

US EPA ARCHIVE DOCUMENT

PD-1056  
T17-992

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BIOLOGICAL EFFECTS OF DIMILIN ON VERTEBRATES  
AND OTHER SELECTED ORGANISMS

USDA

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**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 Station, 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.**

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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

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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 [<sup>3</sup>H] acetate and [<sup>35</sup>S] sulfate into individual glycosaminoglycans. Synthesis of other classes of biopolymers was quantitated

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by measuring the incorporation of [<sup>3</sup>H] leucine into protein, [<sup>3</sup>H] fucose into glycoproteins and [<sup>3</sup>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

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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, 100 ppm 200ppm.

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  $15\mu\text{Ci}$  of  $^{14}\text{C}$  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.

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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 ( $\mu\text{g}$  uronic acid/g tissue) at all dose levels over controls.

Hyaluronate Synthesis

The report states, "Treatment with 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 component of skin. This possibility was beyond the scope of the experiment.

Validation: Core-Guidelines

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**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**

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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.**

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- 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

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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 Station diet. Each kind of diet was administered to two kinds of chickens, a broiler strain (Hubbard) and a Leghorn strain. Day-old male chicks were placed on either of the two diets at the above stated dose levels. Food consumption, bodyweights, testosterone levels were monitored periodically. After sacrifice, heart, kidney, spleen, bursa of Fabricius, liver, testes and comb weights were taken.

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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~~ 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 Station 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 Station 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

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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 competency of EPA entomologists. 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.

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FOLLOWING

Additional 24 studies submitted in June 1977 in support of the  
Dimilin petition by Philips Duphar, Weesp, The Netherlands.

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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.

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Section 2 - Vol I of V

Additional Data Applicable to  
PP MOS 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

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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-pyruvic 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.

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Section 3 - Vol I of V

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

R#962

June 10, 1977

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

Report No 56654/10/77

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

Methodology: Rats were intubated with  $^{14}\text{C}$  diflubenzuron to determine  
rate of excretion and therefore, rate of absorption of  
test material. In one experiment, intact rats were used.

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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

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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

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**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 erythrocytes, packed cell volumes

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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.

Key target is type 1b2. in ↗