



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

007972

JUN 11 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Subject: Clethodim Technical (RE-45601 - Review of Toxicology Data
Submitted by the Registrant to Support Registration of this
Chemical for Food Uses (Terrestrial); Request for Tolerance

Tox Chem. No. 721F
Proj. No. 9-1868
MRID Nos. 410301-01 to 410301-32; 410302-01 and - 02;
409745-06 to 409745-19

From: James N. Rowe, Ph.D. *James N. Rowe*
Review Section I *5/30/90*
Toxicology Branch II
Health Effects Division (H7509C)

To: J. Miller/M. Erumsele, PM Team 23
Herbicide-Fungicide Branch
Registration Division (H7505C)

Thru: Yiannakis M. Ioannou, Ph.D., Head *J.M. Ioannou 5/31/90*
Review Section I
Toxicology Branch II
Health Effects Division (HED) (H7509C)

and

Marcia van Gemert, Ph.D., Chief *Marcia van Gemert 6/4/90*
Toxicology Branch II/HED (7509C)

Registrant: VALENT U.S.A. Corporation; Chevron Chemical Company

Background: Clethodim Technical is a new herbicide for annual and
perennial grass control in cotton and soybeans.

Action Requested: Review toxicology data submitted in support of
registration of Clethodim Technical for terrestrial, food uses.
Consider request for food tolerance.

Recommendations:

DATA GAPS

Based on the evaluation of all data submitted by the
Registrant, the following Guideline toxicology studies have been
identified as data gaps:

1675

1

Technical (RE-45601)81-2

Acute dermal study; submission of information on surface area exposed is required.

82-1

13 week feeding study in dogs; analytical data required.

82-2

21-Day dermal study in rats; submission of information on surface area exposed is required.

82-5

90-Day neurotoxicity study; 40 CFR Section 158.340 requires this study if acute oral, dermal or inhalation studies indicate neurotoxicity or neuropathy. Findings in the acute oral toxicity study (MRID 409745-07) indicate, 1) neurotoxicological signs including salivation, hyperreactivity, decreased motor activity, unsteady gait, clonic convulsions and collapse which occurred within 30 minutes to 3.5 hours after dosing, and 2) neuropathology as evidenced by 2/5 females (1.45 g/kg) with a trace gliosis with/without trace focal vacuolar changes in the spinal nerve in the lower lumbar area.

83-1

One year dog study; analytical data must be submitted.

End-Use Product (SELECT 2.0 EC)81-2

Acute dermal study; information of surface area exposed to test material is required.

81-3

Acute inhalation study; new study on formulation not dilution is required.

81-6

Dermal sensitization test; new study required.

82-2

21-Day dermal study; submission of information on surface exposed to test material is required.

The request for consideration of a food tolerance is subject to submission of the additional information required to complete toxicology data requirements for the technical and end-use products.

LABELING CONSIDERATIONS

Based on the review of the acute toxicity studies, the following wording should be used on the proposed labels:

Technical - The signal word "WARNING" and the precautionary statement, "Causes... skin...irritation..." should appear on the label.

End-Use Product - The signal word "WARNING" and the precautionary statement, "Causes eye...irritation..." should appear on the label.

Review of Data

Toxicology Branch II has completed the review of all toxicology studies submitted by the registrants on Clethodim Technical. Studies reviewed (accompanied by major findings) were as follows:

Guideline 81-1

Acute Oral Toxicity - Rats (MRID 410301-01)

RE-45601 Technical, 83.8% ai; RE-51228, 99.9%, ai.

- LD50 of RE-51228 > 1.4 gm/kg (female rats), Acute Tox Category
III. LD50 of RE-45601 (female rats) cannot be determined.

Classification - Core Minimum, RE-51228
Core Supplementary, RE-45601

Acute Oral Toxicity - Rats (MRID 410301-02)

RE-47719, 98.6% ai.

- LD50 of RE-47719 > 1.4 gm/kg (female rats), Acute Tox Category
III.

Classification - Core Minimum

Acute Oral Toxicity - Rats (MRID 409745-06)

SELECT 2.0 EC, 26.1% RE-45601 (Clethodim Technical) ai.

- LD50: males, 3.61 g/kg; females 2.92 g/kg; Acute Tox Category
III.

Classification: Core Guideline.

Acute Oral Toxicity - Rats (MRID 409745-07)

RE-45601 (Clethodim Technical), 83.3% ai.

- LD50: males, 1.63 g/kg; females 1.36 g/kg; Acute Tox Category
III.

Classification: Core Guideline.

Acute Oral Toxicity - Mice (MRID 409745-08)

RE-45601 (Clethodim Technical), 83.3% ai.

LD50: males, 2.57 g/kg; females 2.43 g/kg; combined sexes,

2.46; Acute Tox Category III.

Classification: Core Guideline.

Guideline 81-2

Acute Dermal Toxicity - Rabbits (MRID 409745-10)

RE-45601 (Clethodim Technical), 83.3% ai.

LD50: > 5.0 g/kg (both sexes); Acute Toxicity Category IV.

Classification: Core Supplementary.

Acute Dermal Toxicity - Rabbits (MRID 409745-11)

SELECT 2.0 EC, 26.0% RE-45601 ai.

LD50: > 5.0 g/kg (both sexes); Acute Toxicity Category IV.

Classification: Core Supplementary.

Guideline 81-3

Acute Inhalation - Rats (MRID 409745-12)

RE-45601 (Clethodim Technical), 83.3% ai.

LC50: > 3.9 mg/L (both sexes); Acute Toxicity Category III.

Classification: Core Minimum.

Acute Inhalation - Rats (MRID 409745-13)

SELECT 2.0 EC, 26.1% ai (Clethodim Technical), in 1.8% mixture in distilled water.

LC50: > 5.4 (4.4 to 5.8) mg/L (both sexes) of mixture (0.33 mg/L of ai); Acute Toxicity Category III for aqueous mixture.

Classification: Core Minimum.

Guideline 81-4

Acute Eye Irritation - Rabbits (MRID 409745-14)

RE-45601 (Clethodim Technical), 83.3% ai.

Clearing of conjunctival irritation after 72 hours; Acute Toxicity Category III.

Classification: Core Guideline.

Acute Eye Irritation - Rabbits (MRID 409745-15)

SELECT 2.0 EC, 26.1% ai (Clethodim Technical).

Cornea and iris significantly affected by test material. All areas of eye had returned to a normal appearance by 14 days. Acute Eye Irritation Category II.

Classification: Core Minimum.

Guideline 81-5

Acute Skin Irritation - Rabbits (MRID 409745-16)

RE-45601 (Clethodim Technical), 83.2% ai.

Slight to severe erythema and slight to moderate edema observed at 72 hours postdosing in the first of two studies. A second study resulted in less severe findings (slight erythema and no edema by 72 hours). Acute Skin Irritation Category II.

Classification: Core Minimum.

Acute Skin Irritation - Rabbits (MRID 409745-17)

SELECT 2.0 EC, 26.0% ai (Clethodim Technical).

Slight to well-defined erythema and edema at 72 hours after topical application. Acute Skin Irritation Category III.

Classification: Core Minimum.

Guideline 81-6

Dermal Sensitization - Rabbits (MRID 409745-18)

RE-45601 (Clethodim Technical), 83.4% ai.

Repeated dermal application (10x) of 5.0% and 0.5% solutions of RE-45601 over a 3 week period did not induce any evidence of dermal sensitization.

Classification: Core Guideline.

Dermal Sensitization - Rabbits (MRID 409745-19)

SELECT 2.0 EC, 26.1% ai (Clethodim Technical).

Repeated dermal application (10x) of 10.0% and 1.0% solutions of SELECT 2.0 EC over 3 weeks followed by a challenge exposure (1.0%) after two weeks did not produce dermal sensitization. However, the severity and persistence of the skin irritation

response observed at the fifth and 10th induction application for the 10% test material strongly suggests a potential dermal sensitization response.

Classification: Core Supplementary.

Guideline 82-1

4-Week Feeding Study - Mice (MRID 410301-03)

RE-45601 Technical, 83.3% ai.

RE-45601 technical was administered to male and female CD-1 mice at dietary levels of 0, 100, 250, 625, 1500 and 4000 ppm for 4 weeks. There was a dose-related decrease in red cell mass at 1500 and 4000 ppm as manifested by decreased erythrocytes, hemoglobin and hematocrit values. Increased liver weights and increased hypertrophy of centrilobular hepatocytes in high dose mice (both sexes) were observed. Increased liver weights were also observed in male mice administered 1500 ppm in the diet. The systemic NOEL was 625 ppm.

Classification - Core Supplementary.

5-Week Feeding Study - Rats (MRID 410301-04)

RE-47719, Clethodim imine sulfone, 99.3% ai.

RE-47719 was fed to male and female rats at nominal concentrations of 0, 100, 1000 or 8,000 ppm for 5 weeks. Compound-related effects (both sexes) in the high dose group included significant increases in serum cholesterol, absolute and relative liver weights and decreases in food consumption (females only). The NOEL is 1000 ppm (calculated to be 871 ppm) and the LOEL is 8000 ppm (calculated to be 7820 ppm).

Classification - Core Supplementary.

5-Week Feeding Study - Rats (MRID 410301-05)

RE-51228 (5-Hydroxy Sulfone), 94.8% ai.

RE-51228 was fed to male and female Sprague-Dawley rats at nominal concentrations of 0, 100, 1000 and 8000 ppm for 5 weeks. No compound-related effects were noted. The systemic NOEL was 8000 ppm (HDT; calculated dose of 7290 ppm).

Classification - Core Supplementary.

13-Week Feeding Study - Dogs (MRID 410301-06)

RE-45601 technical, 83.3% ai.

RE-45601 was fed to male and female dogs at dietary levels of 0, 1, 25, 75 or 125 mg/kg/day for 90 days. Absolute (statistically significant; HDT) and relative liver weights (75, 125 mg/kg/day) were increased in both sexes. Liver lesions (cytoplasmic vesiculation/vacuolation) were increased in severity in high dose groups (both sexes) and mean serum cholesterol and alkaline phosphatase were significantly increased in females in the high dose group. Based upon the increase liver weights associated with histopathological changes of the liver and serum elevations in liver-related enzymes (cholesterol, alkaline phosphatase), the LOEL is 75 mg/kg/day and the NOEL is 25 mg/kg/day.

Classification - Core Supplementary. Analytical data on concentrations and stability of test material in capsules must be submitted.

13-Week Feeding Study - Rats (MRID 410301-07)

RE-45601 Technical, 83.4% ai.

RE-45601 Technical was fed to male and female rats at 0, 50, 500, 2500 or 5000 ppm for 13 weeks followed by a 6-week recovery period. Body weights and body weight gains were depressed (both sexes) at 2500 and 5000 ppm and body weights remained depressed in HDT females throughout recovery. Food consumption was decreased at 5000 ppm (both sexes). Cholesterol, total protein, and globulin levels were increased in 5000 ppm males and the 2500 and 5000 ppm males and females had increases in absolute and relative liver weights. These findings were related to an increase in centrilobular hypertrophy of the liver (both sexes). Liver weights in females remained increased following recovery. The LOEL is 2500 ppm and the NOEL is 500 ppm.

Classification - Core Minimum.

5-Week Feeding Study - Rats (MRID 410301-08)

RE-45601 (SX-1718), high purity, 96.2% ai; RE-45601, process neutrals (SX-1717), 3.2% ai.

RE-45601 (high purity) and RE-45601 (process neutrals) were fed to male and female Sprague-Dawley rats for 5 weeks at dietary levels of 6800 and 1200 ppm, respectively. Body weights (both sexes) were depressed for both treatment groups but to a greater extent with pure material. RBCs, hematocrit and hemoglobin concentrations were decreased with SX-1718 but not SX-1717. Liver weights (absolute, relative) of both treatment groups (females only with process neutrals) were increased and centrilobular hypertrophy was exhibited in both treatment groups (to a greater extent with the higher purity material). The high purity RE-45601 appeared to be more toxic than the process neutrals. No NOEL was established.

Classification - Core Supplementary.

Guideline 82-2

21-Day Dermal - Rats (MRID 410301-09)

RE-45601 Technical, 83.2% ai.

Male and female rats (6/dose group) were exposed topically 6 hours/day for 21 days to concentrations of 0, 10, 100 or 1000 mg/kg/day of RE-45601. On the basis of anogenital discharge and staining of the anogenital fur in both sexes, depressions in food efficiency and body weight gain in males, and increases in absolute and relative liver weights in females dosed at 1000 mg/kg/day, the systemic LOEL is 1000 mg/kg/day and the systemic toxicity NOEL is 100 mg/kg/day. There was dose-related increase in the incidence and severity of skin irritation in both males and females of the 10-, 100- and 1000 mg/kg/day groups, the LOEL for dermal irritation being 10 mg/kg/day; a NOEL was not established.

