

Lung tumors (any grade) again showed increasing incidences with increasing dose levels for male mice that survived the test period with the exception of those animals at the 50 ppm dose level.

Incidences of lung tumors for the group of male mice that survived together with those that succumbed the test period again showed increased values over controls with the exception at the highest dose level.

Comments

No statement was made that the diets were chemically analyzed for the actual concentration of DU 112307 during the 80-week period.

The report states: "At weekly intervals the intake of the test compound (mg/kg/day) was calculated from the food consumption and body weight data." The intake of test material needs to be

calculated from the food consumption and the analytical concentrations of test material in the food to arrive at the actual dosage levels of DU 112307.

The tissues routinely examined were lymph nodes (cervical and mesenteric), liver, spleen, ovary, adrenals and pituitary along with other tissues which on macroscopic examination showed evidence of lesions.

In view of the uniquenss of DU 112307 both from the structural viewpoint as well as to its mechanism of action on chitin, it is recommended that as many tissues as possible be examined histologically. To the 25 tissues listed in the report as preserved (and not examined), I would add tendon, ligaments, synovial membranes, cartilages (articular), xiphoid process, larynx, bursa mucoas, vertebral discs, and eyes, for additional microscopic study.

Clarification is needed why only seven tissues were examined in histopathology routinely from all animals and about 25 tissues were excluded from examination in a study designed to detect oncogenicity.

It was stated that blood and bone marrow smears were taken but no data has been made available.

Clarification is needed in the basis for the choice of seven tissues chosen for histopathology examination.

Clarification is needed why ovaries were chosen to be examined histologically routinely but not testis.

A compound which produces methemoglobinemia and sulfhemoglomia, which when administered to mice produces no clinical effects or splenic tissue effects raises more questions than the experiment is designed to answer. The question arises, as to whether this study was conducted at the maximum tolerated dose level. It would appear that the diet levels of test material are too low to warrant a definitive assessment of oncogenicity.

Validation: The data in the routinely, microscopically examined tissues is considered supplementary.

Recommendations: It is recommended that this study be repeated for two primary reasons.

(1) In an oncogenicity study, it is expected that the number of tissues examined histologically from each animal reflect the tumorigenicity potential of test material. In this experiment, the routine examination of seven tissues does not accomplish the intended purpose of the experiment. The microscopic examination of tissues as a basis for screening is not satisfactory.

(2) The dose levels used in this study were too low to establish definitively oncogenicity potential of DU 112307. It is already remarkable that tumors in number were observed over control

values at a fraction of a mg/kg/day dose levels. It is expected that statistically significiant values may be established for oncogenicity at higher dose levels, if the inferences in this study are real and not apparent. It is therefore recommended that this study be repeated at higher dose levels.

Validation: For the above stated reasons, this study is considered supplementary.

STATISTICS

Summary

The following are tables derived from the 80-week oncogenicity test. The statisitcal methods employed are FISCHER'S EXACT METHOD and the CHI^2 test. These tests were employed considering the non-parametric nature of the data.

Statistical differences can be found in mice with lymphosarcomas at levels equal to or above 95% confidence limits in mice that survived the 80-week period and were sacrificed (maximum exposure) or in the group composed of both those mice that succumbed the test period together with those that survived. The mice with lymphosarcomas that died during test period, while not exceeding 76% confidence limits, nevertheless showed increasing trends for lymphosarcomas with dose.

Lymphoreticular tumors in female mice statistically were different at 16 ppm dose level to confidence limits of 98%.

Lung tumors in male mice rose to 92% confidence limits at the 8 ppm dose level.

All tumors (male mice) were statistically, significantly different at 8 and 16 ppm dose levels at about the 90% confidence limit while females (all tumors) were statistically significant from controls at about the 92% confidence limit at the 16 ppm dose level.

000964

SECTION 33 - VOLUME V OF VII

EFFECTS OF DU 112307 IN DIETARY ADMINISTRATION

TO RATS FOR 104 WEEKS

TEST COMPOUND: DU 112307 - Batch # P7227

TEST SPECIES: SPRAGUE-DAWLEY RATS, CD STRAIN

NUMBER OF RATS: Forty-Five Males and Forty-Five Females Per Test Group In the Main Study for Tumorgenicity Study. Fifteen Males and Fifteen Females Per Group--Satellite Study For Toxicity Study.

ROUTE OF ADMINISTRATION: Dietary

DURATION OF STUDY: 104 Weeks

DIETARY LEVELS: 0 (CONTROLS)
10 ppm
20 ppm
40 ppm
160 ppm

TEST LABORATORY: Huntingdon Research Center,
Huntingdon, Cambridgeshire, England

SPONSOR: B. V. Philips-Duphar, Weesp, Holland

PETITIONER: Thompson Hayward Chemical Company

114

At termination, all surviving rats were killed by CO_2 asphyxiation.

A "carcinogenicity screen" was performed by examining adrenals, thyroids, ovaries, liver, spleen, lymh nodes and pituitary glands plus all tissues which upon macroscopic examination show signs of lesions. Abnormalities found in blood smears were confirmed by examining bone marrow.

A toxicity screen was performed in examining microscopically these seven organs as listed above including kidney and all tissues which macroscopically showed lesions. The mean intake of DU 112307 was calculated from body weight and food consumption data. The dose levels were 10 ppm (Group 2), 20 ppm (Group 3), 40 ppm (Group 4) and 160 ppm (Group 5). The calculated intake for males was 0.35 mg/kg/day (Group 2), 0.70 mg/kg/day (Group 3), 1.43 mg/kg/day (Group 4 and 5.83 mg/kg/day (Group 5) respectively while for females the calculated intake was 0.43 mg/kg/day (Group 2), 0.88 mg/kg/day (Group 3), 1.73 mg/kg/day (Group 4) and 7.05 mg/kg/day (Group 5).

Results: The report states clinically there were no overt signs of reaction treatment. Mortalities and autopsy data appeared to have no relationship to treatment. Food intake changes or body weight gain and food efficiency could not be related to test material ingestion. No evidence was available that water intake was affected by DU 112307. Glucose in urine was found to be high in four control animals at 103 weeks but in only one animal at 160 ppm does level.

Methodology: Six hundred Sprague-Dawley-CD rats were used in this study. Four hundred and fifty were used in the oncogenicity test and the balance were used in a satellite study to determine toxicity. Five groups of animals were composed of 45 rats per sex per dose in the oncogenicity test and 15 rats per sex per dose were used in the toxicity test.

The dose levels were 0, 10 ppm, 20 ppm, 40 ppm, and 160 ppm. Randomization of rats took into consideration litter origin and body weight. Animals were identified by earmark. Rats were housed five to a suspended cage with wire mesh floors. Room temperature and relative humidity were 21*20° C and 50*5% respectively. Lighting was 12 hours light and 12 hours dark. Water was "ad lib." Food was Spratt's Laboratory Diet 2.

Test material was administered in the diet. Dietary administration continued for 104 weeks.

All rats were examined daily for ill-health, signs of toxicity and behavioral changes. Skin lesions, cataracts and palpable growths were recorded. All rats were examined macroscopically to determine cause of death. Body weight and food consumption were recorded weekly. All eyes were examined at 0, 13, 26, 52, 78, and 104 weeks from groups 1 (controls) and from group 5 (160 ppm). Urines were examined as frequently. Blood was also examined at these time intervals from selected number of animals.

which predominated in both studies were pituitary adenomas and mammary fibro-adenomas well distributed throughout the four test groups and controls. A scattering of tumors, one of a type throughout the remaining tissues were observed.

