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

005520

SEP 26 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Experimental Use Permit and Temporary Tolerance for Ignite Herbicide (HOE-39866); EPA Reg. No. 8340-EUP-RN; PP #4G3156; Accession #263026, 263028; Caswell #580I; Tox. PN #2098.

TO: Richard Mountfort (23)
Registration Division (TS-767C)

FROM: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

DSS 9/20/86

THRU: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

LDC 9/26/86
TMF 9/26/86

Action Requested

Review the studies submitted in support of a revised Experimental Use Permit (8340-EUP-RN) and a temporary tolerance (PP #4G3156).

Recommendations

1. Toxicology Branch does not object to the proposed Experimental Use Permit (8340-EUP-RN) and temporary tolerance (PP #4G3156). The proposed use will result in a TMRC of 0.00012 mg/kg/day, which will occupy 28.9% of the PADI (see Discussion).

2. The acute oral toxicity study with Ignite Herbicide 20% WSC (report #85.0203) was classified as Core-Guideline data. The oral LD50 was 2170 mg/kg in males and 1910 mg/kg in females. These values correspond to Toxicity Category III.

2. The acute dermal toxicity study (Report #85.0204) was classified as Core-Guideline data. The dermal LD50 was 1400 mg/kg in males and 1380 mg/kg in females. These values correspond to Toxicity Category II.

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Recommendations (con't)

3. The acute inhalation toxicity study (Report #85.0912) was classified as Core-Guideline data. The inhalation LC50 was 3.22 mg/L in males and 4.31 mg/L in females. These values correspond to Toxicity Category III.

4. The primary eye irritation study (Report #85.0931) was classified as Core-Guideline data. The formulation was shown to be moderately irritating without washing, and only slightly irritating if eyes are washed immediately. All effects were reversible by 7 days. These findings place the formulation in Toxicity Category II based on reversible corneal opacity.

5. The primary skin irritation study (Report #85.0146) was classified as Core-Guideline data. The formulation was shown to be only slightly irritating to the skin, which corresponds to Toxicity Category IV.

6. The 28-day feeding study (Report #86.0012) with HOE 61517 (3-methylphosphinico-propionic acid), a plant and animal metabolite of HOE 39866, was classified as Core-Supplementary data. Only 5 rats/sex/dose were studied, and the duration of treatment was insufficient to satisfy guideline requirements for a subchronic feeding study. This study was apparently intended as a range-finding study for a longer term feeding study. The NOEL was established as 2500 ppm, based on alterations in clinical chemistry in males and females, and elevated liver weights (females only) noted at 5000 ppm, the highest dose tested.

7. The submitted label is consistent with the toxicological hazards identified for this chemical.

Background

Toxicology Branch has previously reviewed a proposed EUP for this chemical (see attached memo Saunders to Mountfort, 8/2/85). The current request is larger in scope than the most recent proposal, and involves the use of a slightly different formulation (18.2% a.i. currently, 19.5% a.i. previously). The total acreage to be treated is 444 acres at 42 sites in 23 states, and a maximum total of 715 lbs. a.i. will be applied.

Three types of uses are to be studied:

1. Soybeans- 20 sites will be studied in 12 states. The treated acreage will range from 20 to 40 acres (400 acres total) and the application rate will be 0.5-1.5 lbs a.i./acre by either aerial (2 studies) or ground (18 studies) application (maximum total of 600 lbs. a.i. applied). A temporary tolerance of 0.05 ppm (limit of detection) was requested for soybeans.

Background (con't)

2. Vine and tree crops- 13 sites will be studied in 9 states. The treated acreage will range from 2 to 6 acres (26 acres total) and the application rate will be 1.0-2.0 lbs a.i./ acre by ground application (maximum total of 52 lbs. a.i. applied). A temporary tolerance of 0.05 ppm (limit of detection) was requested for stone fruits, citrus fruits, pome fruits, and grapes.

3. Non-crop use- 9 sites will be studied in 9 states. The treated acreage will equal 2 acres in each state (18 acres total) and the application rate will be 1.5-3.5 lbs a.i./acre by ground application (maximum total of 63 lbs. a.i. applied).