Classification - Core Supplementary. Information regarding the size of the dermal site of application (cm²) and the percent of the body surface exposed for each dose group must be supplied by the registrant.

21-Day Dermal - Rats (MRID 410302-01)

Select 2.0 EC, 26.3% ai.

Rats were exposed dermally for 6 hours/day to 21 applications of 0, 10, 50 or 100/200 mg/kg/day of Select 2.0 EC. Due to excessive skin irritation in both sexes the top dose was reduced to 100 mg/kg. On the basis of increased reticulocyte count in HDT males and females, the systemic toxicity LOEL is 100 mg/kg/day and the systemic NOEL is 50 mg/kg/day. Due to treatment-related increases in the incidence and severity of skin irritation at all dose-levels, the dermal irritation LOEL is 10 mg/kg/day; a NOEL was not established.

Classification - Core Supplementary. Information regarding the size of the dermal site of application (cm²) and the percent of the body surface exposed for each dose group must be supplied by the registrant.

Guideline 83-1

1-Year Feeding Study - Dogs (MRID 410301-11)

RE-45601 technical, 83.3% ai.

RE-45601 technical was fed to male and female dogs at dose levels of 0, 1, 75 or 300 mg/kg/day for 1 year. Absolute and

relative liver weights were increased in high dose males and females associated with histological changes (centrilobular-to-midzone hepatocellular enlargement) at the high dose level. Increases in total cholesterol, alanine aminotransferase, alkaline phosphatase and triglyceride levels were also seen in both sexes at the HDT. A variety of other hematology and clinical chemistry parameters were affected at the HDT [significant decreases in mean RBC values (both sexes), decreases in hemoglobin and hematocrit (females only)] or mid and high dose levels (decreases in glucose) (females, both sexes, resp.). The LOEL is 75 mg/kg/day based on the increased absolute and relative liver weights in association with histopathological changes and increased liver-related enzymes. The NOEL is 1 mg/kg/day.

Classification - Core Supplementary. Information on the stability and concentrations of test material administered in the capsules must be submitted.

Guideline 83-2

18-Month Oncogenicity Study - Mice (MRID 410301-12)

RE-45601 Technical, 83.3% ai.

RE-45601 was administered in the diet to groups of 60 male and female mice at levels of 0, 20, 200, 1000 or 3000 ppm for 78 weeks. Survival incidence for both sexes at the high dose was significantly lower at week 78. Depressions in hematology parameters were noted at the 3000 ppm dose level. Absolute and relative liver weights were increased in 1000 and 3000 ppm males and 3000 ppm females after 52 and 78 weeks, respectively. Centrilobular hypertrophy, increased pigment and bile duct hyperplasia were noted in both sexes at the mid-high and high doses. Systemic amyloidosis was increased among unscheduled deaths in the HDT. RE-45601 was not oncogenic. Based on treatment-related effects on survival, red cell mass, liver weights and microscopic findings in the lung and liver, the NOEL and LOEL are 200 and 1000 ppm, respectively.

Classification - Core Guideline.

Guideline 83-5

2 year Chronic/Oncogenicity Study - Rats (MRID 410301-21)

RE-45601 technical, 83% ai.

RE-45601 technical was fed to rats for 2 years at dose levels of 0, 5, 20, 500 or 2500 ppm. Mean body weights (10% during first 90 days) and body weight gains were depressed in high dose groups (both sexes; throughout study). Food consumption (both sexes) and food efficiency (males only) were also decreased. Liver weights

were increased in high dose animals (both sexes) and this was accompanied by centrilobular hypertrophy in some rats during the first year on test. Liver weights were not affected at study termination nor were there any compound-related histological changes noted. There were no macroscopic or microscopic findings in other organs that were related to dosing. Clethodim was not oncogenic under the conditions of this bioassay. The LOEL for systemic toxicity is 2500 ppm (100 mg/kg/day), and the NOEL is 500 ppm (19 mg/kg/day).

Classification - Core Guideline.

Guideline 83-3

Pilot Developmental Toxicity/Full Developmental Toxicity Study - Rabbits

(MRID's 410301-13, 410301-15)

RE-45601 technical, 82.6% ai.

New Zealand White rabbits were administered RE-45601 via gavage at 0, 25, 100 or 300 mg/kg/day during gestational days 7-19. Maternal toxicity (mid and high dose groups) was manifested as clinical signs of toxicity and reduced weight gain and food consumption during treatment. Based on these results, the maternal NOEL and LOEL were 25 and 100 mg/kg/day, respectively. Developmental toxicity was not observed in this study and, therefore, the NOEL is 300 mg/kg/day.

Classification - Core Minimum.

Pilot Developmental Toxicity/Full Developmental Toxicity Study - Rats

(MRID's 410301-14, 410301-16)

RE-45601 technical, 82.6% ai.

Groups of mated female rats were administered RE-45601 technical at doses of 0, 10, 100, 350 or 700 mg/kg/day, by gavage, during days 6-15 of gestation. Based upon reductions in body weight gain and clinical signs of toxicity (excessive salivation), the NOEL and LOEL for maternal toxicity were 100 and 350 mg/kg/day, respectively. The NOEL and LOEL for developmental toxicity were 100 and 350 mg/kg/day, based on reductions in fetal body weight and increases in skeletal anomalies at 350 and 700 mg/kg/day.

Classification - Core Minimum.

Developmental Toxicity Study - Rats (MRID 410301-17)

RE-47719, 5-[2-(Ethylsulfonyl)propyl]-3-hydroxy-2-(1-imino-propyl)-2-cyclohexen-1-one, 98.6% pure.

Mated female rats were administered RE-47719 via gavage at dose levels of 0, 10, 100 or 700 mg/kg/day during days 6-15 of gestation. Maternal toxicity includes excessive salivation (highest dose tested) and reduced body weight gain and food consumption in mid and high dose groups. The maternal NOEL and LOEL were 10 and 100 mg/kg/day. Based upon significant reductions in fetal body weight/litter and fetal sternal ossification sites/fetus/litter and significantly increased litter and fetal incidences of cervical rib at the high dose, the NOEL and LOEL for developmental toxicity are 100 and 700 mg/kg/day, respectively.

Classification - Core Supplementary. Inadequate numbers of animals (10/dose group) were used.

Developmental Toxicity Study - Rats (MRID 410301-18)

RE-51228, 5-Hydroxyl sulfone, 99.1% ai.

Female rats were administered RE-51228 via gavage at 0, 10, 100 or 700 mg/kg/day during gestational days 6-15. Neither maternal or developmental toxicity were observed in this study. Therefore, the NOELs were 700 mg/kg/day.

Classification - Core Supplementary. Inadequate numbers of animals (10/dose group) were used and the dose levels did not produce any evidence of toxicity.

Guideline 83-4

Pilot Reproduction/Two Generation (1 Litter) Reproduction Study - Rats

(MRID's 410301-19, 410301-20)

RE-45601 technical, 83.2% ai.

In a reproductive toxicity study, groups of rats were fed diets containing 0, 5, 20, 500 or 2500 ppm of RE-45601 technical for two consecutive generations (1 litter/generation). The NOEL and LOEL for parental toxicity were 500 and 2500 ppm (approximately 51 and 263 mg/kg/day, respectively), based on reductions in body weight, particularly in males, and decreased food consumption during both generations. No effects on fertility, length of gestation, or growth and development of offspring were observed. Therefore, the NOEL for reproductive toxicity was 2500 ppm (HDT).

Classification - Core Minimum.

Guideline 84-2: MUTAGENICITY ASSAYS:

Ames Assay-Salmonella (MRID 410301-22)

RE-45601, 83% ai.

Negative for induced gene reversions in Ames strains of Salmonella treated with/without activation up to cytotoxic levels (10,000 ug/plate).

Classification - Acceptable.

Ames Assay-Salmonella (MRID 410301-23)

RE-45601, 83.3% ai.

Negative for inducing reversions with/without activation in Salmonella TA strains and E. coli WP2 uvrA exposed up to cytotoxic levels (10,000 ug/plate).

Classification - Acceptable.

24) DNA Damage/Repair in vivo/in vitro (mouse UDS) (MRID 410301-

RE-45601, 83.3% ai.

Negative for inducing unscheduled DNA synthesis (UDS) in hepatocytes drawn from mice treated orally up to toxic dose (5000 mg/kg).

Classification - Acceptable.

Chromosomal Damage in vivo - Rat Bone Marrow (MRID 410301-25)

RE-45601, 83.3% ai.

Negative for inducing chromosome aberrations in male and female rats treated by acute oral gavage up to toxic doses (1500 mg/kg).

Classification - Acceptable.

Gene Mutation (Ames) (MRID 410301-26)

RE-51228 (5-hydroxy clethodim sulfone), 99.1%.

Negative for inducing reverse gene mutation in Salmonella TA his- (Ames) and E. coli WP2 uvrA (tryp-) exposed with and without activation up to cytotoxic levels (5000 ug/plate).

Classification - Acceptable.

Gene Mutation (Ames) (MRID 410301-27)

RE-47719 (Clethodim imine sulfone), 98.6%.

Negative for inducing reverse gene mutation in Salmonella TA strains (Ames) and E. coli WP2 uvrA (tryp-) exposed with and without activation up to doses of 10,000 ug/plate.

Classification - Acceptable.

Chromosomal Aberrations in vitro (CHO) (MRID 410301-28)

RE-45601 technical, 81.4% ai.

Positive for increased structural chromosome aberrations (but only in the absence of metabolic activation) at doses near the limit of solubility and/or cytotoxicity (1.0 and 1.2 uL/mL).

Classification - Acceptable.

Chromosome Aberrations in vitro (CHO) (MRID 410301-29)

RE-45601, purified, 96.1%.

Negative for inducing chromosome aberrations in both nonactivated and activated CHO cells exposed up to levels of insolubility (0.3 and 1.0 uL/mL).

Classification - Acceptable.

Chromosome Aberrations in vitro (CHO) (MRID 410301-30)

RE-47719 (clethodim imine sulfone), 99.3%.

Negative for inducing chromosome damage in CHO cells exposed w/without activation to the limit of the test article's solubility (400 ug/mL).

Classification - Acceptable.

Chromosome Aberrations in vitro (CHO) (MRID 410301-31)

RE-51228 (5-hydroxy clethodim sulfone), 99.9%.

Negative for inducing structural chromosome aberrations in CHO cells exposed w/without activation to the limit of the test article's solubility (2500 ug/mL).

Classification - Acceptable.

Guideline 85-1

Metabolism Study - Rats (MRID 410301-32)

Clethodim; [^{14}C]-labeled material was >96% pure; analytical grade Clethodim was 99% pure.

Groups of 5 male and female rats were given by gavage a single oral dose of 4.5 or 450 mg [^{14}C]Clethodim (low and high dose groups), respectively. An additional group of rats (10/sex) received single daily doses of unlabeled Clethodim (4.5 mg/kg/day) for 14 days; 5 animals/sex were chosen randomly to receive 4.5 mg/kg/day of labeled material (repeated-dose group). Another dose group (10 male rats) were given a single oral dose of labeled Clethodim, 450 mg/kg (supplemental dose group), for identification of urinary metabolites.

Clethodim is readily absorbed and eliminated with nearly all (87-92 %) of the [^{14}C] dose recovered from urine within 7 days after compound administration. Smaller amounts were recovered from feces (9-17%) and expired air (less than or equal to 1.0%). Gastrointestinal absorption was estimated at 89-96% (no sex, dose or treatment-related differences). There was no evidence of bioconcentration following multiple exposures; the adrenals at 7 days post-dosing had the highest concentrations of radiolabel (0.07 to 0.22 ppm for low and repeated doses; 5.4 to 13 ppm for high-dose rats). Clethodim was extensively metabolized with <1 percent eliminated as the unchanged parent compound. The predominant metabolite recovered was clethodim sulfoxide (48 to 63 % of administered label after 48 hrs).

Classification - Acceptable.

Guideline 85-3

Dermal Absorption - Rats (MRID 410302-02)

SELECT 2EC (25.6% Clethodim); [^{14}C]-labeled Clethodim was 98.7% radiochemically pure.