Comment: A carcinogenicity study is designed to assess oncogenic potential of test material. A "carcinogenicity screen" performed only on the microscopic examination of seven tissues from each animal plus tissues decreed by visual inspection to have lesions does not satisfy the experimental design or intent.

In the toxicity satellite experiment composed of only 15 rats per sex per dose administered DU 112307 at 0, 10, 20, 40, 160 ppm, histology in seven tissues were performed on 11 rats in control, 12 rats at 10 ppm, 13 rats at 20 ppm, 10 rats at 40 ppm and 13 rats at 160 ppm while macroscopic examinations only were done on the remaining animals. No rationale could be found either in a statement of experimental design or in the inspection of the number and kinds of tissues examined, that a basis exists for the choice and number of tissues subjected to microscopic examination. Tissue chosen for microscopic examination on the basis of visibly observable lesions does not permit an analysis of lesions as to severity or frequency of lesions in any of the treated or control group nor between any treated groups and control group.

Methaemoglobinemia began to increase at 13 weeks in both sexes as observed between group 1 (controls) and group 5 (160 ppm). At 26 weeks, methaemoglobinemia is checked not in group 1 and 5 but between group 1 and 4. Notwithstanding, there is again observed an increase over control values. At 52 weeks and 78 weeks, the increase is still obvious between controls and the 160 ppm dose levels. At 102 weeks, treated males at 160 ppm begin to approach control values while females continue to show an increased methaemoglobinemia.

Other blood parameters seem to be within normal limits considering this specie of animal and age.

Organ weight changes did not show consistent variations that could be attributed to the ingestion of test material.

Oncogenicity-female rats: The incidences of tumors observed in the females rats ranged from 90% to 95% in the four treated groups, on the basis of the ratio of number of rats with tumors to the number of rats examined. However, the meaning of so high a percentage of tumorigenicity is obfuscated by the 98% incidence of tumors in the control female rats. No conclusion, therefore, can be drawn as to the oncogenicity of DU 112307 in female rats when fed DU 112307 at 0, 10, 20, 40 and 160 ppm in the diet for two years.

Generally the same tumor incidences were observed in the 150 rats used to study toxicity in the satellite study. The tumors

which predominated in both studies were pituitary adenomas and mammary fibro-adenomas well distributed throughout the four test groups and controls. A scattering of tumors, one of a type throughout the remaining tissues were observed.

Comment: A carcinogenicity study is designed to assess oncogenic potential of test material. A "carcinogenicity screen" performed only on the microscopic examination of seven tissues from each animal plus tissues decreed by visual inspection to have lesions does not satisfy the experimental design or intent.

In the toxicity satellite experiment composed of only 15 rats per sex per dose administered DU 112307 at 0, 10, 20, 40, 160 ppm, histology in seven tissues were performed on 11 rats in control, 12 rats at 10 ppm, 13 rats at 20 ppm, 10 rats at 40 ppm and 13 rats at 160 ppm while macroscopic examinations only were done on the remaining animals. No rationale could be found either in a statement of experimental design or in the inspection of the number and kinds of tissues examined, that a basis exists for the choice and number of tissues subjected to microscopic examination. Tissue chosen for microscopic examination on the basis of visibly observable lesions does not permit an analysis of lesions as to severity or frequency of lesions in any of the treated or control group nor between any treated groups and control group.

✓

11/29

The incidence of tumors both in female control animals and in the treated female animals at all dose levels are so high (about 90 percent) as to obfuscate the presence of oncogenicity which might be attributable to DU 112307 both in the main oncogenicity study as well as in the satellite toxicity study.

Conclusion: Due to the deficiencies enumerated under comments, no assessment of oncogenicity or long term toxicity can be made from the data submitted in this study.

Validation: This study is invalid unless clarification is forthcoming regarding comments.

000964

Section 34 - Vol. VII of VII

Effect of DU 112307 On Reproductive Function of Multiple Generations In the Rat

Report # PDR 173/7594

Test Compound: DU112307 (Batches P7227, 309181)

Test Animals: Charles River Specific Pathogen Free CD Strain

Number of Animals: Two hundred rats, one hundred males and 100 females

Route of Administration: Dietary

Doses: 0, 10, 20, 40 and 160 ppm, 40 animals per dose, 20 males and 20 females.

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N. V. Philips-Duphar, Weesp, Holland

Methodology: Animals in the FO generation were placed on their respective treatment diets 60 days before mating. The animals were mated to produce a FIA generation. A second mating was permitted producing an F1B generation. Animals from the F1B were used as parents to produce an F2A generation. These were now used to produce the F3A generation.

Results: The animals in the FO generation initially showed a low mating performance hence the F1A litters were sacrificed. The FO generation was again matted to form the F1B group. In the 2d mating of the FO generation there was an occurrence of more than 50% of total litter losses. Of the 17 litters lost in the 3 generations, 10 litters were lost at the 2nd mating of the FO parents. There were no consistent dosage-related trends in regards to food consumption, or body weights when assessed over the three generations. Weight ranges of females during pregnancies and lactation showed no consistent dosage related trends. All other test parameters were not remarkable in respect to control values.

Comments: The study states "the occurrence of more than 50% of the total litter losses at the second mating of the FO generation was considered to be associated with the consequences of the sialodacyroadenitis occurring at the first mating of the FO generation..

The assumption that an inflammatory process of the lacrymal and salivary glands was responsible for 50% litter loss is non-acceptable. The macroscopic examination of these tissues does not verify the existence of an inflammatory process of these tissues.

Validation: Core-Guidelines

000964

Section 35 Vol. VII of VII

Mutagenic Study With TH 6040 In Albino Mice

IBT No. 622-05068
P.O. No. 9973

Test Compound: TH 6040

Test Specie: Charles River strain albino mice

Number of Mice: Thirty-six males, twelve per group

Route of Administration: Single intraperitoneal injection

Doses: 0 (Controls), 1,000 mg/kg (Group T-I). 2000mg/kg (Group T-II)

Testing Laboratory: Industrial Bio-Test Laboratories

Petitioner: Thompson Hayward Chemical Company

Methodology: One group of 12 male mice were treated with a single intraperitoneal injection of TH 6040 at 1000 mg/kg and another group of 12 male mice were treated at 2000 mg/kg test material. Controls were given corn oil. Sequential mating for each male with 3 untreated females per week continued for six weeks. Mutagenic effects in this dominent lethal study were to be detected by examining implantation sites, resorption sites and embryos. Females were sacrified at about one week after mating or about half way through gestation.

Results and Comments: While the report states that two dose levels were employed, TABLE 11 shows seven dose levels were examined. These dose levels were 4,000 mg/kg, 3000 mg/kg, 2,000 mg/kg, 1000 mg/kg, 300 mg/kg, 100 mg/kg and 30 mg/kg. It is reported that dose levels of 4,000 mg/kg, 3,000 mg/kg and 2,000 mg/kg produced excessive grooming, hypoactivity, and ruffled fur. These signs diminished with decreasing dose levels down to 2,000 mg/kg level. This report does not report data at the three lower dose levels. Hypoactivity at 4,000 mg/kg was deemed responsible for the lack of mating activity. Hypoactivity began 15 minutes after injection and lasted for about 3 days.