Discussion

The submitted data do not affect the overall toxicological assessment of this chemical (see previous memos Saunders to Mountfort 4-18-85 and 2-7-86). In the original assessment of this EUP, a number of potentially serious considerations regarding the ability of HOE 39866 to interfere with endogenous biochemical processes were raised by Toxicology Branch. The Registrant agreed to conduct a research program to further elucidate these potential effects. Although Toxicology Branch opposes any permanent food uses until this issue is resolved, the modest proposed EUP and temporary tolerance was not opposed. In subsequent discussions with the Registrant, the Agency was informed that the requested studies were in progress, and no adverse effects had been noted. A summary of progress to date, included in the present submission, affirms that no adverse effects have been noted. A copy of this summary is attached to this memorandum. Actual data, however, have not been submitted.

The PADI was previously based on a NOEL of 8 ppm from a 90-day rat feeding study, and a 2000x safety factor. The PADI should be revised to reflect a 1000x safety factor, in accordance with current Toxicology Branch policy. The current PADI for HOE 39866 is therefore:

$$\frac{8 \text{ ppm } (0.05 \text{ mg/kg/ppm})}{1000} = 0.0004 \text{ mg/kg/day}$$

The proposed temporary tolerance request for 0.05 ppm in or on citrus, pome and stone fruit, grapes, and soybeans would result in a TMRC of 0.0001 mg/kg/day, which would occupy 28.9% of the PADI under the "Food Factor" system (see attached printout).

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TOXICOLOGY BRANCH ADI PRINTOUT Date: 09/09/86

Ignite (HOE 39866) 90d feeding- rat PADI = 0.000400 mg/kg/day
Caswell #580I NOEL = 0.4000 mg/kg Safety Factor = 1000
CFR No. 180. LEL = 3.2000 mg/kg
Status: ADI HAS NOT BEEN VERIFIED BY TOX OR AGENCY RED COMMITTEES.

DRAFT

RESIDUE CONTRIBUTION OF PUBLISHED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY

No published tolerances listed in file.

RESIDUE CONTRIBUTION OF TOX-APPROVED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY

No tox-approved tolerances are listed in the file.

TMRC %PADI
0.000000 mg/kg/day (60kg BW, 1.5kg diet) 0.000000

RESIDUE CONTRIBUTION OF NEW (PENDING) TOLERANCES

	CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
33	Citrus fruits	0.050	4G3156	3.81	0.002857500
66	Grapes, including raisins	0.050	4G3156	0.49	0.000367500
126	Pome fruits	0.050	4G3156	2.79	0.002092500
148	Soybeans (oil)	0.050	4G3156	0.92	0.000690000
151	Stone fruits	0.050	4G3156	1.25	0.000937500

TMRC %PADI
0.000116 mg/kg/day (60kg BW, 1.5kg diet) 28.937500

HOE 039866

Mode of Action

Status Report

At the meeting with EPA on April 11th, 1986 we discussed the mode of action of HOE 039866 in rats and dogs. The chemical Hoe 039866 is a close structural analogue of the amino acid glutamate. Due to the fact that glutamate may play a pivotal role in a large number of biochemical and neurochemical reactions, a research program including acute and subchronic treatment of rats and dogs was established. The experimental phase is complete. The results are under evaluation and will be reported within the next few months. A brief outline of the results is listed below.

A) Neurobehavioral studies indicated an effect only at extremely high doses. The low and medium levels used in the chronic studies did not show any changes in behavior in rats or dogs.

B) Inhibition of amino acid glutamine synthesis was observed. A clear threshold level could be established and it was shown that the inhibition of glutamine synthetase was clearly reversible.

C) The pharmacokinetics and the distribution of the test substance and its metabolites in the different organ systems were examined.

D) There was no major interference with amino acid or protein metabolism in several organ systems.

E) There was no interaction with oxidative metabolism.

F) There was no interaction with other neurotransmitters. The different neuroreceptors were not influenced by the test compound.