Application to a 10 cm² area of the rat's back resulted in the following percent absorption (blood, urine, feces, carcass and cage wash):

Exposure time(hrs)	0.05 mg/rat	0.5 mg/rat	5.0 mg/rat
2	8.5	4.4	2.0
10	28.4	14.4	6.7
24	42.8	34.1	29.7

Classification - Acceptable.



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

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MAY 29 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Chemical (Caswell) No.: 721F
RD Record No.: 239,859/860/861
HED Project No.: 9-18682

MEMORANDUM

SUBJECT: Clethodim - Review and Evaluate Toxicity Data
(Mutagenicity) Under MRID Nos. 410301-22, -23, -24,
-25, -26, -27, -28, -29, -30, and -31
EPA ID No. 239-EANb

FROM: Irving Mauer, Ph.D., Geneticist *Irving Mauer* 5-18-90
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: James Rowe, Ph.D. *James Rowe*
Toxicology Branch II - Herbicide, Fungicide and
Antimicrobial Support
Health Effects Division (H7509C)

THRU: Karl P. Baetcke, Ph.D., Chief *Karl P. Baetcke* 5/25/90
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: Chevron Chemical, Richmond, CA

Request

At your request, the following mutagenicity studies were reviewed for HFAS/HED:

1. Vol. 24: Microbial/Mammalian Microsome Mutagenicity Plate Incorporation Assay with RE-45601 (83% Purity, SX1688) (Ames), performed by Chevron Environmental Health Center, Richmond, CA, Lab. Project S-2760, Final Report dated January 13, 1986 (EPA MRID No. 410301-22).

2. Vol. 25: Microbial/Mammalian Microsome Mutagenicity Plate Incorporation Assay with RE-45601 Technical (83.3%, SX1688) (Ames), performed by Chevron Environmental Health Center, Richmond, CA, Lab. Project S-2859, Final Report dated April 28, 1986 (EPA MRID No. 410301-23).
3. Vol. 26: In Vivo-In Vitro Hepatocyte DNA Repair Assay: In Vitro Evaluation of Unscheduled DNA Synthesis (UDS) Following Oral Administration of Chevron RE-45601 Technical to B6C3F1 Mice (UDS), performed by SRI, Menlo Park, CA, Lab. Project S-2762, Final Report dated August 7, 1986 (EPA MRID No. 410301-24).
4. Vol. 27: Cytogenetics Assay in Bone Marrow Cells of Rats Following Acute Oral Exposure to RE-45601 Technical (Rat BM), performed by Microbiological Associates, Bethesda, MD, Lab. Project No. S-2864, Final Report dated February 11, 1987 (EPA MRID No. 410301-25).
5. Vol. 28: Microbial/Mammalian Microsome Plate Incorporation Mutagenicity Assay With RE-51228 (Ames), performed by Chevron Environmental Health Center, Richmond, CA, Lab. Project S-3111, Final Report dated December 7, 1987 (EPA MRID No. 410301-26).
6. Vol. 29: Microbial/Mammalian Microsome Plate Incorporation Mutagenicity Assay With RE-47719 (SX-1800) (Ames), performed by Chevron Environmental Health Center, Richmond, CA, Lab. Project No. S-3155, Final Report dated October 19, 1988 (EPA MRID No. 410301-27).
7. Vol. 30: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with Chevron RE-45601 Technical (CHO), performed by Microbiological Associates, Bethesda, MD, Lab. Project S-2761, Final Report dated July 22, 1986 (EPA MRID No. 410301-28).
8. Vol. 31: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells With Purified Chevron RE-45601 (CHO), performed by Microbiological Associates, Bethesda, MD, Lab. Project S-2865, Final Report dated December 15, 1986 (EPA MRID No. 410301-29).

9. Vol 32: Chromosome Aberration in Chinese Hamster Ovary (CHO) Cells: RE-47719. Final Toxicology Report (CHO), performed by Microbiological Associates, Bethesda, MD, Lab. Project S-3156, Final Report dated November 3, 1968 (EPA MRID No. 4103C1-30).
10. Vol. 33: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells: RE 51228 (CHO), performed by Microbiological Associates, Bethesda, MD, Lab. Project S-3160, Final Report dated November 7, 1988 (EPA MRID No. 410301-31).

TB Conclusions

[See detailed reviews appended to this memorandum.]

Vol./Study (MRID No.)	Reported Results	TB Evaluation
24/Ames (410301-22)	Negative w/without MA at cytotoxic doses (10,000 ug/plate) in Ames strains.	ACCEPTABLE
25/Ames (410301-23)	Negative w/without MA at cytotoxic doses (10,000 ug/plate) in Salmonella and E. coli.	ACCEPTABLE
26/UDC (410301-24)	Negative for inducing unscheduled DNA synthesis (UDS) in mice treated orally up to lethal levels (5000 mg/kg).	ACCEPTABLE
27/BM/CA (410301-25)	Negative for chromosome aberrations in rats treated acutely (oral gavage) up to toxic doses (1500 mg/kg).	ACCEPTABLE
28/Ames (410301-26)	Negative for inducing gene mutation in bacterial cultures treated up to cytotoxic doses (5000 ug/plate), w/without activation.	ACCEPTABLE
29/Ames (410301-27)	Negative for inducing gene mutations in bacterial cultures treated up to cytotoxic doses (10,000 ug/mL), w/vithout activation.	ACCEPTABLE

Vol./Study (MRID No.)	Reported Results	TB Evaluation
30/CHO/CA (410301-28)	Positive in the absence of activation for induced structural chromosome damage at high concentrations (1.0 and 1.2 uL/mL).	ACCEPTABLE
31/CHO/CA (410301-29)	Negative for inducing chromosome aberrations in CHO cells treated up to levels of insolubility (0.3 and 1.0 uL/mL).	ACCEPTABLE
32/CHO/CA (410301-30)	Negative for inducing chromosome aberrations in CHO cells treated up to solubility limit (400 ug/mL).	ACCEPTABLE
33/CHO/CA (410301-31)	Negative for inducing chromosome damage in CHO cells treated to the limit of solubility (2500 ug/mL).	ACCEPTABLE

Attachments (DERs)

Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IRS (#7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (#7509C)

Irving Mauer
5-18-90

Karl P. Baetcke
5/25/90

DATA EVALUATION REPORT

007972

I. SUMMARY

MPID (Acc) No.: 410301-22
ID No.: 239-FANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-1868Z

Study Type: Mutagenicity - Gene mutation in bacteria
(Ames Assay)

Chemical: Clethodim

Synonyms: PE-45601

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: (Chevron) Environmental Health Center
(CEHC), Richmond, CA

Title of Report: Microbial/Mammalian Microsome Mutagenicity
Plate Incorporation Assay with PE-45601
(83% Purity, SX-1698).

Author: M.L. Machado

Study Number: S-2760

Date of Issue: January 13, 1986

TB Conclusions:

Negative for induced gene reversions in Ames strains
of Salmonella treated with/without activation up to
cvtotoxic levels (10,000 μ g/plate).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RF-45601 Technical

Description: Dark amber liquid
 Batch (Lot): SX-1688
 Purity (%): 83
 Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: Salmonella typhimurium LT2
 Strain: TA98, TA100, TA1535, TA1537 (his-)
 Source: B.N. Ames, UCal, Berkeley, CA

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of clethodim when administered in vitro to Salmonella TA strains, according to standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Triplicate cultures of each of the four Ames strains of Salmonella were exposed at 37 °C for 48 hours to test compound at dose levels ranging from 100 to 10,000 ug/plate (based on previous cytotoxicity testing), both in the absence and presence of a mammalian metabolic system consisting of liver microsomal enzymes (S9) from male Sprague-Dawley rats injected with the PCB mixture, Aroclor 1254, plus NADP(H)-generating cofactors (S9 Mix). In addition to concurrent solvent (DMSO) controls, mutagens specific for each bacterial strain* served as positive controls.

<u>*Strain</u>	<u>Activation</u>	<u>Positive Controls</u>	<u>Amt/Plate</u>
TA98	+	2-Aminoanthracene	2 <u>ug</u>
TA98	-	2-Nitrofluorene	10 <u>ug</u>
TA100	+	2-Aminoanthracene	2 <u>ug</u>
TA100	-	Sodium Azide	1 <u>ug</u>
TA1535	+	2-Aminoanthracene	2 <u>ug</u>
TA1535	-	Sodium Azide	1 <u>ug</u>
TA1537	+	2-Aminoanthracene	2 <u>ug</u>
TA1537	-	9-Aminoacridine	50 <u>ug</u>

At harvest (48-hour incubation), revertent (his⁺) colonies were counted automatically (Artek 880) or by hand (Quebec Darkfield Counter), and the quality of background lawn evaluated under low magnification (28X). Standard criteria for both judging whether an assay was valid (page 4 of the Final Report), as well as determining the nature and degree of mutagenic response (Appendices B to D) were provided.

E. Results:

[NB: Data from previous dose-selection cytotoxicity testing mentioned in the text (SQCALs 2410, 2455, 2465, 2476) were not provided in this Report.]

The test material was not completely miscible in tissue culture medium at 3300 ug/plate and above, and was slightly toxic (as evidenced by reduction in his⁺ revertents) to TA100 under activated conditions (+S9) at both 3300 and 10,000 ug/plate, as well as to nonactivated (-S9) cultures of TA100 and TA1537 at the HDT (Report Table 1, appended to this DEP). At no dose, however, did the test substance increase the incidence of revertents over DMSO controls. By contrast, all positive controls responded as expected, with increases ranging from 5 to over 100 times solvent values.

The author concluded that RE-45601 Technical was not mutagenic in this assay.

F. TB Evaluation: ACCEPTABLE

This assay was conducted under adequate conditions and appropriate controls such that the negative result reported is judged valid.

Attachment (Data Table)

007972

Attachment I
Data Table

CLETHODIM

Page 24 is not included in this copy.

Pages _____ through _____ are not included.

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- ☐ Identity of product inert ingredients.
 - ☐ Identity of product impurities.
 - ☐ Description of the product manufacturing process.
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 - ☐ Identity of the source of product ingredients.
 - ☐ Sales or other commercial/financial information.
 - ☐ A draft product label.
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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Irving Mauer
5-18-90
Karl P. Baetcke
5/25/90

DATA EVALUATION REPORT

007972
007972

I. SUMMARY

MRID (Acc) No.: 410301-23
ID No.: 239-EANh
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-18682

Study Type: Mutagenicity - Gene mutation in bacteria
(Ames Assay)

Chemical: Clethodim

Synonyms: RE-45601

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: (Chevron's) Environmental Health Center
(CEHC), Richmond, CA

Title of Report: Microbial/Mammalian Microsome Mutagenicity
Plate Incorporation Assay with RE-45601
(83.3% Purity, SX-1688).

Author: M.L. Machado

Study Number: S-2859

Date of Issue: April 28, 1986

TB Conclusions:

Negative for inducing reversions with/without activation
in Salmonella TA strains and *E. coli* WP2 uvrA exposed up to
cytotoxic doses (10,000 μ g/plate).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

007972

A. Test Material - RE-45601

Description: Dark amber liquid
Batch (Lot): SX-1688
Purity (%): 83.3
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacteria

Species: (1) Salmonella typhimurium LT2; and
(2) Escherichia coli WP2
Strains: (1) TA1535, TA1537, TA98, TA100 (his⁻)
(sal); and
(2) uvrA⁻ (trv⁻)
Source: (1) R.N. Ames, UCal, Berkeley, CA;
(2) S. Haworth, Microbiological Associates,
Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of clethodim when administered in vitro to histidine-deficient (his⁻) cultures of Salmonella (TA1535, TA1537, TA98, TA100) and trvtonphane-deficient E. coli (WP2 uvrA), according to standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - After cytotoxicity testing (with TA100 only exposed to test article at doses ranging from 3 to 10,000 μ g/plate), triplicate cultures of all five bacterial strains were exposed for 48 hours at 37 °C to RE-45601 at doses ranging from 100 to 10,000 μ g/plate, both in the absence and presence of a mammalian metabolic activation system consisting of liver microsomal enzymes (S9) from male Sprague-Dawley rats pretreated with the PCB mixture, Aroclor 1254, together with NADP(H)-generating cofactors (S9 Mix). In addition to solvent (DMSO) controls,

mutagens specific for each bacterial strain* were employed concurrently as positive controls.

At harvest (48 hours incubation), revertent colonies (his⁺) were counted (by Artek 880 or Quebec Parkfield Counters), and the background lawn evaluated under low magnification (25X).

Standard criteria for judging the validity of an assay for evaluation of response (page 4 of Final Report), as well as those for determining the nature and degree of mutagenic response (Report Appendices B to D) were both provided.