Conclusion: Treating male mice with 1,000 mg/kg i.p. (12 males) or 2,000 mg/kg (12 males) with TH6040 did not result in an increase ~~in an increase~~ in dominent lethal mutations following sequential mating of each male with three untreated females per week for six consecutive weeks.

Validation: IBT Study. Results need to be validated by the sponsor.

BIOLOGICAL EFFECTS OF DIMILIN ON VERTEBRATES AND OTHER SELECTED ORGANISMS

USDA

000992

Table of Contents

Biological Effects of Dimilin On Vertebrates And Other Selected Organisms

Section 11

Glycosaminoglycan Biosynthesis

Effect of Dimilin (TH 6040) On The Hyaluronic Acid Concentration In Chicken Combs

Test Compound: TH 6040

Test Specie: Chickens, broilers and layers

Dose: TH 6040 250 ppm in diet for 98 days

Route of Administration: In feed

Number of Animals: No mention

Test Laboratory: Veterinary Toxicology and Entomology Research Laboratory College Stattion, Texas

Methodology: Two groups of chickens, broilers and layers were treated with Dimilin for 98 days. Four groups of broilers and four groups of layers were fed Dimilin respectively at 0, 2.5, 25 and 250 ppm. At 21, 28, 42, 56, and 98 days, the combs were excised, weighted, wrapped in foil and frozen until hyaluronic acid analysis was performed. The analytical procedure was essentially that of Bergmeyer's Method of Enzymatic Analysis, Verlage Chemie Weinheim page 1157-1164.

Results: Hyaluronic acid content of the combs increased in time due to maturation and increase in size. Dimilin (TH 6040) fed in the diet of chicken for 98 days up to a level of 250 ppm in the diet had no effect upon hyaluronic acid synthesis or deposition in the comb.

Validation: Core-Guidelines

Biological Effects Of Dimilin On Vertebrates And Other Selected Organisms

June 8, 1977

Section 11

Effect of Dimilin On Glycosaminoglycan Biosynthesis in Rat Glial Cells

Contract No. 12-14-5001-269 May 10, 1977

Purpose: These experiments have been devised to determine the effects of Dimilin on rat RGC-6 cell cultures in regards to cell morphology, cell adhesiveness, rates of cell division and cell viability, and on the effects of Dimilin on biosynthesis of glycosaminoglycans including hyaluronic acid.

Test Laboratories: Biochemical Division
Eunice Kennedy Shriver Center for Mental Retardation, Inc. and Department of Neurology
Massachusetts General Hospital

Results: The report states: "Dimilin neither inhibited the rate of cell division nor did it induce discernable changes in cell morphology. Comparative biosynthesis studies showed that Dimilin neither inhibited the net production of glycosaminoglycans nor altered the pattern of incorporation of [^{3}H] acetate and [^{35}S] sulfate into individual glycosaminoglycans. Synthesis of other classes of biopolymers was quantitated

by measuring the incorporation of [^{3}H] leucine into protein, [^{3}H] fucose into glycoproteins and [^{3}H] glucosamine into glycoproteins and glycolipids in the presence and absence of the test material." The report states, "Dimilin had no effect."

Validation: Core-Guidelines

13~

Biological Effects of Dimilin on Vertebrates
and Other Selected Organisms

Section 11

The Effect of Dimilin On Glycosaminoglycan Biosynthesis In Mouse Tissue

USDA Contract 12-14-5001-268

Purpose: This experiment is designed to determine the effect of Dimilin on glycosaminoglycan biosynthesis in mouse tissue through the inhibition of labelled glucose into either hyaluronic acid or chondroitin sulfate (glycosaminoglycans).

Test material: TH6040 technical grade 99% pure/DMSO varying concentrations

Doses: 50 ppm, 200 ppm, 400ppm, 1000ppm 2000ppm.

Route: Dietary administration to mice.

Testing Lab: Dept. of Biochemistry
University of Oregon Medical School
Portland, Oregon

Method: Six groups of fifteen mice per group were fed 0, 50 ppm, 200 ppm, 400 ppm, 1000 ppm and 2000 ppm of test material respectively, for 30 days. Mice were frequently weighed and observed for signs of toxicity, After 30 days, they were injected i,p. with 15μCi of ^{14}C glucose and killed eight hours later. Skins were removed, weighed and frozen. Hyaluronic acid and chondroitin sulfate were isolated.

Results: The animals eating 1000 and 2000ppm showed signs of cynosis. Blood was examined spectrophotometrically. Methemoglobin and sulfhemoglobin were detected. Sulfhemoglobin increased with increasing doses of TH 6040.

Sulfhemoglobin reached levels of 13% of total hemoglobin. After three weeks after the experiment with animals on normal diet, the sulfhemoglobin disappeared.

Results: Chondroitin Sulfate Biosynthesis

The experiment tended to show that there was an increased amount of chondroitin sulfate present in the skin (μg uronic acid/g tissue) at all dose levels over controls.

Hyaluronate Synthesis

The report states, "Treatment eith TH 6040 at 400 ppm and 1000 ppm appears to stimulate the biosynthesis of hyaluronate. There is, however, a noticeable decrease at the highest dose level of 2000 ppm".

Comment: The report does not hesitate to mention that the increases in chondroitin sulfate and of hyaluronate may be relative due to a decrease in some other componenet of skin. This possibility was beyond the scope of the experiment.

Validation: Core-Guidelines

Biological Effects Of Dimilin On Vertebrates And Other

Selected Organisms

U.S. Department of Agriculture

Western Regional Research Center

Berkeley, California

and

Stanford Research Institute

Menlo Park, California

III

Mutagenicity

Test Compound: 1. Technical Dimilin 99% pure (Lot # PP 312)

2. 25% wettable powder
Dimilin W-25
TH 6040,Lot # PP278

(a) Ames Salmonella/Mammalian Microsome Mutagenicity Test

(b) Micronucleus test in Mice

(c) Forward Mutation in Cultured Mammalian Cells

A. Tests have been completed on the Ames Salmonella Mutagenicity Test using 10, 100, 1000 ug/plate of technical material and levels of 19, 186, 1860 ug/plate of TH6040 25% wettable powder in strains TA 98, TA 100, TA 1537 and TA 1535. Tests were done with and without an in vitro rat liver metabolizing system.

Results: There is no indication, according to the report, of a mutagenic effect nor of growth inhibition of the tester strain. Diflubenzuron appears to be non-mutagenic in the Ames test.

Comment: Since the solubility of test material is poor, the question is raised whether the test actually reflects mutagenic potential, test substance.

125

B. The In Vivo Micronucleus Test in mice used oral doses of 15, 150 and 1500 mg/kg of technical Diflubenzuron in Swiss-Webster mice.

Results: Technical Diflubenzuron did not appear mutagenic nor alter the ratio of mature/polychromatic erythrocytes in bone marrow.

C. In the Forward Mutation in Cultured Mammalian Cells involving the gene controlling thymidine kinase in cultured L 5178Y mouse lymphoma cells, no evidence of a mutagenic effect could be found at concentrations up to and exceeding the limits of solubility of Diflubenzuron either with or without an in vivo mouse liver metabolizing system.