G) Following intoxication with HOE 039866 at sublethal and lethal doses it was possible to antagonize the convulsions with phenobarbital. An inhibition of acetyl cholinesterase could be ruled out as the initiator of the neurochemical reaction since Atropine and 2-PAM or toxogonin proved to be ineffective.

H) The determination of effects on other biochemical reactions that use glutamic acid as a substrate were investigated. Glutathione synthesis was not affected.

The parameters examined in order to demonstrate possible interference with the test compound are too numerous to list and therefore we would like to propose an extensive discussion of the results with representatives of EPA at the time the final reports will be available.

Although the evaluation of the results are in a preliminary state, in summary it can be concluded that comparable 'No observable effect levels' which were generated in the subchronic and chronic feeding studies in rats and dogs were supported. After initial exposure to the test material the animals adapts establishing a new homeostasis within the organism.

Signed
Dr. K-H Leist
Head of Plant Protection
Toxicology
Hoechst AG

DATA EVALUATION RECORD

STUDY TYPE: Acute oral toxicity (formulation)

ACCESSION NUMBER: 263026 MRID NUMBER: 159838

TEST MATERIAL: HOE 39866 water soluble concentrate (200 g/L) (Caswell #580I)

SYNONYMS: Ignite herbicide.

STUDY NUMBER: 84.0454

REPORT NUMBER: 85.0203

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 39866 - Liquid, Water-soluble Concentrate (200 g/L). Testing for Acute Oral Toxicity in Male and Female Wistar Rats."

AUTHORS: Markert, M. and Leist, K.H.

REPORT ISSUED: 5-3-85

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: Oral LD_{50} = 2170 mg/kg (males)
= 1910 mg/kg (females)

Toxicity Category III.

Classification: Core-Guideline

Materials and Methods

A. Materials: (1) Test material- HOE 39866 Water-soluble Concentrate (200 g/L), code OH SL18 A502, 18.6% a.i.

(2) Test Species: Male and female Wistar rats, obtained from Hoechst AG breeding colony.

B. Study Design

Rats were dosed by gavage with a 25% w/v preparation of the test material (diluted in deionized water) as follows:

Dose (mg/kg)	Volume (ml/kg)	Number Males	Number Females
1600	6.4	-	4
1800	7.2	-	5
2000	8.0	5	5
2240	9.0	5	-
2500	10.0	5	5

Rats were observed daily for mortality and clinical signs of toxicity, and body weights were recorded weekly. At the end of the 14-day observation period, surviving animals were sacrificed and examined for gross macroscopic changes.

Results/Discussion

The effect of treatment on mortality is depicted in Table 1. Based on these results, the oral LD50 was calculated to be 2170 mg/kg in males (95% c.i. 1890-2410 mg/kg) and 1910 mg/kg in females (95% c.i. 1720-2200 mg/kg). These findings correspond to Toxicity Category III.

Table 1. Mortality[a]

Dose (mg/kg)	Mortality Males	Mortality Females
1600	-	0/4
1800	-	1/5
2000	1/5	4/5
2240	4/5	-
2500	4/5	5/5

[a]data excerpted from submitted study.

Clinical signs noted on the day of treatment included "squatting, pilo-erection, and reduced activity", noted mainly at the higher doses. Other signs noted on succeeding days included "convulsions, agitated condition, staggering gait, and flanks contracted".

Animals that survived the observation period did not exhibit any clear effect on body weight gain. Rats that died lost body weight prior to death.

Gross necropsies of rats that died revealed reddened small intestine and filled with yellow, foamy fluid or a black mass, discolored liver, adrenals, spleen and pancreas, and bloody anal region. No abnormalities were noted in rats that survived the 14-day observation pericd.

Classification: Core-Guideline

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DATA EVALUATION RECORD

STUDY TYPE: Acute dermal toxicity (formulation).

ACCESSION NUMBER: 263026 MRID NUMBER: 159839

TEST MATERIAL: HOE 39866 water soluble concentrate (200 g/L) (Caswell #580I)

SYNONYMS: Ignite herbicide.