- E. Results - In preliminary cytotoxicity testing, "slight toxicity was observed at 10 mg/plate" (the HDT - data not shown). In the first trial of the mutagenesis assay per se, insolubility of the test article was noted at 3300 ug/plate and above, and (again) slight cytotoxicity in TA100 cultures at the HDT (Report Table 1, appended to this DER). However there were no increases over control in revertents at any dose in any of the five bacterial strains (Tables 1 and 2).

In the second experiment, no induced increase in revertents was found, except for a singular instance of a twofold increase in nonactivated TA98 cultures (46 ± 7 vs. 22 ± 6) treated at the highest dose. Since a comparable increase was not observed in the first trial, the author considered this result of uncertain biological significance.

Hence, the investigator concluded that RE-45601 was not mutagenic in four TA strains of Salmonella or in the WP2 uvrA strain of E. coli.

*Strain	Activation	Positive Controls	Amt/Plate
TA98	+	2-Aminoanthracene	2 <u>ug</u>
TA98	-	2-Nitrofluorene	10 <u>ug</u>
TA100	+	2-Aminoanthracene	2 <u>ug</u>
TA100	-	Sodium Azide	1 <u>ug</u>
TA1535	+	2-Aminoanthracene	2 <u>ug</u>
TA1535	-	Sodium Azide	1 <u>ug</u>
TA1537	+	2-Aminoanthracene	2 <u>ug</u>
TA1537	-	9-Aminoacridine	50 <u>ug</u>
WP2-uvrA	+	2-Aminoanthracene	80 <u>ug</u>
WP2-uvrA	-	1-Ethyl-2-nitro-3-nitrosoquinidine	2 <u>ug</u>

007972

F. TB Evaluation - ACCEPTABLE.

Since these independent assays were conducted under adequate procedures and controls, we agree with the conclusion that the test article is nonmutagenic.

Attachments (Data Tables)

007972

Attachment I
Data Tables

CLETHODIM

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IPS (H7509C)
Secondary Reviewer: Karl P. Raetcke, Ph.D., Chief
Toxicology Branch I - IPS (H7509C)

Irving Mauer
5-18-90

Karl P. Raetcke
5/25/90

DATA EVALUATION REPORT

007947

I. SUMMARY

MRID (Acc) No.: 410301-24
ID No.: 239-FANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-1868Z

007972

Study Type: Mutagenicity - DNA damage/repair in vivo/in vitro (mouse UDS)

Chemical: Clethodim

Synonyms: RE-45601

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: SRI, Menlo Park, CA

Title of Report: In vivo/in vitro Hepatocyte DNA Repair Assay: In Vitro Evaluation of Unscheduled DNA Synthesis (UDS) Following Oral Administration of Chevron RE-45601 Technical to B6C3F1 Mice.

Authors: Karen L. Steinmetz and Jon C. Mirsalis

Study Number: S-2762

Date of Issue: August 7, 1986

TB Conclusions:

Negative for inducing unscheduled DNA synthesis (UDS) in hepatocytes drawn from mice treated orally up to toxic doses (5000 mg/kg).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RE-45601 Technical (from Chevron)

Description: Dark amber liquid
 Batch (Lot): SX-1688
 Purity (%): 83.3
 Solvent/Carrier/Diluent: Carboxymethylcellulose (CMC)/Tween-80

B. Test Organism - Rodents

Species: Mice
 Strain: B6C3F1
 Age: 5 weeks
 Weights - Males (only): 21 to 26 g
 Source: Charles River, Kingston, NY

C. Study Design (Protocol) - This study was designed to assess the genotoxic (DNA damage/repair) potential of clethodim when administered by oral gavage to mice, and unscheduled DNA synthesis measured (as cellular uptake of tritiated thymidine) in perfused hepatocytes in vitro, according to referenced methodology.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Male mice were gavaged once with a range of doses and sacrificed at two sampling times, according to the following schedule:

<u>Group</u>	<u>Dose Level (mg/kg bwt)</u>	<u>Number of Treated Males Per Group</u>	
		<u>2 hr</u>	<u>16 hr</u>
I	Vehicle Control	0	3
II	RE-45601 (5000)	3	5
III	RE-45601 (1000)	3	3
IV	RE-45601 (100)	3	3
V	DMN (10)	0	3

In addition to the vehicle control (3 mice given CMC/Tween-80), a fifth group of three males was dosed with the mutagen dimethylnitrosamine (DMN, 10 mg/kg), as positive control.

At the specified sacrifice times (2 and 16 hours posttreatment), hepatocytes were perfused, and established as primary monolayer cultures on coverslips in Linbro culture dishes (up to 2 hours after perfusion). All cultures were then incubated for 4 hours in culture medium containing 10 μ Ci/mL tritiated thymidine (^{3} H-TdP, spec. act. 80 Ci/mM), followed by 14 to 18 hours exposure to 0.25 mM unlabeled thymidine (TdR-"chase"). The cells were then washed, swelled in 1% (hypotonic) sodium citrate, fixed in acetic acid/ethanol and washed thoroughly in DW. The coverslip cell preparations were then mounted on standard microscope slides, which were dipped in a photographic emulsion (Kodak NTB-2), and stored in light-tight microscope boxes at -20 °C for 7 days. Following this cold storage, the preparations were stained in 1% methyl-green Pyronin Y and sealed.

Thirty morphologically normal cells were selected at random per slide (3 slides/animal) for quantitative autoradiographic silver grain counting by automatic counters scoring nuclear labeling (silver grains) from which highest counts from adjacent cytoplasmic (background) areas were subtracted to give net grains per nucleus (NG). In addition, the percent of cells in repair was determined, given as cells with at least 5 NG.

A positive is declared when the grain count (indicating extent of UDS) in test cultures markedly exceeds the solvent control. A positive dose-response, increases in percent repair and reproductivity are all desirable adjuncts in evaluation of a positive response. When none of these criteria obtain, the test article is considered negative for UDS.

- E. Results - In the single UDS experiment, three of the five mice scheduled for sacrifice 16 hours after dosing with 5000 mg/kg were found dead. No abnormal signs were evident in the survivors. However, none of the survivors exhibited increased grain counts over solvent values (with less than 2% of cells in repair), compared to the significant increase in DMN-treated mice (Report Table 3, appended to this DER).

Hence, the investigators concluded that RE-45601 Technical did not induce UDS in hepatocytes drawn from mice treated up to orally toxic levels (5000 mg/kg).

007972

F. TB Evaluation - ACCEPTABLE

Within the constraints of a newer genotoxic assay, this study was performed with adequate controls to support the negative result obtained.

Attachment (Data Table)

007972

Attachment I
Data Table

CLETHODIM

Page 39 is not included in this copy.

Pages _____ through _____ are not included.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Josephine
5/12/90
Karl P. Baetcke
5/29/90

DATA EVALUATION REPORT

007972

007972

I. SUMMARY

MRID (Acc) No.: 410301-25
IP No.: 239-EANb
PD Record No.: 239,850/860/861
Caswell No.: 721F
Project No.: 9-18682

Study Type: Mutagenicity - Chromosome damage in vivo
(Rat BM)

Chemical: Clethodim

Synonyms: RE-45601

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: Microbiological Associates, Bethesda, MD

Title of Report: Cytogenetics Assay in Bone Marrow Cells of
Rats Following Acute Oral Exposure to
RE-45601 Technical.

Author: Donald L. Putnam

Study Number: S-2864

Date of Issue: February 11, 1987

TB Conclusions:

Negative for inducing chromosome aberrations in male
and female rats treated by acute oral gavage up to toxic
doses (1500 mg/kg).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RF-45601 Technical (from Chevron)

Description: Clear, amber, semi-viscous liquid
 Batch (Lot): SX-1682
 Purity (%): 83.3
 Solvent/Carrier/Diluent: Carboxymethylcellulose,
 sodium salt (0.7% CMC)/
 Tween-80 (1.0%)

B. Test Organism - Rodent

Species: Rat
 Strain: Sprague-Dawley (S-D)
 Age: (Not stated)
 Weights - Males: 254-364 g
 Females: 189-266 g
 Source: Charles River, Raleigh, NC

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-damaging) potential of clethodim when administered by oral gavage to S-D rats by standardized methodology (presented as Appendix I, pages 26 to 37, of the Final Report).

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary toxicity tests for the purpose of critical dose-selection (a range of 5 doses from 600 to 5000 mg/kg), groups of five male and five female rats received single oral doses of 0 (vehicle control), 150, 500 or 1500 mg/kg test article, and were sacrificed 12, 24 or 48 hours later. Two to four hours prior to killing, animals were injected with the mitotic-arresting agent, colchicine (0.25 mg/mL). A known clastogen, triethylemelamine (TEM, 0.5 mg/mL) was administered to a group of five males and five females, which were sacrificed at 48 hours (positive control).

At scheduled sacrifice times, femoral bone marrow cells obtained from each animal were prepared for microscopy on glass slides by standard cytological techniques, and stained with 4% Giemsa before permanent mounting. A minimum of 50 metaphases per animal were scored on coded slides for the conventional array of structural and numerical chromosome aberrations. Mitotic indices

(indirect measures of cytotoxicity) were calculated from the ratio of cells in mitosis per 500 cells counted times 100.

A positive for this laboratory is considered if a test agent induces a significantly increased incidence of aberrations over solvent control ($p < 0.05$, by Student's t-test). The control frequency should not exceed 6 percent.

- E. Results - Based on increased mortality, weight loss, and clinical signs beginning at 1200 mg/kg of the preliminary toxicity trials, 1500 mg/kg was selected as the MTD for the main study, with two lower doses anticipated to produce little if any toxicity. In addition to the 15 high-dose males and 15 high-dose females scheduled (5/sex at 12, 24, and 48 hours), another group of five males and five females were treated at 1500 mg/kg, anticipating replacements in the event any scheduled high-dose animals died.

In the main assay, 5/20 high-dose males and 3/20 females did succumb before scheduled sacrifice, and survivors showed reduced weight gain and clinical signs (prostration, lethargy, tremors, etc.). At lower doses only transient lethargy was evident. However, at no dose level or sampling time in either sex were there recorded any significant changes from control values for chromosome number, incidence or type of structural aberration or mitotic indices in any test slide. By contrast, the positive control (TEM) induced an average of 2.312 aberrations/cell with 32.4 percent of cells analyzed containing one or more aberrations in males, and 1.896 aberrations/cell with 31.2 percent of cells containing 1+ aberrations in females (Report Tables 3 to 10, appended to this DER).

The author concluded that this lot of RE-45601 Technical was not clastogenic in rats treated up to toxic doses.

F. TB Evaluation: ACCEPTABLE

The study was carried out with all due regard for adequate procedures and appropriate controls such as to warrant acceptance of the negative result as valid.

Attachments (Data Tables)

007972

Attachment I
Data Tables

CLETHODIM

Page _____ is not included in this copy.

Pages 44 through 52 are not included.

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- ____ Identity of product inert ingredients.
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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IPS (H7509C)
Secondary Reviewer: Karl P. Raetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Joe Weaver
5-18-90
Karl P. Raetcke
5/25/90

DATA EVALUATION REPORT

007972

I. SUMMARY

MRID (Acc) No.: 410301-26 007972
ID No.: 239-FANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-1868Z

Study Type: Mutagenicity - Gene mutation in bacteria
(Ames)

Chemical: 5-Hydroxyclethodim sulfone

Synonyms: RE-51228

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: (Chevron's) Environmental Health Center
(CFHC), Richmond, CA

Title of Report: Microbial/Mammalian Microsome Plate
Incorporation Mutagenicity Assay with
RE-51228.

Author: M.L. Machado

Study Number: S-3111

Date of Issue: December 7, 1987

TB Conclusions:

Negative for inducing reverse gene mutation in
Salmonella TA his⁻ (Ames) strains and E. coli WP2 uvrA
(tryp⁻) exposed with and without activation up to cytotoxic
levels (5000 ug/plate).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RF-51228

Description: White/beige powder
Batch (Lot): (Not stated)
Purity (%): 99.1
Solvent/Carrier/Diluent: Acetone

B. Test Organism - Bacterial cultures

Species: (1) Salmonella typhimurium LT2; and
(2) Escherichia coli WP2
Strains: (1) TA98, TA100, TA1535, TA1537 (his⁻); and
(2) uvrA (tryp⁻)
Source: (1) R.N. Ames, UCal, Berkeley, CA;
(2) S. Haworth, Microbiological Associates,
Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of RE-51228 when administered in vitro to four TA strains of Salmonella and the uvrA (tryp) strain of E. coli, by standardized (published and referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following dose-selection cytotoxicity testing with TA98 and TA100 (a range of doses up to 10,000 ug/plate), triplicate cultures of all five bacterial strains were exposed for 48 hours to five concentrations of test article (ranging from 33.3 to 3300 ug/plate in the first of two assays, and 100 to 5000 ug/plate in the second), both in the absence and presence of a mammalian metabolic activation system, consisting of liver microsomal enzymes (S9) from rats pretreated with Aroclor 1254, plus NADP(H)-generating cofactors.