Comment: What is the limit of solubility of the test material in this system?

Validation: Core-Guidelines

136

000992

Biological Effects of Dimilin on Vertebrates and Other Selected Organisms

Agricultural Research Service
U.S. Department of Agriculture

Section IV

Testosterone and Organ Weights (Chickens)

Test Compound: Diflubenzuron (Dimilin)
Technical 99.0%, Lot # PP305

Test Specie: Leghorn Chickens
Hubbard Chickens

Number of Animals: 992 Chickens- all males

Doses: 2.5,25, and 250 ppm Dimilin in basal diets.

Route of Administration: Dietary

Test Laboratories: Beltsville Agricultural Station
Beltsville, Maryland

Methodology: Three levels of Dimilin and one control group were used in this experiment. The three doses of Dimilin were 2.5, 25, and 250 ppm fed in the diets. One experiment used basal diet called the Beltsville Diet, the other experiment used College Stattion diet. Each kind of diet was administered to two kinds of chickens, a broiler strain (Hubbard) and a Leghorn strain. Day-old make chicks were placed on either of the two diets at the above stated dose levels. Food consumption, bodyweights, testosterone levels were monitered periodically. After sacrifice, heart, kidney, spleen, bursa of Fabricus, liver, testes and comb weights were taken.

137

Results: The Leghorn chickens both on the Beltsville diet and on the College Station diet showed a dose related decrease in testicular weights at 8 weeks. The male Hubbard chicks showed statistically significant decreased testicular weights on the Beltsville diet at 14 weeks on the 2.5 and 250 ppm dose level. The Hubbards showed increased testicular weight statistically significant at 14 weeks at the 250 ppm dose level. The Leghorn chicks showed decreased comb weight statistically significant at 14 weeks in all treated groups on the Beltsville diet and the Hubbard chicks showed decreased comb weights at 14 weeks on the Beltsville diet.

Spleen weights show a dose related decrease in Leghorns on the Beltsville diet becoming statistically significant at 25 and 250 ppm while the Leghorns on the College Stattion diet show a decrease statistically significant from controls at 25 and 250 ppm at 8 weeks.

Testosterone levels were markedly decreased with dose levels in all treated Leghorns already at 4 weeks and again at 8 weeks - for chickens on the Beltsville diet. The Leghorns showed decreased testosterone levels at 25 ppm at 14 weeks on the College Stattion diet.

Comment: The report in part states, "Growing birds fed Dimilin had a general depression in the mean concentration of serum testosterone at all treatment levels in both strains of birds until the 56th day of life." Also, the report states, "Data from this and other experiments indicated that Dimilin fed at 2.5, 25 and 250 ppm has a depressing effect of serum testosterone levels in the developing rooster until the 56th day of life".

Validation: Core-Guidelines

000992

Agricultural Research Service

U. S. Department of Agriculture

Biological Effects of Dimilin On Vertebrates and Other Selected Organisms

June 8, 1977

Section V

DNA Synthesis and Reproductive Organs In The Boll Weevil

This study is within the jurisdiction and competancy of EPA entomolcgists. Note: The report states that the decrease noted in DNA synthesis in treated males and females may not be due to a direct effect of Dimilin upon DNA.

000992

FOLLOWING

Additional 24 studies submitted in June 1977 in support of the

Dimilin petition by Philips Duphar, Weesp, The Netherlands.

IVD
14

000992

Section 1 - Vol 1 of V

Addendum Report To The Chronic Studies With DU 112307

A. Dietary Administration To Rats For 104 Weeks

B. Dietary Administration To Mice For 80 Weeks

Athor: O.R. Offringa

The addendum information does not change the conclusions arrived at in the previous submission.

000992

Section 2 - Vol I of V

Additional Data Applicable to
PP #OS 6F1773, 6F1832, and 7F1898

R#962

June 10, 1977

Report #174/74198

Preliminary Assessment of the Toxicity to Male Mice
in Dietary Administration for Six Weeks

Test Compound:	DU112307
	(Batch P7227)
Test Species:	Mice - CFPL Strain
Number of Animals:	3 groups of 8 males
Route of Administration:	Dietary
Doses:	0, 16 ppm, 50 ppm
Testing Laboratory:	Huntingdon Research Center Huntingdon, England

Methodology: Twenty-four male mice were divided into three groups, receiving 0, 16 and 50 ppm DU112307 respectively in the diets for six

weeks. Signs of toxicity, food consumption, body weight, organ weights (5 organs) were monitored. Thirty-five tissues were preserved in formalin but only one tissue, the liver was processed for microscopic examination. The consumption of DU112307 corresponds to 2.0 mg/Kg/day for the group on the 16 ppm diet and 6.1 mg/Kg/day for the group on the 50 ppm diet.

Results: No signs of toxicity were observed clinically. There were no mortalities. Food consumption and body weight changes reflected no ill effects. Blood chemistry was normal insofar as serum glutamic-pyruyic transaminase levels were concerned in all mice. Macroscopically, no changes were observed related to treatment. Spleen weights decreased with increasing dose levels. Necrotic hepatocytes were seen in the liver of three of the eight mice of the 50 ppm level.

Conclusion: It is not possible to evaluate the toxic effects of DU112307 at 16 or 50 ppm in the diet of mice when only one organ (liver) has been examined microscopically.

Validation: Invalid.

000992

Section 3 - Vol I of V

Additional Data Applicable to

PP NOS 6F1773, 6F1832 and 7F1898

R#962

June 10, 1977

Diflubenzuron: Instestinal Absorption In The Rat In Relation to Dosage Level

Report No 56654/10/77

Test Compound:	^{14}C Diflubenzuron
Test Species:	Wistar Rats, males and females
Route of Administration:	Gavage
Doses:	First study: 4, 16 and 128 mg/kg.
	Second study: up to 1g/kg
Testing Laboratory:	Duphor B.V., Weesp The Netherlands

Methodology: Rats were intubated with 14C diflubenzuron to determine rate of exerction and therefore, rate of absorption of test material. In one experiment, intact rats were used.

and in a second experiment, rats were prepared to determine biliary excretion as well as urinary and faecal excretion patterns over periods of time.

Results: It was established that percentage-wise excretion of test material decreases as the dose increases, as the report states.

Comment: While the amount of test material decreases as a percentage of the increasing administered dose, the total amount of absorbed test material in absolute value increases with increasing dose levels.

Validation; Core - Guidelines

000992

Section 4 - Vol I of V

Additional Data Applicable to

PP NOS 6F1773, 6F1832, and 7F1898

R#962

June 10, 1977

Preliminary Assessment of The
Effect of DU112307 on the Rat

Report No. 243/77208

Test Compound:	DU112307
	Batch # FL 44/605201
Test Specie:	Pathogen Free Rat - CFY Strain
Number of Animals:	50 Rats, 5/sex/dose
Doses:	0; 800 ppm; 4,000 ppm; 20,000 ppm; 100,000 ppm.
Route of Administration:	Diet
Test Laboratory:	Huntingdon Research Center Huntingdon, England

140

Methodology: Fifty rats were divided into five groups, with five males and five females per group. The test material DU112307 was administered in the diets at 0; 800; 4,000; 20,000 ppm and 100,000 ppm respectively for the five groups. This experiment lasted four weeks. Animals were weighted initially and then two times per week for the four weeks. Food intake, and body weight were recorded. Hematological examinations were performed along with macroscopic pathology at the end. No microscopic pathology was performed on any tissue. Adrenals, kidneys, liver, ovaries, spleen and testes were weighted.