STUDY NUMBER: 84.0455

REPORT NUMBER: 85.0204

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 39866 - Liquid, Water-soluble Concentrate (200 g/L). Testing for Acute Dermal Toxicity in Male and Female Wistar Rats."

AUTHORS: Markert, M. and Leist, K.H.

REPORT ISSUED: 2-27-85

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: Dermal LD_{50} = 1400 mg/kg (males)
= 1380 mg/kg (females)

Toxicity Category II.

Classification: Core-Guideline

Materials and Methods

A. Materials: (1) Test material- HOE 39866 Water-soluble Concentrate (200 g/L), code OH SL18 A502, 18.6% a.i.

(2) Test Species: Male and female Wistar Hoe:WISKf(SPF71) rats, obtained from Hoechst AG breeding colony.

B. Study Design

Five rats/sex/dose were treated with dermal applications of 1000, 1500, or 2000 mg/kg of the undiluted test material for 24 hours to shaved, intact skin. The application site was occluded by aluminum foil and a elastic bandage. At the end of the 24 hour application period, the treatment site was washed with warm water.

Animals were observed daily for mortality and clinical signs of toxicity, and body weights were recorded weekly. Animals that died as a result of treatment, and rats that survived to the end of the 14-day observation period were sacrificed, necropsied and examined for macroscopic changes.

Results/Discussion

The effect of treatment on mortality is depicted in Table 1. Based on these results, the dermal LD50 was calculated to be 1400 mg/kg in males and 1380 mg/kg in females. These findings correspond to Toxicity Category II.

Table 1. Mortality After Dermal Application[a]

Dose (mg/kg)	Mortality	
	Males	Females
1000	1/5	1/5
1500	3/5	2/5
2000	4/5	5/5

[a]data excerpted from submitted study.

No clinical signs were noted on the day of treatment. Signs noted on the day after treatment included "squatting, pilo-erection, reduced respiration, flanks contracted, and convulsions", noted mainly at the higher doses.

No effect of treatment on body weight gain was apparent in animals that survived the observation period, and all surviving rats gained body weight over the 14-day observation period. Rats that died lost body weight prior to death.

Findings after gross necropsies of rats that died included small intestine and filled with yellow, foamy fluid or a black mass, peritoneum reddened, discolored spleen and lungs, and urinary bladder taut with blood and urine. No abnormalities were noted in rats that survived the 14-day observation period.

Classification: Core-Guideline

DATA EVALUATION RECORD

STUDY TYPE: Acute inhalation toxicity (formulation).

ACCESSION NUMBER: 263026 MRID NUMBER: 159840

TEST MATERIAL: HOE 39866 water soluble concentrate (200 g/L) (Caswell #580I)

SYNONYMS: Ignite herbicide.

STUDY NUMBER: 84.0483

REPORT NUMBER: 85.0912

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Abteilung fur Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 39866 - Liquid, Water-soluble Concentrate (200 g/L). Testing for Acute Aerosol Inhalation Toxicity in Male and Female SPF Wistar Rats - 4 Hour LC50."

AUTHORS: Hollander, H. and Weigand, W.

REPORT ISSUED: 9-16-85

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: 4 Hour LC_{50} = 3.22 mg/L (males)
= 4.31 mg/L (females)

Toxicity Category III.

Classification: Core-Guideline

Materials and Methods

A. Materials: (1) Test material- HOE 39866 Water-soluble Concentrate (200 g/L), code OH SL18 A505, 18.2% a.i.

(2) Test Species: Male and female Wistar Hoe:WISKf(SPF71) rats, obtained from Hoechst AG breeding colony.

B. Study Design

Five rats/sex/dose were treated by 4-hour inhalation exposures to 0.99, 1.76, 2.36, 4.12, and 4.67 mg/L of the undiluted test material. Chamber concentrations were determined by gas chromatography, and particle sizes were assessed by an "APS 33 Aerodynamic Particle Sizer" (TSI Inc., St. Paul, MN).