007972

Appropriate and strain-specific mutagens* were run concurrently in both assays as positive controls.

- E. Results - The test substance was toxic to TA98 and TA100 at the highest doses tested. In the first mutagenicity trial (Experiment 1), a weak but statistically significant increase over solvent control was recorded in nonactivated TA100 (129 vs. 101) at the HDT (3333 μ g/plate) (Report Table 1, appended here), which was not confirmed at this dose level in the second trial (Experiment 2, Report Table 3, appended here). In Experiment 2, statistically significant responses were recorded in TA100 (-S9) as well as in TA1537 (+S9), but only at mid-dose levels. No increases over solvent were found in either trial with the E. coli strain (Report Tables 2 and 4, appended). All positive controls responded as expected in both experiments, with highly significant increases ranging from 10 to over 100 times the solvent control values.

The author concluded that RF-51278 was not mutagenic to Salmonella TA (his⁻) strains or to E. coli WP2 uvrA (trp⁻) in repeat trials up to cytotoxic levels (5000 μ g/plate).

F. TR Evaluation - ACCEPTABLE.

This study was performed adequately, with appropriate controls, such that the negative results obtained are supportable.

Attachments (Data Tables)

<u>*Strain</u>	<u>Activation</u>	<u>Positive Controls</u>	<u>Amt/Plate</u>
TA98	+	2-Aminoanthracene	2 μ g
TA98	-	2-Nitrofluorene	10 μ g
TA100	+	2-Aminoanthracene	2 μ g
TA100	-	Sodium Azide	1 μ g
TA1535	+	2-Aminoanthracene	2 μ g
TA1535	-	Sodium Azide	1 μ g
TA1537	+	2-Aminoanthracene	2 μ g
TA1537	-	ICR-191	2 μ g
WP2 <u>uvrA</u>	+	2-Aminoanthracene	80 μ g
WP2 <u>uvrA</u>	-	ICR-191	50 μ g

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Attachment I
Data Tables

CLETHODIM

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Pages 57 through 60 are not included.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IRS (#7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (#7509C)

J. Mauer
5-18-90

K. Baetcke 007972

5/25/90

DATA EVALUATION REPORT

007972

I. SUMMARY

MRID (Acc) No.: 410301-27
ID No.: 230-FANb
RD Record No.: 230,850/860/861
Caswell No.: 721F
Project No.: 9-1868Z

Study Type: Mutagenicity - Gene mutation in bacteria (Ames)

Chemical: Clethodim-imine-sulfone

Synonyms: RE-47719

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: (Chevron's) Environmental Health Center
(CEHC), Richmond, CA

Title of Report: Microbial/Mammalian Microsome Plate
Incorporation Mutagenicity Assay with
RE-47719 (SX-1800).

Author: M.L. Machado

Study Number: S-3155

Date of Issue: October 19, 1988

TB Conclusions:

Negative for inducing revertants in Salmonella (Ames)
TA strains and *E. coli* WP2 uvrA exposed in the presence
and absence of activation to doses up to 10,000 μ g/plate.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RE-47719

Description: White solid
Batch (Lot): SX-1800
Purity (%): 98.6
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: (1) Salmonella typhimurium LT2;
(2) Escherichia coli
Strains: (1) TA98, TA100, TA1535, TA1537 (his⁻);
(2) WP2 - uvrA (trp⁻)
Source: (1) B.N. Ames, UCal, Berkeley, CA;
(2) S. Haworth, Microbiological Associates,
Pockville, MD

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of RE-47719 when administered in vitro to bacterial strains by standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following cytotoxicity testing for dose selection, triplicate cultures of all five bacterial strains were exposed for 48 hours to test article in repeat (separate) assays at doses ranging from 100 to 10,000 ug/plate, both in the absence and presence of a mammalian metabolic activation system, consisting of liver microsomal enzymes (S9) from rats stimulated to enhanced enzyme activity by treatment with Aroclor 1254, plus appropriate NADP(H)-generating cofactors (S9 Mix).

Concurrent with solvent (DMSO) controls, strain-specific mutagens* were employed as positive controls.

- E. Results - The test material was considered slightly cytotoxic in nonactivated TA100 treated at 10,000 μ g/plate. A statistically significant increase in revertents was calculated in Trial 1 for activated TA1537 cultures treated at the HDT (Report Table 1, appended here), a result not confirmed in the repeat assay (Report Table 2, appended). In no other test culture in either experiment were increases over solvent values registered, in contrast to the expected responses of the positive controls.

F. TB Evaluation - ACCEPTABLE

This study was conducted with appropriate standards of procedures and controls, and the negative results are judged valid.

Attachment (Data Tables)

<u>*Strain</u>	<u>Activation</u>	<u>Positive Controls</u>	<u>Amt/Plate</u>
TA98	+	2-Aminoanthracene	2 μ g
TA98	-	2-Nitrofluorene	10 μ g
TA100	+	2-Aminoanthracene	2 μ g
TA100	-	Sodium Azide	1 μ g
TA1535	+	2-Aminoanthracene	2 μ g
TA1535	-	Sodium Azide	1 μ g
TA1537	+	2-Aminoanthracene	2 μ g
TA1537	-	ICR-I91	2 μ g
WP2 uvra	+	2 Aminoanthracene	80 μ g
WP2 uvra	-	ICR-I91	50 μ g

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Attachment I
Data Tables

CLETHODIM

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Review Section I, Toxicology Branch I - IPS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IPS (H7509C)

Irving Mauer
5-18-93

007972

0-18-93

DATA EVALUATION REPORT

I. SUMMARY

MRID (Acc) No.: 410701-28
ID No.: 239-FANb
PD Record No.: 239,859/860/861
Caswell No.: 721P
Project No.: 9-1268

Study Type: Mutagenicity - Chromosome aberrations in vitro
(CHO)

Chemical: Clethodim

Synonyms: RE-45601

Sponsor: Chevron Chemical Company, Richmond, CA

Testing Facility: Microbiological Associates, Bethesda, MD

Title of Report: Chromosome Aberrations in Chinese Hamster
Ovary (CHO) Cells with Chevron RE-45601.

Author: Donald L. Putnam

Study Number: S-2761

Date of Issue: July 22, 1986

TB Conclusions:

Positive for increased structural chromosome aberrations
(but only in the absence of metabolic activation) at doses
near the limit of solubility and/or cytotoxicity (1.0 and
1.2 μ L/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED PFVIFW

A. Test Material - RE-45601 Technical

Description: Clear amber liquid
Batch (Lot): SX-1688
Purity (%): 81.4
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established mammalian cell line

Species: Chinese hamster (ovary)
Strain: K1
Source: American Type Culture Collection (CCL
#61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential of RE-45601 when administered in vitro to Chinese hamster ovary (CHO) cells, according to standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary toxicity (dose-selection) testing (9 test concentrations up to 1 μ L/mL, in the presence of bromodeoxuridine to evaluate cell cycle kinetics), duplicate cultures of CHO cells were exposed in separate (initial and confirmatory) cytogenetic trials to four concentrations of test article, for 8 hours in the absence of activation, but for only 2 hours (with an additional 6 hours in fresh medium) in the presence of a mammalian metabolic activation system, consisting of the microsomal fraction of liver homogenates (S9) from adult male Sprague-Dawley rats pretreated with the PCB mixture, Aroclor 1254, plus NADP(H)-generating cofactors. In addition to concurrent solvent (DMSO) controls, other cultures were also treated with the clastogens, triethylenemelamine (TEM, 1 μ g/mL) and cyclophosphamide (CP, 100 μ g/mL) for, respectively, the nonactivated and S9-supplemented test series. At the end of the treatment period, the mitotic-arresting alkaloid, Colcemid (0.1 μ g/mL) was added to each culture, cells harvested 2 to 3 hours later by mitotic shake-off, and prepared for microscopy on glass slides by standard cytological techniques.

Fifty metaphases per coded slide (100 per treatment) were scored for the conventional array of structural chromosome changes as well as pulverization. In addition, the incidence of numerical alterations (polyploidy, polysomy, endoreduplication, etc.) was recorded (per 100 cells).

Cytotoxicity was also recorded for each treatment (relative to the solvent control), and the percent cells with structural aberrations as well as the frequency of such aberrations per cell calculated for each test group, and compared to solvent control values (which should be less than 4% for a valid assay) for statistical significance by Student's t-test. Chi-square analysis (2 x 2 contingency) was employed to ascertain any statistically significant differences between treatment and control groups in numerical aberrations.

- F. Results - In preliminary toxicity testing, the test article was partially insoluble at concentrations of 1.0 and 0.3 $\mu\text{L/mL}$ (and completely soluble at all other dose levels), but no evidence of cytotoxicity (reduced cell survival, or cell cycle delay) was found at any dose level. Hence, dose levels of 0.03, 0.1, 0.3, and 1.0 $\mu\text{L/mL}$ were selected for the initial main assay, and a harvest time of 10 hours (to assure all cells would be in the first posttreatment cell division, i.e., M1 metaphases).

In the first of two cytogenetic assays, an increased incidence of structural aberrations was recorded in nonactivated test cultures treated at the HDT, 1.0 $\mu\text{L/mL}$ (11% aberrant cells and 0.31 aberrations/cell vs. control values of 2 percent and 0.02, $p < 0.05$), but not at any dose level in activated cultures (Report Tables 4 vs. 5, both appended to this DER).

In the confirmatory assay (using slightly higher dose levels of 0.4, 0.6, 0.8, 1.0, and 1.2 $\mu\text{L/mL}$, but with the same harvest time), statistically increased incidences of structural aberrations ($p < 0.05$) were again found in nonactivated cultures at the two higher doses (1.0 and 1.2 $\mu\text{L/mL}$, the latter accompanied by slight toxicity to the cell monolayer), but not under activation conditions (Report Tables 6 and 7, also appended here). In neither assay was there any increase in numerical aberrations.

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In both assays, both positive controls (TEM and CP) responded as expected, with highly significant increases ($p < 0.01$) in structural aberrations, but not in numerical changes.

The author concluded that Chevron PF-48601 Technical was positive in inducing dose-related and reproducible increased structural aberrations at high doses in CHO cells, but only in the absence of activation.

F. TB Evaluations - ACCEPTABLE

These repeat studies were conducted with appropriate procedures and adequate controls such that the results obtained are judged valid.

Attachments (Data Tables)

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Attachment I
Data Tables

CLETHODIM

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Irving Mauer
5-18-90

Karl P. Baetcke
5/25/90

DATA EVALUATION RECORD

007972

I. SUMMARY

MRID (Acc) No.: 410301-29
ID No.: 239-EANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-18682

Study Type: Mutagenicity - Chromosome damage in vitro (CHO)

Chemical: Clethodim

Synonyms: RE-45601

Sponsor: Chevron Chemical, Richmond, CA

Testing Facility: Microbiological Associates, Bethesda, MD

Title of Report: Chromosome Aberrations in Chinese Hamster
Ovary (CHO) Cells with Purified Chevron
RE-45601.

Author: Donald L. Putnam

Study Number: S-2865

Date of Issue: December 15, 1986

TB Conclusions:

Negative for inducing chromosome aberrations in both
nonactivated and activated CHO cells exposed up to levels of
insolubility (0.3 and 1.0 μ L/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Chevron RE-45601, purified

Description: Amber liquid
Batch (Lot): SX-1718
Purity (%): 96.1
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Mammalian cell line

Species: Chinese hamster (ovary)
Strain: K1
Source: American Type Culture Collection (CCL
No. 61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential of clethodim when administered in vitro to CHO cells, according to standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following cytotoxicity testing (measuring cell growth at doses up to 1.0 μ L/mL, and in the presence of bromodeoxyuridine, to determine effects on cell cycle kinetics), duplicate cultures of CHO cells were exposed to test article at four concentrations in repeat assays, for 8 hours in the absence of activation, but only for 2 hours (plus an additional 6 hours in fresh medium) in the presence of a mammalian metabolic activation system consisting of the microsomal fraction from homogenates of livers (S9) from adult male Sprague-Dawley rats pretreated with Aroclor 1254, plus NADP(H)-generating cofactors. In addition to concurrent solvent (DMSO) controls, other sets of cell cultures were treated with the mutagens triethylenemelamine (TEM, 1 μ g/mL) and cyclophosphamide (CP, 100 μ g/mL) as positive controls for, respectively, the nonactivation and S9-supplemented series. Two hours prior to harvest, all cultures were exposed to the mitotic-inhibitor alkaloid, Colcemid (0.1 μ g/mL).