Results: There were no mortalities during the four week test period. There were no clinical signs of toxicity except for the colors of the stools due to the color of the diet high in DU112307. Bodyweights were within normal ranges with the exception of periods of food withdrawal for two consecutive nights prior to blood sampling (changes in food consumption appeared unrelated to treatment). All treated animals had "sulphemoglobin values" significantly higher than control animals. Methemoglobin values were significantly different from controls in males at all dose levels while methemoglobin values in females were statistically significant at doses 4,000 ppm and above. In hematology, statistically significant decreases were found for erythocytes, packed cell volumes

000992

and hemoglobin in all animals fed test compound at 100,000 ppm.

Comments: It is presumed that since this is a preliminary study no histopathology was contemplated.

Validation: Core - Guidelines

→ There is a general dose-related increase in absolute and relative spleen and liver weights.

may forget to type this in —

14F

22

BB-1056
TKL-991

000991

Section 5 - Vol I of V

Additional Data Applicable To

PP NOS 6F1773, 6F1832, and 7F1898

Toxicology Data

R# 962

June 10, 1977

Report No. 56654/8/77

Diflubenzuron: Analysis of Metabolites
Connected With Methaemoglobinemia

Test Compound:	Dimilin (Diflubenzuron)
Test Specie:	Male Wistar Rats
Number of Animals:	45 rats, 15 per test group, one control group
Route of Administration:	Dietary
Dose:	7.8 grams/kg - mean daily dose - for four days
Testing Laboratory:	Philips - Duphar, B.V. Weesp, The Netherlands

1039

000991

Methodology: The evaluation of the chemical analytical methods used to analyze for 4-chloroaniline, 4,4'dichloro azobenzene and 4,4'dichloro-azoxybenzene are within the competancy and jurisdiction of Chemistry Branch.

Results: The plasma levels of 4-diloroaniline in rats averaged about 30 ng/ml while levels at 4-chloroaniline in red blood cells were 30 µg/gram of cells explaining the mechanism for the formation at methaemoglobinemia.

Validation: Core - guideline

000991

Section 6 - Vol 1 of V

Additional Data Applicable to

PP NOS 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report Number 56645/2/77

The Methaemoglobin and Sulphaemoglobin Forming Properties of DU112307 In Male Rabbits After Prolonged Dietary and Dermal Administration

Experiment 1

Test Compound:	DU 112307 (technical; Batch # 405093
Test Specie:	Male rabbits
Number of Animals:	15 treated, 15 controls
Route of Administration:	Diet
Dose:	640 ppm for 21 days

Experiment 2

A.	Test Compound:	DU 112307 (analytically pure) Batch # 601141
	Test Specie:	Male rabbits

Experiment 2 A. Cont'd.

	Number of Animals:	15 treated, 15 controls
	Dose:	640 ppm for 18 days
	Route:	Dietary
B.	Test Compound:	DU 112307 (technical)
	Test Specie:	Male rabbits
	Number of Animals:	Two groups of 15 treated rabbits each. One control group treated with vehicle only.
	Route:	Dermally
	Dose:	70% test material concentration, 1.5 ml/kg for 18 days. (One of the two groups with Formo-Cibazol 1000 ppm coccidiostat and the other without the coccidiostat).

Methodology - Experiment 1

A group of 15 male rabbits was administered DU 112307 (technical) at dose level of 640 ppm for 21 days. A control group of 15 rabbits received only basal diet. Methemoglobin levels and sulphemoglobin levels were examined in blood samples at 20, 17 days and one hour before treatment; and at five and 24 hours and at 4, 9, 14 and 21 days after treatment.

Methodology - Experiment 2

A. A group of 15 male rabbits was administered DU 112307 (analytically pure) in the diet at 640 ppm for 18 days. A control group received just diet.

000991

B. Two groups of 15 male rabbits were treated dermally with technical DU 112307 at 70%, 1.5 ml/kg for 18 days. One of these groups was given the coccidiostat Formo-Cibazol at a dose of 1000 ppm before and during treatment and recovery. A control group was dermally treated with vehicle and received no coccidiostat.

After the treatment period, there was a recovery period of 21 days for the group fed test material and 4 days for those dermally treated. Methemoglobin values were measured in blood at 31 and one day before treatment and on days 1, 4, 11, 18 after treatment for the orally dosed group and on days 1, 2, 4, 7, 14 and 21 days after treatment and day one and four of the recovery period for animals dermally treated.

Results: Experiment 1.

The report states, "According to the t-Test of Student at days 4, 9, 14 and 21 the methemoglobin levels and after five hours and at 9, and 14 days the 'SulphHb' levels of the compound treated groups were significantly although marginally increased."

Comment: Sulphemoglobin is not a normal constituent of blood. Clarification is needed regarding the presence of sulphemoglobin values in control animals.

Results: Experiment 2. Dermal Testing.

In group 2 (with coccidiostat) there is an increasing trend with time in metHb levels from day 4 of treatment to day 18 with day 11 being

statistically significant over its corresponding control values. Control values from day 4 to 18 also show an increasing trend. Group 3 (without coccidiostat) again shows an increasing trend with again statistical significance evident at day 11.

Results: Experiment 2. Dietary Testing

According to the T test, the group 5 (640 ppm) was significantly increased over controls on days 11 and 18 of treatment and on days 1, 2, 4 and 7 of recovery.

Conclusion: It can be concluded that DU 112307 is a methemoglobin-forming agent in rabbits either orally or dermally when administered at the above stated dose levels.

000991

Section 7 - Vol. I of V

Additional Data Applicable to
PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

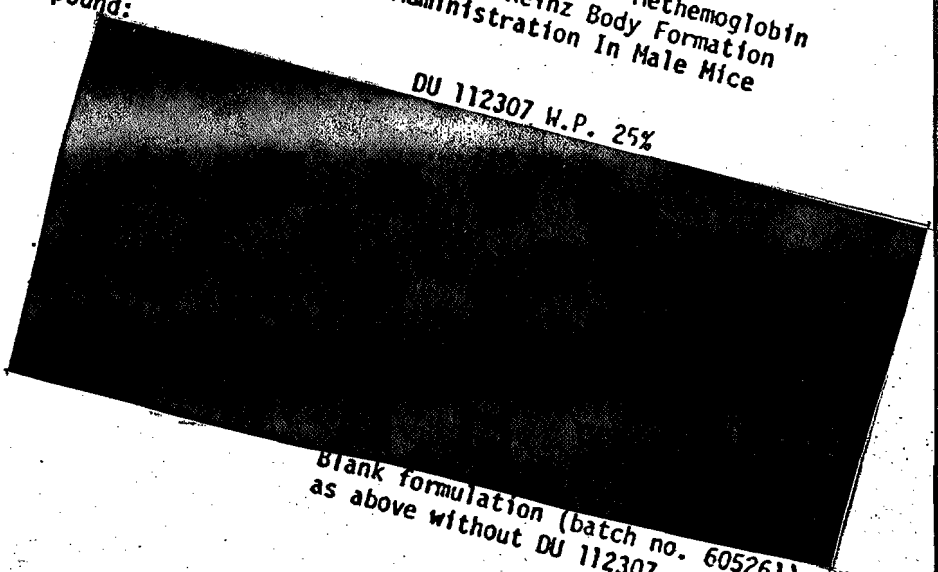
June 10, 1977

Report No. 56645/12/77

The Effect of DU 112307 W.P. 25% On The Methemoglobin And Sulphemoglobin Levels and Heinz Body Formation After A Single Oral Administration In Male Mice

Test Compound:

DU 112307 W.P. 25%



Blank formulation (batch no. 605261) as above without DU 112307.