Animals were observed daily for mortality and clinical signs of toxicity, and body weights were recorded weekly. Animals that died as a result of treatment, and rats that survived to the end of the 14-day observation period were sacrificed, necropsied and examined for macroscopic changes.

Results/Discussion

The effect of treatment on mortality is depicted in Table 1. Based on these results, the inhalation LC50 was calculated to be 3.22 mg/L in males (95% c.i. 2.04-7.07 mg/L) and 4.31 mg/L in females (95% c.i. 2.77-13.7 mg/L). These findings correspond to Toxicity Category III.

Table 1. Mortality By Inhalation[a]

Dose (mg/L)	Mortality Males	Females
0.99	1/5	0/5
1.76	2/5	0/5
2.36	1/10*	2/10*
4.12	3/5	3/5
4.67	4/5	3/5

[a]data excerpted from submitted study.
*this dose was repeated due to an error in analytical measurement.

Common clinical signs noted during treatment included "irregular respiration, uncoordinated gait, nasal discharge, squatting position, pilo-erection, cornea dull, and weakened reflexes". Signs noted on the days succeeding treatment included "squatting, pilo-erection, jerky respiration, sneezing, cornea dull, and weakened reflexes".

Body weight gain appeared to be reduced in animals exposed to the highest concentration who survived the observation period, as compared to surviving rats exposed to the lower concentrations.

Findings after gross necropsies of rats that died included pink lungs, occasionally with red foci, and stomach dark red. No abnormalities were noted in rats that survived the 14-day observation period.

Classification: Core-Guideline

DATA EVALUATION RECORD

STUDY TYPE: Primary eye irritation (formulation).

ACCESSION NUMBER: 263026 MRID NUMBER: 159841

TEST MATERIAL: HOE 39866 water soluble concentrate (200 g/L) (Caswell #580I)

SYNONYMS: Ignite herbicide.

STUDY NUMBER: 84.0890

REPORT NUMBER: 84.0931

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 39866 - Liquid, Water-soluble Concentrate (200 g/L). Testing for Primary Eye Irritation in Rabbits."

AUTHORS: Ebert, E. and Leist, K.-H.

REPORT ISSUED: 1-22-85

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: Moderately irritating without washing, only slightly irritating if eyes are washed immediately. All effects were reversible by 7 days. Toxicity Category II based on findings of reversible corneal opacity.

Classification: Core-Guideline

Materials and Methods

A. Materials: (1) Test material- HOE 39866 Water-soluble Concentrate (200 g/L), code OH SL18 A502, 18.6% a.i.

(2) Test Species: New Zealand Albino rabbits, obtained from Hoechst AG breeding colony, Kastengrud.

B. Study Design

Test material (0.1 ml undiluted) was applied to the conjunctival sac of the left eye of each of 9 rabbits. The right eye of each rabbit served as control. One minute after application, the eyes of 3 rabbits were washed with saline for 1 minute, whereas the eyes of the remaining 6 animals received no further treatment. Eyes were examined 1, 24, 48 and 72 hours after treatment according to the technique of Draize. Lesions were scored on the basis of area involved and severity (cornea), degree of irritation (iris), and redness, chemosis and discharge (conjunctivae). The maximum combined score was 110; the substance was classified as a non-irritant if the score was 0-10, slight irritant for 11-25, moderate irritant for 25-56, and a severe irritant for 57-110.

Results

In unwashed eyes, mean irritation scores of 32, 33.5, and 30 were obtained after 1, 24, and 48 hours. The effects that were noted included chemosis, redness and discharge from the conjunctivae in all rabbits, slight irritation to iris in 2/6 rabbits, and "scattered or diffuse" opacity that involved the majority of the cornea in all 6 rabbits. These effects were fully reversible, as all eyes were judged normal after 7 days.

Washing of the eyes after 1 minute of treatment greatly attenuated this response, as a maximum mean irritation score of 13.3 was obtained after 24 hours. The effects noted were mainly irritation to the conjunctivae, with only minimal corneal involvement in 1/3 rabbits. All washed eyes were also judged normal after 7 days.