At harvest, metaphase cells were collected (by mitotic shake-off) and prepared for microscopy by standard cytological techniques. Fifty Giemsa-stained metaphases per coded slide (100 per treatment) were scored for the conventional array of structural chromosome aberrations,

and pulverization. In addition each slide was scanned for the presence of numerical changes (polyploidy, endoreduplication, etc.). Relative cell growth (a measure of cytotoxicity) and the incidence of aberrations were calculated and compared to background (solvent and/or untreated controls, which should not exceed 4%) by Student's t-test (for structural aberrations) or Chi-square analysis (for numerical changes).

- E. Results - No evidence of decreased growth or cell cycle delay was found in preliminary toxicity testing, although the test article was only partially soluble at test doses of 0.3 and 1.0 $\mu\text{L/mL}$ (Report Tables 1 and 2). Hence, test dose levels of 0.03, 0.1, 0.3, and 1.0 $\mu\text{L/mL}$ were selected for the initial assay, and harvest time set for 10 hours, in order to assure all cells would be in the first posttreatment cell division (i.e., M-1 metaphases).

In the first assay, the test substance was partially insoluble at the two highest dose levels. A statistically increased level of aberrations (0.03 aberrations/cell) was found only at the 0.1 $\mu\text{L/mL}$ dose under activation when compared to the solvent control (0.00 aberrations/cell), but not when compared to the untreated control value (0.01) (Report Tables 3 and 4 appended to this DER). The author did not consider this solitary increase biologically significant since it did not recur in the confirmatory assay at any dose (Report Tables 5 and 6, also appended here). By contrast both mutagens induced highly significant increased chromosome damage ($p < 0.01$) in both assays. No increases in numerical aberrations were found for any treatment in either assay. The author concluded that purified RE-45601 was not clastogenic under conditions of these studies.

- F. TB Evaluation - ACCEPTABLE. These studies were conducted with all appropriate controls under such adequate conditions as to render the negative results valid.

Attachments (Data Tables)

007972

ATTACHMENT I

Data Tables

CLETHODIM

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

5-18-90
5/25/90

DATA EVALUATION RECORD

007972
007972

I. SUMMARY

MRID (Acc) No.: 410301-30
ID No.: 239-EANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-18682

Study Type: Mutagenicity - Chromosome damage in vitro (CHO cells)

Chemical: Clethodim imine sulfone (metabolite)

Synonym: RE-47719

Sponsor: Chevron Chemical, Richmond, CA

Testing Facility: Microbiological Associates, Rockville, MD

Title of Report: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells: RE-47719. Final Toxicology Report.

Authors: D.L. Putnam and M.J. Morris

Study Number: S-3156

Date of Issue: November 3, 1988

TB Conclusions:

Negative for inducing chromosome damage in CHO cells exposed w/without activation to the limit of the test article's solubility (400 μ g/mL).

Classification (Core Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RE-47719

Description: White, crystalline solid
Batch (Lot): SX-1800
Purity (%): 99.3
Solvent/Carrier/Diluent: Acetone

B. Test Organism - Mammalian cell line

Species: Chinese hamster (ovary)
Strain: K1
Source: American Type Culture Collection (CCL No. 61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential of RE-47719 when administered in vitro to CHO cells, according to standardized (referenced) procedures.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary toxicity testing (9 doses ranging from 0.13 to 1250 $\mu\text{g/mL}$, measuring mitotic index, as well as cell cycle kinetics using bromodeoxuridine), duplicate cultures of CHO cells were exposed to four dose levels of test article, for 10 hours in the absence of activation, but only for 2 hours (followed by a further 4- or 8-hour incubation in fresh culture medium) in the presence of a mammalian metabolic activation system consisting of the microsomal fraction of liver enzyme homogenates from adult male Sprague-Dawley rats pretreated with Aroclor 1254 (S9), plus NADP(H)-generating cofactors. In addition to concurrent solvent (acetone) and untreated controls, other cultures were exposed to the mutagens, triethyl-enemelamine (TEM, 0.5 $\mu\text{g/mL}$) and cyclophosphamide (CP, 50 $\mu\text{g/mL}$) as positive controls for, respectively, the nonactivated and S9-supplemented series.

At the end of the treatment periods, all cells were exposed for 2 hours to the mitotic-arresting alkaloid Colcemid (0.1 $\mu\text{g/mL}$) before harvest. Harvested cultures were prepared for microscopy on glass slides by standard cytological techniques. A minimum of 100 Giemsa-stained metaphases per culture flask (= 200 per treatment) were scored on coded slides for the conventional array of

chromosome aberrations, and 500 cells scanned per flask for mitotic index (Ml. as percent metaphases).

Aberration data were analyzed by Fishers Exact test for pairwise statistical significance between test and negative control groups. If a positive were found, Cochran-Armitage was applied to determine trend (dose-responsiveness).

- E. Results - The preliminary toxicity screen revealed partial insolubility of test article at 380 $\mu\text{g/mL}$ and the HDT, 1250 $\mu\text{g/mL}$ (Report Tables 1 and 2), accompanied by minimal toxicity (reduced relative Ml = 28%) and only a slight delay in cell cycle traverse (increased number over control of Ml cells: 15% vs. 4%/-S9; 15% vs. 9%/+S9). Hence, the four dose levels selected for the main study were 50, 100, 200, and 400 $\mu\text{g/mL}$, and harvest times of 12 hours for nonactivated cultures and either 8 and 12 hours for S9-activated cells.

In the single cytogenetic assay, no increases in aberrations were recorded in RE-47719-treated cultures, with or without activation (Tables 3 to 5, appended to this DER). By contrast, the mutagen-treated cultures responded as expected, with increased aberration rates 16 (CP) and 40 (TEM) times background.

The authors concluded that RE-47719 was negative in CHO cells for induced clastogenic activity.

- F. TB Evaluation - ACCEPTABLE. This study was conducted with appropriate controls and procedures to warrant confidence in the negative result obtained.

Attachments (Data Tables)

007972

ATTACHMENT I
Data Tables

CLETHODIM

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

IRB *K. Baetcke*
5-18-90

K. P. Baetcke
5/25/90

DATA EVALUATION RECORD

007972

007972

I. SUMMARY

MRID (Acc) No.: 410301-31
ID No.: 239-EANb
RD Record No.: 239,859/860/861
Caswell No.: 721F
Project No.: 9-18682

Study Type: Mutagenicity - Chromosome damage in vitro (CHO)

Chemical: 5-Hydroxyclethodim sulfone (metabolite)

Synonym: RE-51228

Sponsor: Chevron Chemical, Richmond, CA

Testing Facility: Microbiological Associates, Rockville, MD

Title of Report: Chromosome Aberrations in Chinese Hamster
Ovary (CHO) Cells: RE-51228.

Authors: D.L. Putnam and M.J. Morris

Study Number: S-3160

Date of Issue: November 7, 1988

TB Conclusions:

Negative for inducing structural chromosome aberrations
in CHO cells exposed w/without activation to the limit of
the test article's solubility (2500 μ g/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - RE-51228 (tech)

Description: Off-white solid
Batch (Lot): SX-1796
Purity (%): 99.9
Solvent/Carrier/Diluent: Acetone

B. Test Organism - Mammalian cell line

Species: Chinese hamster (ovary)
Strain: K1
Source: American Type Culture Collection (CCL No. 61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential of RE-51228 when administered in vitro to CHO cells, according to standardized (referenced) practices.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing (evaluating mitotic index relative to solvent control, as well as cell cycle kinetics using bromodeoxyuridine), duplicate cultures of CHO cells were exposed to four dose levels of test article, for 10 hours in the absence of activation, but for only 2 hours (plus additional 4- or 8-hour incubations in fresh medium) in the presence of a mammalian metabolic activation system, consisting of the S9 (microsomal) fraction of liver homogenates from adult male S-D rats pretreated with Aroclor 1254, plus NADP(H)-generating cofactors. In addition to concurrent solvent (acetone) and untreated negative controls, other sets of CHO cultures were exposed to the mutagens triethylenemelamine (TEM, 0.5 ug/mL) and cyclophosphamide (CP, 50 ug/mL) as positive controls for, respectively, the nonactivation and S9-supplemented test series.

At the completion of the treatment/incubation periods, all cultures were treated with the mitotic-arresting alkaloid Colcemid (0.1 ug/mL) and harvested 2 hours later. Metaphase cells were then collected and prepared for microscopy on glass slides according to standard cytological techniques. At least 100 Giemsa-stained metaphases per culture (200 per treatment) were scored

on coded slides for the conventional array of structural chromosome aberrations, and mitotic index determined (number cells in mitosis per 500 cells counted x 100).

Statistical analysis of aberration data was by Fisher's Exact test, comparing pairwise each test group against solvent control for significance ($p \leq 0.05$). When Fisher was positive, a Cochran-Armitage Test was applied to determine trend (dose-responsiveness). Standard criteria for assay validity and judging test results were applied.

- E. Results - In preliminary screening, cells were treated at nine doses ranging from 0.25 to 2500 $\mu\text{g/mL}$. The test article was partially insoluble in culture medium at 250 $\mu\text{g/mL}$ and above (750 and 2500 $\mu\text{g/mL}$), produced a moderate degree of cytotoxicity in nonactivated cultures at the HDT (= 50%) but relatively no cell cycle delay (Report Tables 1 and 2). Hence the authors selected 313, 625, 1250, and 2500 $\mu\text{g/mL}$ test article for the main assay.

In the single cytogenetic assay, mitotic indices were slightly reduced relative to solvent, but at no test dose in either series was the percent aberrations statistically increased over control (Report Tables 3 to 5, appended to this DER). By contrast, both positive controls responded as expected, with statistical increases 7 (CP) to 20 (TEM) times solvent values.

The authors concluded that RE-51228 was negative for clastogenic activity in this assay with CHO cells.

- F. TB Evaluation - ACCEPTABLE. This study was conducted with adequate procedures under appropriate controls to warrant the negative result valid.

Attachments (Data Tables)

007972

ATTACHMENT I
Data Tables

96

31

CLETHODIM

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REVIEWED BY: James N. Rowe, Ph.D.
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90

J.M.F. 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Acute oral (81-1), rats

CHEM. NO.: 721F

MRID NO.: 409745-06

TEST MATERIAL: SELECT 2.0 EC

007972

SYNONYMS: SX-1721

STUDY NUMBER: S-2852

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, California 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, California 94804-0054

TITLE OF REPORT: The Acute Oral Toxicity of Select 2.0 EC (CC-14900) (SX-1721) In Adult Male And Female Rats

AUTHORS: J.H. Hedgecock

REPORT ISSUED: February 2, 1987

CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of Select 2.0 EC. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 3.61(2.35-5.53) g/kg	1.71(1.07-2.75)
Females 2.92(1.42-6.00) g/kg	1.61(0.77-3.37)

This places this formulation in Acute Toxicity Category III.

CLASSIFICATION: Core Guideline.

A. MATERIALS:

007972

1. **TEST COMPOUND:** Select 2.0 EC (CC-14900)
DESCRIPTION: Amber liquid
BATCH #: SX-1721
PURITY: 26.1% RE-45601 (Clethodim)
2. **TEST ANIMALS:**
SPECIES: Rats
STRAIN: Sprague-Dawley Crl:CD (SD) BR
AGE: Males, 72-105 days; females, 112 days (time of dosing)
WEIGHT: Males, 294-384 gms; females, 170-257 (time of dosing)
SOURCE: Charles River Breeding Laboratories, Inc.,
Wilmington, MA

B. STUDY DESIGN:

Young male and female rats were conditioned for a period of 22-42 days prior to dosing. Animals were housed individually in wire-bottom cages. The temperature ranged from 20.5-20.8°C and the relative humidity ranged from 71.1-72.3%. A 12 hour light/dark cycle was maintained and the animals were given water and food ad libitum except during the night prior to dosing when only water was provided.