000991

Test Specie:	Male Swiss Mice
Number of Animals:	30 mice, 15/test group
Route of Administration:	Gastric intubation
Dose:	10,000 mg/kg
Testing Laboratory:	Philips-Duphar B. V. Weesp, The Netherlands

Methodology: A group of 15 male mice were administered a one percent tragacanth suspension of test formulation at a single dose of 10,000 mg/kg body weight. Animals were housed in stainless steel cages, five per cage. Animals were weighed before dosing and 24 hours post treatment. A group of 15 male mice were treated the same but administered 7,500 mg/kg of blank formulation. Blood samples were taken at 4 hours and 24 hours after treatment. Methemoglobin and sulphemoglobin values and Heinz Bodies were sought.

Results: There were no effects seen in bodyweight after 24 hours. No Heinz bodies could be detected at the two sampling intervals, 4 and 24 hours.

At 4 hours, there was a statistically significant increase in methemoglobinemia in the treated group, as compared to the 4 hour control group.

No appreciable increase was noted for sulphemoglobin at 4 hours or at 24 hours as compared to controls.

8/56

000991

Comment: Sulphemoglobin is not a normal constituent of blood. The existence of sulphemoglobin in mouse blood in control animals found at 4 hours and at 24 hours requires classification.

Validation: Core - Guidelines tentative to clarification under comment.

9 /17

000991

Section 8 - Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

June 10, 1977

Report # 56645/13/77

The Effect Of Du 112307 W.P. 25% On The Methaemoglobin And Sulphaemoglobin Levels And Heinz Body Formation After A Single Dermal Application In Rabbits

Test Compound:	DU 112307 W.P. 25% (Batch No. Fl. 44/703111)
Test Specie:	Male New Zealand White Rabbits
Number of Animals:	30 rabbits, 15 per test group, two test groups
Route of Administration:	Dermal
Dose:	0 - controls and 4,640 mg/kg test group
Test Laboratory:	Philips-Duphar B. V. Weesp, The Netherlands

Methodology: Animals were weighed, housed in stainless steel cages. Room temperature was 18-29°C, relative humidity 70-80%. The day prior to testing, the animals were clipped free of hair around the trunk. Each animal was wrapped in sticking plaster covered with aluminum foil. Test material was introduced between skin and plaster using blunt needle. After 24 hours, coverings were removed and skin was washed with soap and water then dried. Blood was drawn from ear vein at 4 and 24 hours after application to measure methaemoglobin, sulphaemoglobin levels and to stain for Heinz bodies.

Results: No methaemoglobin or sulphaemoglobin levels were detected. No Heinz bodies were found on the prepared slides.

Conclusion: DU 112307 25% W.P. when applied to New Zealand White rabbits at 4,640 mg/kg dermally does not produce methemoglobin or sulphaemoglobin or Heinz bodies according to the methods employed for detection.

Validation: Core - Guidelines

000991

Section 9 Vol. I of V

Additional Data Applicable to PP NOS. 6F1773,

6F1832, and 7F1898

R # 962

June 10, 1977

Report No. 56645/15/77

The Methemoglobin and Sulphemoglobin and Heinz Body Forming Properties of DU 112307 After Oral Administration To Male Rats During 8 Days

Test Compound:	DU 112307 (technical) in one percent tragacanth vehicle
Test Specie:	Male rats, Wistar Strain
Number of Animals:	30 rats, 15 per test group
Route of Administration:	Oral intubation
Dose:	5,000 mg/kg per day for eight days

Methodology: One group of 15 rats were administered DU 112307 (technical) at 5,000 mg/kg by gastric intubation daily for eight days in a volume of 15 ml/kg bodyweight. Blood samples were taken two days before administration of test material and

at 4 and 24 hours after the first dosing and at 2, 3, 4 and 8 days after start of the repeated dosing and at day 3, 4 hours after dosing that day. Heinz bodies were sought in blood samples taken 4 hours, 24 hours and 8 days after initial treatment.

Results: No Heinz bodies were observed in any of the above sample periods. At one day the methemoglobin values and at day 2, 3, 4, 8 the methemoglobin and sulphemoglobin values were increased in respect to control values.

Conclusion: When DU 112307 (technical) is administered at 5,000 mg/kg p.o. to male Wistar rats, methemoglobin and sulphemoglobin is detected in blood levels statistically significant over control values.

Validation: Core - Guidelines

000991

Section 10 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report No. 56645/4/77

Acute Oral Toxicity Study With DU 112307 (technical) in Mice

This report submits the laboratory test data information needed to validate report no. 56645/14/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

This information in this report no. 56645/4/77 now validates report no. 56645/14/73 in regards to the results in mice.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

000991

Section 11 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report No. 56645/3/77

Acute Oral Toxicity Study With DU 112307 W.P. 25%
In Mice and Rats

This report submits the laboratory test data information needed to validate report no. 56645/15/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report no. 56645/3/77 now validates report no. 56645/14/77.

Test Laboratory: Philips-Duphar B.V.
Weesp, The Netherlands

163

000991

Section 12 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

June 10, 1977

Report # 56645/2/77

Acute Toxicity In Rats of DU 112307 (Technical)
After Dermal Application

Test Material:	DU 112307 (technical) Batch # 405093
Test Specie:	Male and female rats - SPF Wistar
Number of Animals:	3 groups of 5 males and 5 females
Route of Administration:	Dermal
Doses:	4,640 mg/kg and 10,000 mg/kg
Testing Laboratory:	Philips Duphar, B.V. Weesp, The Netherlands

Methodology: Three groups of SPF Wistar rats, 5 males and 5 females were placed on test. The rats were shaved with clippers around the flanks and back. The clipped trunks were

covered with a strip of plaster. The rats were not abraded. One group received DU 112307 at 10,000 mg/kg in a volume of 20 ml/kg dermally while a second group received 4,640 mg/kg of test material also dermally. The third group received only vehicle-1% tragacanth solution. Blood was taken from the orbital vein before test material administration and at 5 and 24 hours after test material administration. The animals were observed for a subsequent period of two weeks after initiation of test for signs of toxicity.

Results: The LD_{50} is greater than 10,000 mg/kg of bodyweight of DU 112307 (technical) and no increase in methemoglobin or sulphemoglobin were detected under these conditions in rats, during test material administration or during the subsequent two week period.

Validation: Core - Guidelines

000991

Section 13 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Acute Dermal Toxicity Study with DU 112307 (Technical) in Rats

Test Material: DU 112307 Technical

Batch No. 405093

Test Specie: Male and female Wistar rats

Number of Animals: 5 per sex per dose

Route of administration: Dermal

Doses: 4,640 and 10,000 mg/kg

Testing Laboratory: Philips Duphar, B.V.