Therefore, the test material is considered to be a moderate eye irritant, and is in Toxicity Category II, based on reversible corneal opacity.

Classification: Core-Guideline

DATA EVALUATION RECORD

STUDY TYPE: Primary dermal irritation (formulation).

ACCESSION NUMBER: 263026 MRID NUMBER: 159842

TEST MATERIAL: HOE 39866 water soluble concentrate (200 g/L) (Caswell #580I)

SYNONYMS: Ignite herbicide.

STUDY NUMBER: 84.0913

REPORT NUMBER: 84.0146

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 39866 - Liquid, Water-soluble Concentrate (200 g/L). Testing for Primary Dermal Irritation in Rabbits."

AUTHORS: Markert, M. and Leist, K.-H.

REPORT ISSUED: 2-14-85

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: Slightly irritating to the skin (Toxicity Category IV).

Classification: Core-Guideline

Materials and Methods

A. Materials: (1) Test material- HOE 39866 Water-soluble Concentrate (200 g/L), code OH SL18 A502, 18.6% a.i.

(2) Test Species: New Zealand Albino rabbits, obtained from Hoechst AG breeding colony, Kastengrud.

B. Study Design

The test material was applied to intact, shaved skin, by means of a cellulose patch containing 0.5 ml of the undiluted test material. After 4 hours of treatment the patches were removed. Skin was examined at 1, 24, 48, and 72 hours after removal of patches. Irritation to the skin was evaluated by the method of Draize. Scores for irritation were as follows:

0.0-0.5	Non-irritant
0.6-3.0	Slight irritant
3.1-5.0	Moderate irritant
5.1-8.0	Severe irritant

Results

A maximum mean irritation score of 2.3 was obtained 1 hour after treatment, and was characterized by slight to "well-defined" erythema in 6/6 rabbits, and slight edema in 4/6 rabbits. Irritation scores declined with time, so that by 7 days only slight erythema was noted in 3/6 rabbits, and 4/6 rabbits had dry, chapped skin and/or coarse peeling. All skin appeared normal by 14 days after treatment. The test material is therefore considered a "slight" skin irritant (Toxicity Category IV).

Classification: Core-Guideline

DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study (28 day) on a plant metabolite.

ACCESSION NUMBER: 263028 MRID NUMBER: 159916

TEST MATERIAL: HOE 61517 technical (Caswell #580I)

STUDY NUMBER: 34.0704

REPORT NUMBER: 86.0012

SPONSOR: American Hoechst Corporation
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxicologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "HOE 61517 - Active Technical Ingredient: Testing for Subacute Oral Toxicity - Dose Range Finding - (28 day study) in SPF Wistar Rats".

AUTHORS: Ebert, E.; Leist, K.-H.; Mayer, D.; and Langer, K.-H.

REPORT ISSUED: 1-14-86

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch

Conclusion: Treatment-related changes were restricted to high dose rats: increased blood uric acid (males), serum triglycerides (females), and increased absolute and relative liver weights (females). No effect on the microscopic appearance of liver or other organs was noted.

The submitted study was apparently conducted as a range-finding study for a longer term feeding study. The study does not conform to existing guidelines for subchronic feeding studies, as only 5 rats/sex/dose were studied (10/sex/dose recommended), and the duration of treatment was only 28 days, rather than the recommended 90 days.

NOEL = 2500 ppm
LEL = 5000 ppm Altered clinical chemistry in males and females, increased liver weights in females.

Classification: Core-Supplementary Deficiencies as noted.

Materials and Methods

A. Materials: (1) Test material- HOE 61517 Technical, 3-methylphosphinico-propionic acid (a metabolite of HOE 39866), code OQ ZC99 0002, 99% a.i.

(2) Test Species: Male and female Wistar (Hoe: WISKf [SPF71]) rats, obtained from Hoechst AG breeding colony, Kastengrud.