The animals were randomly assigned to treatment groups prior to testing by the VAX 11/750 computer using the Tausssky-Todd overflow procedure to eliminate statistically significant differences between mean body weights among the test groups. Each animal was individually identified by a cage card.

Groups of five fasted animals (both sexes) were administered via gavage doses of 1.34, 2.08, 3.23 and 5.00 gm/kg of test material. Additional males were dosed at 4.00 and 6.00 g/kg. Ten fasted animals (5/sex) which were not dosed were used as controls. Animals were dosed approximately 4 hours after the onset of the light cycle and were observed frequently for clinical signs of toxicity on the day of dosing. Thereafter, the animals were observed once in the morning and evening for 13 days. The LD50, slope and 95% confidence limits were determined using the method of Berkson.

Animals were weighed prior to dosing, at 3, 7 and 14 days after treatment. Animals that died and all survivors killed at study termination were examined for gross necropsy. The brain, spinal cord and any abnormal tissues and corresponding control tissues were examined histologically.

Signed and dated statements of quality assurance and adherence to the EPA GLPs were included.

C. RESULTS:

Signs of toxicity reported in males and females that died during the study include, "...urine stain, ataxia, tremors, convulsions, collapse, absent toe and tail pinch response, absent righting and placing reflexes, absent startle response, splayed hindlimbs and moribundity.". Signs of toxicity seen only in treated surviving animals included "...green or black anogenital discharge/stain, orange urine, black feces, reduced amount of feces, circling behavior, abnormal respiratory sounds, and abnormal gait.". A number of additional clinical signs of toxicity were also reported in treated animals including salivation, decreased motor activity, reduced food intake, decreased body temperature, lacrimation, sluggish pupil response, etc.

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 3.61(2.35-5.53) g/kg	1.71(1.07-2.75)
Females 2.92(1.42-6.00) g/kg	1.61(0.77-3.37)

Mean body weights were not statistically significantly different from the controls in either sex at any time after dosing.

Gross necropsy of treated animals indicated that Select 2.0 EC produced pathological changes in the stomach and possibly the small and large intestines at the higher dose levels tested (3.23 and 5.00 g/kg, females; 4.0, 5.0 and 6.0 g/kg, males). These changes included the presence of discolored fluid/contents, apparent blood and mucosal thinning or sloughing. Microscopic examination of those animals with apparent abnormal tissues revealed apparent compound-related lesions (severe) in the glandular and non-glandular stomach of males and females. The lesions of the glandular stomach included erosion and/or hemorrhage. Lesions of the non-glandular stomach included erosion, necrosis, ulcer, chronic gastritis and/or hyperkeratosis.

D. CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of Select 2.0 EC. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 3.61(2.35-5.53) g/kg	1.71(1.07-2.75)
Females 2.92(1.42-6.00) g/kg	1.61(0.77-3.37)

This places this formulation in Acute Toxicity Category III.

REVIEWED BY: James N. Rowe, Ph.D. *James N. Rowe 5/24/90*
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *MI 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral (81-1), rats

CHEM. NO.: 721F

MRID NO.: 409745-07

TEST MATERIAL: RE-45601 Technical

007972

SYNONYMS: SX-1688

STUDY NUMBER: S-2764

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, California 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, California 94804-0054

TITLE OF REPORT: The Acute Oral Toxicity of RE-45601 Technical
(SX-1688) In Adult Male And Female Rats

AUTHORS: M.D. Easter, B.S.

REPORT ISSUED: July 14, 1986

CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of RE-45601. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.86-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

This places this compound in Acute Toxicity Category III.

CLASSIFICATION: Core Guideline.

A. MATERIALS:

1. **TEST COMPOUND:** RE-45601 Technical
DESCRIPTION: Dark amber liquid coded SX1688
BATCH #:
PURITY: 83.3% RE-45601 (Clethodim)
2. **TEST ANIMALS:**
SPECIES: Rats
STRAIN: Sprague-Dawley Crl:CD (SD) BR
AGE: Males, 65 days; females, 72 days (time of dosing)
WEIGHT: Males, 258-281 gms; females, 190-211 (time of dosing)
SOURCE: Charles River Breeding Laboratories, Inc.,
Wilmington, MA

B. STUDY DESIGN:

Young male and female rats were conditioned for a period of 14 days prior to dosing. Animals were housed individually in wire-bottom cages. The temperature ranged from 21.4-22.5°C and the relative humidity ranged from 24.4-66.3%. A 12 hour light/dark cycle was maintained and the animals were given water and food ad libitum except during the night prior to dosing when only water was provided.

The animals were randomly assigned to treatment groups prior to testing by the IBM 370 computer using the Tausky-Todd overflow procedure to eliminate statistically significant differences between mean body weights among the test groups. Each animal was individually identified by a cage card.

The test material was suspended in 0.7% CMC and 1.0% Tween 80 (w/w) in distilled water. Samples of the test material were taken following dosing and analyzed for stability and homogeneity. Groups of five fasted animals (both sexes) were administered via gavage doses ranging from 1.05 g/kg to 2.50 g/kg for males and 0.80 g/kg to 2.00 g/kg for females. Ten fasted animals (5/sex) were dosed with 10.00 g/kg of 0.7% CMC and 1.0% Tween 80 (w/w) in distilled water as controls. Animals were dosed approximately 6.5-8 hours after the onset of the light cycle and were observed frequently for clinical signs of toxicity on the day of dosing. Thereafter, the animals were observed once in the morning and evening for 13 days; on weekends the animals were observed once daily. The LD50, slope and 95% confidence limits were determined using the method of Berkson.

Animals were weighed prior to dosing, at 7 and 14 days after treatment. Animals that died and all survivors killed at study termination were examined for gross necropsy. The brain, spinal cord and any abnormal tissues and corresponding control tissues were examined histologically.

Signed and dated statements of quality assurance and adherence to the EPA GLPs were included.

C. RESULTS:

Homogeneity and stability of RE-45601 dosing mixtures ranged from 96.1-100% and 98.6-103%, respectively.

In males, salivation, hyperreactivity, decreased motor activity, unsteady gait, clonic convulsions and collapse were seen with test treatment (especially at the higher dose levels) as well as lacrimation, hunched and trembling gait, red stained fur, yellow anogenital stain and reduced food consumption. Similar effects were noted in treated females.

There was a dose-related increase in mortality in both sexes with all male deaths and most female deaths occurring within 2 to 17 hours post treatment. Females in the 1.45 g/kg group died within 17 hours to 3 days after treatment. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.86-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

Mean body weights were not statistically significantly different from the controls in either sex at any time after dosing, except for males in the 1.86 g/kg group at 7 days post treatment (313 g/treated vs 344 g/control, $p < 0.01$).

Gross necropsy findings in treated animals included the presence of dark gelatinous material beneath the meninges of males and females at 1.45 g/kg (2/5 females), 1.86 g/kg (3/5 males), 2.00 g/kg (4/5 females) and 2.50 g/kg (4/5 males). One of 5 males also had reddened meninges in the 2.00 g/kg dose group. Reddened or darkened lungs in 4/5 HDT males or females as well as 2/5 males and 1/5 females. Three of 5 HDT animals of both sexes had foam/froth in their tracheas.

Two of 5 females (1.45 g/kg) had a trace gliosis in the lower lumbar region of the spinal cord and one of these same females also reported a trace vacuolar change.

D. CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of RE-45601. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.86-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

This places this formulation in Acute Toxicity Category III.

REVIEWED BY: James N. Rowe, Ph.D. *James N. Rowe 5/24/90*
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *JMF 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral (81-1), rats

CHEM. NO.: 721F

MRID NO.: 409745-07

TEST MATERIAL: RE-45601 Technical

007972

SYNONYMS: SX-1688

STUDY NUMBER: S-2764

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, California 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, California 94804-0054

TITLE OF REPORT: The Acute Oral Toxicity of RE-45601 Technical
(SX-1688) In Adult Male And Female Rats

AUTHORS: M.D. Easter, B.S.

REPORT ISSUED: July 14, 1986

CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of RE-45601. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.35-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

This places this compound in Acute Toxicity Category III.

CLASSIFICATION: Core Guideline.

007972

A. MATERIALS:

1. TEST COMPOUND: RE-45601 Technical
DESCRIPTION: Dark amber liquid coded SX1688
BATCH #:
PURITY: 83.3% RE-45601 (Clethodim)
2. TEST ANIMALS:
SPECIES: Rats
STRAIN: Sprague-Dawley Crl:CD (SD) BR
AGE: Males, 65 days; females, 72 days (time of dosing)
WEIGHT: Males, 258-281 gms; females, 190-211 (time of dosing)
SOURCE: Charles River Breeding Laboratories, Inc.,
Wilmington, MA

B. STUDY DESIGN:

Young male and female rats were conditioned for a period of 14 days prior to dosing. Animals were housed individually in wire-bottom cages. The temperature ranged from 21.4-22.5°C and the relative humidity ranged from 24.4-66.3%. A 12 hour light/dark cycle was maintained and the animals were given water and food ad libitum except during the night prior to dosing when only water was provided.

The animals were randomly assigned to treatment groups prior to testing by the IBM 370 computer using the Taussky-Todd overflow procedure to eliminate statistically significant differences between mean body weights among the test groups. Each animal was individually identified by a cage card.

The test material was suspended in 0.7% CMC and 1.0% Tween 80 (w/w) in distilled water. Samples of the test material were taken following dosing and analyzed for stability and homogeneity. Groups of five fasted animals (both sexes) were administered via gavage doses ranging from 1.05 g/kg to 2.50 g/kg for males and 0.80 g/kg to 2.00 g/kg for females. Ten fasted animals (5/sex) were dosed with 10.00 g/kg of 0.7% CMC and 1.0% Tween 80 (w/w) in distilled water as controls. Animals were dosed approximately 6.5-8 hours after the onset of the light cycle and were observed frequently for clinical signs of toxicity on the day of dosing. Thereafter, the animals were observed once in the morning and evening for 13 days; on weekends the animals were observed once daily. The LD50, slope and 95% confidence limits were determined using the method of Berkson. *

Animals were weighed prior to dosing, at 7 and 14 days after treatment. Animals that died and all survivors killed at study termination were examined for gross necropsy. The brain, spinal cord and any abnormal tissues and corresponding control tissues were examined histologically.

Signed and dated statements of quality assurance and adherence to the EPA GLPs were included.

C07972

C. RESULTS:

Homogeneity and stability of RE-45601 dosing mixtures ranged from 96.1-100% and 98.6-103%, respectively.

In males, salivation, hyperreactivity, decreased motor activity, unsteady gait, clonic convulsions and collapse were seen with test treatment (especially at the higher dose levels) as well as lacrimation, hunched and tremoring gait, red stained fur, yellow anogenital stain and reduced food consumption. Similar effects were noted in treated females.

There was a dose-related increase in mortality in both sexes with all male deaths and most female deaths occurring within 2 to 17 hours post treatment. Females in the 1.45 g/kg group died within 17 hours to 3 days after treatment. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.86-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

Mean body weights were not statistically significantly different from the controls in either sex at any time after dosing, except for males in the 1.86 g/kg group at 7 days post treatment (313 g/treated vs 344 g/control, $p < 0.01$).

Gross necropsy findings in treated animals included the presence of dark gelatinous material beneath the meninges of males and females at 1.45 g/kg (2/5 females), 1.86 g/kg (3/5 males), 2.00 g/kg (4/5 females) and 2.50 g/kg (4/5 males). One of 5 males also had reddened meninges in the 2.00 g/kg dose group. Reddened or darkened lungs in 4/5 HDT males or females as well as 2/5 males and 1/5 females. Three of 5 HDT animals of both sexes had foam/froth in their tracheas.

Two of 5 females (1.45 g/kg) had a trace gliosis in the lower lumbar region of the spinal cord and one of these same females also reported a trace vacuolar change.

D. CONCLUSIONS:

There was a dose-related increase in mortality in both sexes with most deaths occurring during day 1 post treatment with a single oral administration of RE-45601. The LD50, 95% C.L.s and slopes are presented below:

LD50 (95% C.L.)	Slope (95% C.L.)
Males 1.63(1.05-2.55) g/kg	1.35(0.86-2.11)
Females 1.36(0.82-2.23) g/kg	1.39(0.84-2.30)

This places this formulation in Acute Toxicity Category III.