Weesp, The Netherlands

Methodology: As in report no. 56645/2/77-Section 12-Vol 1 of V

Results: The LD_{50} is greater than 10,000 mg/kg when administered dermally to Wistar rats as a single application of test material suspended in a 1% tragacanth solution.

Validation: Core - Guidelines

000991

Section 14 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Report No. 56645/6/77

Acute Dermal Toxicity Study with DU 112307 W.P. 25% In Rabbits

This report submits the laboratory test data information needed to validate report #56645/17/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report #56645/6/77 now validates report #56645/17/73.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

000991

Section 15 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1838 and 7F1898

R# 962

June 10, 1977

Report Number 56645/5/77

Acute Intra-Peritoneal Toxicity Study With DU 112307 (Technical) In Mice

This report submits the laboratory test data information needed to validate report #56645/1/74 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report #56645/5/77 now validates report #56645/1/74.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

000991

Section 16 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Tumorigencity of DU 112307 To Mice-Dietary Administration For 80 Weeks

(Reevaluated Pathology Data)

Addendum to Report PDR 170/75685

Results: The individual animal pathology data submitted on two mice does not change the results of the report PDR 170/75685 submitted in 1976.

Test Laboratory: Huntingdon Research Center
Huntingdon, England

000991

Section 17 of Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Effects of DU 112307 in Dietary Administration to Rats for 104 Weeks

(Reevaluated Pathology Data)

Results: The individual animal pathology data submitted in this report does not change the results of the report PDR 171/75945 submitted in 1976.

Test Laboratory: Huntingdon Research Center
Huntingdon, England

000991

Section 18 Vol 11 of V

Additional Data Applicable to PP Nos 6F1773, 6F1832 and 7F1898

R# 962

Submitted June 10, 1977

Report

Macroscopic and Microscopic Examination of Tissues of Sheep and Swine Administered

Diflubenzuron

Laboratory #7E-5790

Diflubenzuron

Pathology Report

Test Compound: Diflubenzuron

Test Species: Swine and Sheep

Number of Swine: 9 female, 2 male controls

6 female, 2 males treated

Number of Sheep: 10 female, 2 male controls

6 females, 3 males treated

Route of Administration: Dietary

Doses: Diflubenzuron - 100 ppm

No duration of time given

Testing Laboratory: Cannon Laboratories, Inc.

Sponsor: Thompson - Hayward Chemical Company

Results: All 24 tissues examined both in control and treated animals showed no macroscopic or microscopic evidence of toxicity attributable to the administration of test material.

000991

Section 19 - Vol. II of V

Effects of Feeding Dimilin (TH-6040)

Upon Reproduction and Residues In

Sheep and Swine

Progress Report, February 1977

Test Compound: Dimilin (TH-6040)

Test Specie: Columbia-Rambouilet ewes + rams, Dorsets and Hampshire

Number of Sheep: 51 ewes, 6 rams

Number of Swine: 37 gilts, 4 boars

Route of Administration: Dietary

Doses: 100 ppm

Testing Labortors: Department of Animal Science

University of Maryland

College Park, Maryland 20742

Sponsor: Thompson - Hayward Chemical Co.

Methodology: Sheep Project

Fifty - one Columbia - Rambouilet ewes and six ram (two Dorsets and four Hampshire) were weighed, treated for parasites, given vitamins and ear tagged before starting experiment. The ewes were divided into four groups, two control and two treated groups. Each control group had 8 ewes and there were 12 ewes in the treated groups. The rest of the animals were used as replacements, five were "control replacements" and six were treated replacements.

Diets was corn, cobmeal, ground alfalfa hay and 1.4% propionic acid (as preservative). Treated food has "approximately" 100 ppm of TH6040. Rams were rotated with ewes, matting treated rams with treated ewes and control rams with control ewes. Blood samples were taken for analysis. Liver, muscle, fat, spleen and kidney were taken from some experimental sheep. Fetuses and uteri of pregnant ewes were frozen and other simples were placed in dry ice for analysis at Thompson - Hayward. Three control and six treated sheep were lost due to poor physical condition. It would appear, to date, that none of these deaths were caused by "intoxication as evidenced by post mortems.

Results: From the information available to date, there would not appear to be appreciable differences between control ewes and treated ewes or in the progeny of treated or control ewes.

25 ~~23~~

Comments: Tissues were taken for sampling but no data is made available. Blood samples were taken for chemical analysis but no data is available.

Validation: Core - Guidelines - tentative to the termination of experiment.

000991

Section 20 - Vol. II of V

DU 112307

Thirteen Weeks Oral Toxicity Study

In The Sheep

Submitted - June - 1977

PDR 229/77226

Test Compound: Dimilin 90% w/w

Batch no. FL 11/508141

Diflubenzuron pre - mix 90% No. 508141

Test Specie: Sheep

Number of Animals: 24 Dorset Horn Sheep

12 males, 12 females

Route of Administration: Dietary

Doses: 500; 2,500; 10,000 ppm and controls

Testing Laboratory: Huntingdon Research Center

Huntingdon, England

Sponsor: Philips-Duphar, B.V., Weesp, The Netherlands

27 (83)
175

Methodology: Twenty-four sheep, 12 males, 12 females were divided into four groups for study. Group one was controls, group two was administered test material in feed at 500 ppm, group three was administered test material at 2,500 ppm and group four at 10,000 ppm. Body weights were recorded weekly. Plasma testosterone and Oestradrol were assayed at 13 weeks. Laboratory investigations and eye examinations were don at 0,4,8 and 13 weeks. Post-mortem examination included organ weight determinations, macroscopic examination and histophathology.

Results: There were no signs of toxicity or abnormal behavior. Animals consumed all the diet offered. Bodyweight gain was within normal limits. Ophthalmological examinations revealed no treatment related abnormalities.

Hematology: Hematological results showed statistical significance at weeks in a decreased hemoglobin at 500 ppm and 2500 ppm with diminished red blood all counts at 500, 2500 and 10,000 ppm. At this time period there was decreased packed cell volume at 2,500 ppm and dimished mean corpuscular volume was also statistically evident at 10,000 ppm. At 8 weeks, mean corpuscular hemoglobin concentration was dimished at the 2500 and 10,000 ppm dose levels.

At 13 weeks, the 2,500 ppm level shows a decreased packed cell volume and hemoglobin content and at 10,000 ppm a decreased mean corpuscular volume. The report states that these statistically

significant values showed no clear pattern or a dose - related response.

Methemoglobinemia: Already at 4 weeks, the first period taken for blood studies, methemoglobinemia values showed increasing values in all treated groups over controls. At 8 weeks, treated groups showed increased values becoming statistively significant at the 2,500 ppm dose level and at 10,000 ppm dose level. At 13 weeks only the 2500 ppm dose level and 10,000 ppm dose level were increased over controls but not significantly.

OD_2 Blood Values: An additional blood dyscrasia was noted appearing with methemoglobinemia. This blood dyscrasia is statistically real and dose related throughout the study appearing at the first period fo blood examinations at 4 weeks (reflected by OD_2 value). There is an increasing trend with dose at the first time period of bolld study-4 weeks at the 500 ppm level with statistical significance at the 2,500 ppm dose level and the 10,000 ppm dose level. The increasing trend with dose is again seen at 8 weeks becoming statistically significant at 10,000 ppm dose level. At thirteen weeks, all treated groups are markedly and statistically increased over controls.