B. Study Design: Rats (5/sex/dose) were fed diets containing 0, 50, 500, 2500 or 5000 ppm of the test material for 28 days. The diets were sampled after each preparation for content of the active ingredient. At the end of the 28-day feeding period, rats were sacrificed and subjected to complete necropsies and histopathological examinations.

Methods and Results

A. Diet Stability and Homogeneity- Analysis of the test diets indicated that diets were within 80-90% of their designated nominal values, and remained stable for at least 35 days after preparation.

B. Clinical Signs and Mortality- Animals were observed twice each day (once/day on weekends and holidays) to assess general health and behavior. Animals were examined once each week to assess neurological function, and effects on the eye, oral mucosa, and teeth.

Results- No treatment-related effects on clinical signs were noted. No mortalities occurred during the 28-day treatment period.

C. Body Weights and Food Consumption- Rats were weighed twice each week. Food consumption was measured twice each week, and was calculated per animal per 24 hour period. Water consumption was measured once per week, and was calculated per 100 grams body weight per 16 hour period.

Results- Body weight change was not affected by treatment, as weight gain was similar among all test groups (Table 1). Food and water consumption were similarly unaffected by treatment (data not shown).

Table 1. Body Weights[a]

Group	Week 1	Week 2	Week 3	Week 4
1M	97±5	160±11	199±11	243±16
2M	120±5	179±11	216±12	257±14
3M	119±8	171±13	204±16	239±19
4M	115±5	169±8	205±11	240±12
5M	125±4	171±4	202±6	237±10
1F	128±9	149±10	161±15	167±9
2F	125±6	149±8	160±10	168±10
3F	132±7	156±9	170±9	174±9
4F	125±5	145±8	158±9	166±11
5F	126±9	149±9	160±11	171±12

[a]data excerpted from submitted study. Values are in grams, mean ± std. dev., calculated by the investigators. M = male, F = female; 1 = 0 ppm, 2 = 50 ppm, 3 = 500 ppm, 4 = 2500 ppm, 5 = 5000 ppm.
*p<0.05, **p<0.01, ***p<0.001.

D. Clinical Pathology: (1) Hematology- At study termination, blood was obtained from each rat and the following parameters were assessed (* indicates parameters measured only for control and high dose groups):

- Hemoglobin (HGB)
- Hematocrit (HCT)
- Differential blood count*
- Platelet count*
- Mean corpuscular volume (MCV)
- Coagulation time
- Methemoglobin*
- Heinz bodies*
- Reticulocyte count*
- RBC count
- Total leukocyte count
- Mean corpuscular HGB (MCH)
- Mean corpuscular HGB content (MCHC)
- Thromboplastin time
- Activated Partial thromboplastin time (PTT)

Results- No effect of treatment on hematology parameters was apparent.

(2) Clinical Chemistry- Blood was obtained at sacrifice, and the following parameters were determined:

- Blood urea nitrogen (BUN)
- Cholesterol
- Alkaline phosphatase
- Glucose
- Lactic dehydrogenase
- Alanine aminotransferase
- Aspartate aminotransferase
- gamma-Glutamyl transpeptidase
- RBC, serum and brain ChE
- total lipids
- Total bilirubin
- Creatinine
- Uric acid
- Calcium
- Sodium
- Potassium
- Inorganic phosphorus
- Chloride
- Triglycerides

Results- An apparent treatment-related increase in the concentration of uric acid in blood of about 30% was noted in high dose males, and was statistically significant ($p<0.05$). Individual animal data indicated that 4 of 5 high dose male values were higher than the highest control male value, therefore this apparent effect cannot be attributed to an outlier. An increase in triglyceride concentration of about 200% was noted in high dose females that was statistically significant ($p<0.05$). Although other female treatment groups did not appear to be affected, the effect in the high dose group appeared to be treatment-related as 4 of 5 high dose female values were higher than the highest control female value. Total blood lipids were not affected by treatment. Mean serum cholesterol levels were increased in high dose females by about 15%, and although the change was not statistically significant, 4 of 5 values were higher than the highest female control value.

Other clinical chemistry parameters were unaltered by treatment.

(3) Urinalysis- Urine was collected from each animal for about 16 hours on the day prior to sacrifice. The following parameters were determined (* indicates parameters measured only for control and high dose groups):

Appearance	Bilirubin
Color	pH
Protein	Ascorbic Acid
Glucose	Ketone bodies
Hemoglobin	Nitrite
Sediment*	

Results- Urinalysis parameters were not affected by treatment.

(4) Glutamine Synthetase Activity- At sacrifice, livers were immediately excised, and portions were weighed and frozen and liquid nitrogen. Samples of control and high dose livers were later thawed and assayed for glutamine synthetase activity.

Results- No effect of treatment on liver glutamine synthetase activity was noted in high dose males or females.

E. Necropsy Data- Rats were sacrificed by cervical dislocation, and were exsanguinated. Gross examinations of skin, orifices, eyes, teeth, oral mucosa, and internal organs were conducted. The following tissues were removed, fixed, and examined for macroscopic and microscopic changes in control and high dose animals (* indicates tissues that were weighed): Abnormalities, Adrenals*, Bone marrow smear (femoral), Brain*, Colon, Epididymides, Eyes (with optic nerves), Heart*, Jejunum, Kidneys*, Liver*, Lungs*, Ovaries*, Pancreas, Pituitary*, Prostate, Seminal vesicle, Spleen*, Stomach, Testes*, Thymus, Thyroid*, Urinary bladder, and Uterus. Treatment-related changes noted in high

dose rats were also evaluated in low and mid dose tissues, as well as any grossly-visible abnormalities.

The following tissues, recommended by the 1982 Pesticide Assessment Guidelines, were not examined: Aorta, Caecum, Duodenum, Esophagus, Ileum, Lymph nodes, Parathyroid, Peripheral nerve, Rectum, and Salivary glands.

Results- (1) Organ Weights- The only apparent effect of treatment on organ weights was noted in the liver of high dose females. Absolute liver weights were increased by about 11% (6.48 ± 0.56 g control vs. 7.21 ± 0.53 g high dose). Relative liver weights were increased by a similar amount in high dose females (3.87 ± 0.13% control vs. 4.23 ± 0.07% high dose). Only the relative organ weight change was statistically significant ($p<0.05$).

Other organ weights were unaffected by treatment in males or females.

(2) Gross Observations- No treatment-related macroscopic changes were noted. Changes unrelated to treatment included dilated renal pelvis (1 male and female each of the 2500 ppm group, 1 male of the 5000 ppm group).

(3) Microscopic observations- No treatment-related microscopic changes were apparent. The grossly-observed renal pelvis dilation was confirmed by light microscopy, however no parenchymal alterations were noted in those kidneys. Other changes unrelated to treatment, noted at similar incidence in control and treated rats, included isolated Kupffer cell nodules and fat deposits in isolated hepatocytes (liver) and hemosiderosis of the spleen.

Discussion

Dietary exposure of 28 days to 50, 500, 2500, and 5000 ppm produced no effect of treatment on clinical signs, survival, body weight gain, hematology, urinalysis, or organ pathology.

Treatment-related changes were restricted to high dose males and females, and included increases in uric acid (males), triglycerides (females), and increases in absolute and relative liver weights (females). However, no effect on the microscopic appearance of liver or other organs was noted. Although the list of tissues examined did not conform exactly to the number recommended by the 1982 Pesticide Assessment Guidelines, all major organs were examined, and in the opinion of this reviewer, the histopathological examination was adequate.

The submitted study was apparently conducted as a range-finding study for a longer term feeding study. The study does not conform to existing guidelines for subchronic feeding studies, as only 5 rats/sex/dose were studied (10/sex/dose recommended), and the duration of treatment was only 28 days, rather than the recommended 90 days.

NOEL = 2500 ppm
LEL = 5000 ppm Altered clinical chemistry in males and females, increased liver weights in females.

Classification: Core-Supplementary Deficiencies as noted.

DSS:TOX/HED:8-9-86:FILE EUPHOE