Urine: Specific gravity of urine increased at 8 weeks for both the 500 ppm dose level and the 2,500 ppm level. These increases were

also statistically significant for all treated groups at the 13 weeks period.

Testosterone: Mean plasma testosterone levels at 13 weeks period were 12.13 ng/ml in controls rising to 2.27 ng/ml at the 500 ppm dose level to 5.67 ng/ml at the 2,500 ppm dose with a drop below control values to 1.63 ng/ml at the 10,000 dose level.

Organ Weights: There would appear to be a dimination in thyroid weight in treated groups as compared to control values.

Comments: The obvious blood dyscrasia (measured by optical density) but unidentified as to type needs to be identified. The optical density technique for measurement may not be as sensitive as needed to determine the smallest dose level of DU 112307 that may be ingested that will produce a no-effect level either for methemoglobinemia or other hemoglobinopathy (OD_2 values).

Conclusion: DU 112307 appears to produce methemoglobinemia and other(s) unidentified hemoglobinopathy when fed to sheep at 500; 2,500; and 10,000 ppm in feed for a period of 13 weeks. Other effects observed were decreased thyroid weights, increased and unexplained specific gravity of urine and a normal effect of increasing testostrone levels with dose with a makred decrease of testosterone at the highest dose level.

Validation: Core - guidelines

Comment: No statement has been submitted regarding duration of treatment.

Validation: Core - Guidelines _ Tentative to submitted of duration of study.

000991

Section 21 Vol 11 of V

Additional Addendum Studies Submitted June 1977

Red Blood Cell and Plasma Cholinesterase Value Following Six Weeks Inclusion of DU 112307 In The Diet of Sheep

Report No.: PDR 229A/77225

Test Compound: DU 112307 - Dimilin
Pre Mix 90% w/w

Test Specie: Sheep

Number of Animals: Twenty-four sheep -
12 males, 12 females
3 males, 3 females/dose level

Route of Administration: Diet

Doses: 0, 500, 2,500, 10,000 ppm

Duration: Six weeks

Laboratory: Huntingdon Research Center
Huntingdon, England

Methodology: Sheep were divided up into four groups, 3 males and 3 females per dose level. Doses were 0, 500, 2,500, 10,000 ppm for a period of six weeks. Cholinesterase values were determined in R.B.C. and plasma for each animal.

Results: Plasma cholinesterase did not differ substantially between groups. R.B.C. cholinesterase did not differ as much between groups as between animals in each group. The variations that exist cannot be attributed to the administration of test material.

181

Comment: An experiment of this nature is best designed when each animal acts as its own control also.

Validation: Core - Guidelines

000991

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

Toxicology Data

Section 22 Vol III of V Addendum Studies

R# 962

June 10, 1977

Effects of DU 112307 (Technical) After Dietary Administration To Male Hubbard Broiler Chickens for 14 Weeks

Test Compound: 1. DU 112307-Technical (Batch No. 405093) purity 99.6%

2. DU 112307-Technical (Batch No. 2866) purity 98.5%

Test Specie: Male Hubbard Broiler Chickens

Number of Animals: 1,700 day old male chickens

Route of Administration: In diet

Doses: 0, 2.5, and 250 ppm

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

Methodology: One thousand seven hundred male chickens were divided into four groups, two control groups and two treated groups. The basic diet in this study was not constant throughout the study. During the first 56 days the vitamin/mineral mix 188MK was used and from 57 days to end of the test the vitamin/mineral mix 158/MK/158 was used. Mortality, body-weight and food consumption, testosterone and oestradiol values, organ weights, tibia weight and length, comb and

wattle development, gross pathology, microscopic examination and the evaluation of the pictures of the total birds, head and skinned legs were noted.

Results: Not any of the above perameters appeared exceptionally different from controls. However, because of equivocal results from other studies with chickens in regards to testosterone levels, it will be mentioned that the mean test weight at the low level of DU 112307 or at 2.5 ppm was significantly lower $p > 0.05$ than controls at 4 weeks. At this period, mean testes weight at the high dose level was also lower than controls but not statistically significant. Mean testosterone levels were lower than controls in the high dose group also at 4 weeks.

Statistically ((T test) there would appear to be $p > .001$) a significant statistical difference between low dose and control dose C_1 and again a statistical difference between high dose and control dose C_2 but there is also a statistical difference between the two control groups C_1 and C_2.

Comment: Due to the large variations in the control values, it is difficult to ascertain that DU 112307 has some effect or no effect upon testosterone level or testes weight in growing chickens in this experiment.

Validation: Core - Supplementary data

164

000991

Section 23 Vol V of V

Additional Addendum Studies Submitted June 1977

Isolation and Characterization of Proteoglycans from Chick Limb Bud Chondrocytes Grown in Vitro

Purpose: To determine whether Dimilin significantly intervenes in the incorporation of precursors into preteoglycans.

Test Compound: Dimilin desolved in DMSO/Control DMSO

Test Material: Chick limb bud mesenchymal cells - grown in culture medium facilitating chondrogenic development

Testing Laboratory: Dental Research Institute
Departments of Oral Biology and Biological Chemistry
University of Michigan
Ann Arbor, Michigan 48104

Methodology: The biochemical methodology used is that extensively described in

* "Isolation and Characterization of Proteoglycans from Chick limb Bud. Chondrocytes grown in Vitro by Hascull, Oegema and Brown."

Results: The initial conclusion in the report states "The compound, when administered to the cultures as described above, does not appear to alter significantly the ability of the cells to incorporate 35S into chondriotin sulfate chains and 3H-serine into apparently normal cartilage proteoglycans over a 5 hr. incubation time --."

---

* The Journal of Biological Chemistry, Vol 251, No. II.

000991

Section 24 Vol V of V

Additional Addendum Studies Submitted June 1977

Biochemical Effects of Diflubenzuron On Mouse Embryos

Purpose: To determine:

1. Effect of Diflubenzuron (Dimilin) on Mucopolysaccharide synthesis in mouse limb cartilage.
2. Transfer of diflubenzuron across mouse embryonic membranes.
3. Uptake of Diflubenzuron by suckling mice and teratogenic effects.

Test Compound: Diflubenzuron (Dimilin) 99.6% purity

Test Specie: White Swiss mice

Number of Animals: Forty-six mice

Route of Administration: Dietary

Dose: 50 ppm Diflubenzuron

Test Laboratory: Department of Zoology
Brigham Young University
Provo, Utah 84602

Methodology: Female mice were mated with male mice until vaginal plug was observed. Each pregnant female mouse was individually housed and fed Diflubenzuron and ^{14}C Diflubenzuron in feed at 50 ppm. At 17 days, some pregnant females were sacrificed and embryos prepared for residue analysis. The remaining females were permitted to give birth. These lactating females were kept on treatment and permitted to

suckle their young for 13 days at which time the embryos were sacrificed for residue analysis.

Results:

1. Mucopolysaccharide synthesis in embryonic cartilages was normal.
2. Diflubenzuron did not pass through mouse embryonic membranes.
3. Suckling mice did not pick up Diflubenzuron from treated lactating females.
4. Diflubenzuron does not cause teratogenesis in developing mice.