REVIEWED BY: James N. Rowe, Ph.D.
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90
Jill 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Acute oral (81-1), mice

CHEM. NO.: 721F

MRID NO.: 409745-08

TEST MATERIAL: RE-45601 Technical

007972

SYNONYMS: SX-1688

STUDY NUMBER: S-2732

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, California 94804-0010

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22180

TITLE OF REPORT: Acute Oral Toxicity In Mice With Chevron RE-45601
Technical Final Report

AUTHORS: R.H. Cox, T. Zoetis, R. Quander, K.J. Vargas, P.L. Burlew

REPORT ISSUED: September 16, 1986

CONCLUSIONS:

Oral administration of single doses of 0-3.5 g/kg of RE-45601 to groups of 5 mice (both sexes) produced LD50 and 95% C.L. values of:

Sex	LD50 g/kg	Confidence Limits (Lower)	(Upper)
male	2.5730	2.1149	3.1304
female	2.4300	1.9564	3.0183
combined sexes	2.4639	2.1295	2.8509

This places this compound in Acute Toxicity Category III.

CLASSIFICATION: Core Guideline.

A. MATERIALS:

1. TEST COMPOUND: RE-45601 Technical
DESCRIPTION: Dark brown viscous liquid
LOT #: SX1688
PURITY: 83.3%
2. TEST ANIMALS:
SPECIES: Mice
STRAIN: Charles River CD-1
AGE: Stated in protocol as no less than 6 or more than 8 weeks old
WEIGHT: Males, 24.9-29.6 gms (first range-finding study)
Males, 26.8-33.0 gms (second range-finding and main study)
Females, 20.8-25.2 gms (first range-finding study)
Females, 24.1-26.5 gms (second range-finding and main study)
SOURCE: Charles River, Raleigh, North Carolina

007972

B. STUDY DESIGN:

Two shipments of mice were received for the first and second range-finding study and the LD50 study. The mice were acclimated to laboratory conditions for at least 7 days prior to study initiation. Mice were identified by ear tags and individually housed in stainless steel cages. They were fed and watered ad libitum except for pre-dose fasting. During the study the following temperature and humidity ranges were reported:

Study	Temperature(°F)	Humidity (%)
First range-finding	70 - 76	48 - 70
Second range-finding	72 - 74	43 - 70
LD50	71 - 75	38 - 69

A 12 hour light/dark cycle was maintained.

For the LD50 study 5 animals (per sex) per dose group, with doses ranging from 0 to 3.0 g/kg, were used. Only the definitive study is presented in detail in this review. Animals were gavaged with a single dose of test material suspended in CMC (carboxymethyl cellulose, Tween 80 and water). Samples were sent to the sponsor for analysis. Animals were food-fasted 1 hour pre-dose.

All surviving mice were observed for signs of toxicity and mortality at 1 and 3 hours postdose and once daily for 14 days after dosing. Mortality was checked twice daily. Individual body weights were measured and recorded prior to treatment and on days 3, 8 and 15, or when found dead. Pupillary response was measured on days 2, 8 and 15 of study.

All animals used on the range-finding studies were discarded without necropsy. All animals in the LD50 study were examined grossly including an examination of all cavities and organs in

situ. In addition, the kidneys were sliced and cut surfaces examined. Mortality data were analyzed using the Probit method of Finney (1952). Absolute body weights and body weight changes of the control group were statistically compared to the treated groups using Levene's test of homogeneity of variances, analysis of variance, and Dunnett's test for significance of multiple dose groups vs controls.

Signed and dated statements of quality assurance and adherence to the EPA GLPs were included.

C. RESULTS:

Homogeneity of dosing formulations (160 and 500 mg/kg) averaged 99.2% and 98.3% of theoretical, respectively. Analytical results from the range-finding study (4 dose levels) gave a range of 91-102% of the theoretical concentration. In the definitive study, (6/3/86 and 6/10/86) at 3 and 2 dose levels tested, respectively, the % of theoretical concentrations ranged from 92.8-99.2 and 83.6-90.6%.

Compound-related deaths occurred primarily within 1 to 3 hours postdose in both sexes. Cumulative mortality was as follows, 0/10 at 0 mg/kg, 0/10 at 1.5 g/kg, 3/10 at 2.0 g/kg, 4/10 at 2.5 g/kg, 8/10 at 3.0 g/kg and 4/5 (males only dosed at this level). LD50 and 95% C.L. values are presented below:

Sex	LD50 g/kg	Confidence Limits	
		(Lower)	(Upper)
male	2.5730	2.1149	3.1304
female	2.4300	1.9564	3.0183
combined sexes	2.4639	2.1295	2.8509

Clinical signs reported for both males and females were hypoactivity, rough coat, hunched position, ataxia, urine stains, tremors, salivation and labored respiration. These signs were noted essentially during days 1 and 2 postdosing. Pupillary responses were stated as being normal for all surviving mice. Mean body weights and body weight changes among the treated groups were not statistically significantly different from control values for either sex.

All mice dying during the course of the study were reported to have slightly dark-red lungs and compound-like material in stomach and intestines. No other gross necropsy findings were reported.

007972

D. CONCLUSIONS:

Oral administration of single doses of 0-3.5 g/kg of RE-45601 to groups of 5 mice (both sexes) produced LD50 and 95% C.L. values of:

Sex	LD50 g/kg	Confidence Limits	
		(Lower)	(Upper)
male	2.5730	2.1149	3.1304
female	2.4300	1.9564	3.0183
combined sexes	2.4639	2.1295	2.8509

REVIEWED BY: James N. Rowe, Ph.D. *James N. Rowe 5/24/90*
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *J.M.I. 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity (rats); 81-1 TOX CHEM NO.: 721F

MRID NO.: 410301-01

007972

TEST MATERIAL: RE-45601 and RE-51228

SYNONYMS: SX-1688 and SX-1796, respectively

STUDY NUMBER: S-3159

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Company
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
P.O. Box 4054
15299 San Pablo Avenue
Richmond, CA 94804-0054

TITLE OF REPORT: The Comparative Acute Oral Toxicity of RE-51228
(SX-1796) and RE-45601 Technical (SX-1688) in Adult Female Rats

AUTHORS: K.K. Dougherty, Ph.D.

REPORT ISSUED: 11/9/88

CONCLUSIONS:

RE-51228 did not display any overt forms of acute toxicity when administered at 1.4 gm/kg b.wt to young adult female rats. RE-45601 Technical (Clethodim) administration at 1.4 gm/kg b.wt resulted in 100% acute oral lethality (5/5 rats) within 3 days with a variety of clinical signs of poisoning. The LD50 of RE-51228 is > 1.4 gm/kg and is placed in Acute Toxicity Category III. The Acute Toxicity Category for RE-45601 cannot be determined from this study.

CLASSIFICATION: Core Minimum for RE-51228
Core Supplementary for RE-45601

007972

A. MATERIALS:

1. **TEST COMPOUND:** RE-51228
 DESCRIPTION: white powder
 BATCH #:
 PURITY: 99.9%

 TEST COMPOUND: RE-45601 Technical
 DESCRIPTION: amber oil
 BATCH #:
 PURITY: 83.8%
2. **TEST ANIMALS:**
 SPECIES: rats (females)
 STRAIN: Sprague-Dawley Crl:CD@ BR
 AGE: 68 days at time of dosing
 WEIGHT: 183-202 gms at time of dosing
 SOURCE: Charles River Laboratories, Inc., Portage MI

B. STUDY DESIGN:

Female rats were allowed an 8 day conditioning period prior to dosing. Animals were housed individually in air-conditioned rooms with temperatures ranging from 20-23 °C and relative humidities from 55-78% during the study. A 12 hour light/dark cycle was maintained and animals were fed ad libitum except when fasted overnight prior to dosing.

Animals were randomized in each treatment group by a computer program (Taussky-Todd overflow procedure on a VAX 11/750) and identified via cage card. Test materials were prepared with 140 mg/gm of a suspension of either RE-51228 or RE-45601. Vehicle was a solution of 0.7% carboxymethylcellulose (CMC) and 1.0% TWEEN 80 in distilled water. Dosing suspensions were prepared with a Polytron Tissue Homogenizer; suspensions were stirred continuously while withdrawing doses. Single doses (10 g/kg b.wt.) were administered to fasted female rats.

After dosing, rats were observed frequently during the day of dosing for clinical signs of toxicity and at least once each day for 14 days after treatment. Body weights were determined prior to dosing, and at 2, 7 and 14 days. Necropsy was performed on all animals and the brains, kidneys, liver, lungs, spinal cord and any abnormal tissues were processed for histopathology slides. Lungs were infused with formalin and paired organs were collected and fixed. However, no tissue was evaluated for histopathology.

C. RESULTS:

No rats dosed with RE-51228 showed signs of toxicity or died during the 14 day observation period. All rats treated with RE-45601 died within 3 hours to 3 days. Rats dosed with the technical displayed various symptoms including salivation, decreased motor activity, collapse, hyperreactivity, tremors, reduced food

consumption, diarrhea, dehydration, and nasal, ocular, oral and anogenital discharges. Body weight gains were normal for the RE-51228 animals but were depressed in the rats dosed with RE-45601 (between days 0 and 2).

No gross pathology was observed in the any of the RE-51228 treatment group. Macroscopic pathology was observed in the lung (discoloration, 1/5), brain (meningeal blood, 2/5), spleen (discoloration, black, 1/5), stomach (discoloration, black, 1/5), small intestine (fluid-filled, 1/5) and cecum (fluid-filled, 1/5) of RE-45601 rats. These tissues were not examined histopathologically since the author stated that similar lesions were observed and evaluated in a more comprehensive study of RE-45601 (SOCAL 2498).

D. CONCLUSIONS:

RE-51228 did not display any overt forms of acute toxicity when administered at 1.4 gm/kg b.wt to young adult female rats. RE-45601 Technical (Clethodim) administration at 1.4 gm/kg b.wt resulted in 100% acute oral lethality (5/5 rats) within 3 days with a variety of clinical signs of poisoning. The LD50 of RE-51228 is > 1.4 gm/kg and is placed in Acute Toxicity Category III. The Acute Toxicity Category for RE-45601 cannot be determined from this study.

REVIEWED BY: James N. Rowe, Ph.D.
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90
J.M.I. 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity (rats); 81-1 TOX CHEM NO.: 721F

MRID NO.: 410301-02

TEST MATERIAL: RE-47719

007972

SYNONYMS: SX-1800

STUDY NUMBER: S-3154

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Company
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
P.O. Box 4054
15299 San Pablo Avenue
Richmond, CA 94804-0054

TITLE OF REPORT: The Comparative Acute Oral Toxicity of RE-47719
(SX-1800) and RE-45601 Technical (SX-1688) in Adult Female Rats

AUTHORS: K.K. Dougherty, Ph.D.

REPORT ISSUED: 11/8/88

CONCLUSIONS:

RE-47719 resulted in the death of 1/5 young female rats when administered at 1.4 gm/kg b.wt. Therefore, the LD50 of RE-47719 is > 1.4 gm/kg and the material is placed in Acute Toxicity Category III.

CLASSIFICATION: Core Minimum

A. MATERIALS:

1. TEST COMPOUND: RE-47719
DESCRIPTION: white powder
BATCH #: 007972
PURITY: 98.6%
2. TEST ANIMALS:
SPECIES: rats (females)
STRAIN: Sprague-Dawley Crl:CD@ BR
AGE: 75 days at time of dosing
WEIGHT: 184-215 gms at time of dosing
SOURCE: Charles River Laboratories, Inc., Portage MI

B. STUDY DESIGN:

Female rats were allowed a 14 day conditioning period prior to dosing. Animals were housed individually in air-conditioned rooms with temperatures ranging from 20-22 °C and relative humidities from 68-79% during the study. A 12 hour light/dark cycle was maintained and animals were fed ad libitum except when fasted overnight prior to dosing.

Animals were randomized in each treatment group by a computer program (Taussky-Todd overflow procedure on a VAX 11/750) and identified via cage card. Test materials were prepared with 140 mg/gm of a suspension of RE-47719. Vehicle was a solution of 0.7% carboxymethylcellulose (CMC) and 1.0% TWEEN 80 in distilled water. Dosing suspensions were prepared with a Polytron Tissue Homogenizer; suspensions were stirred continuously while withdrawing doses. Single doses (10 g/kg b.wt.) were administered to fasted female rats.

After dosing, rats were observed frequently during the day of dosing for clinical signs of toxicity and at least once each day for 14 days after treatment. Body weights were determined prior to dosing, and at 2, 7 and 14 days. Necropsy was performed on all animals and the brains, kidneys, liver, lungs, spinal cord and any abnormal tissues were processed for histopathology slides. Lungs were infused with formalin and paired organs were collected and fixed. However, no tissue was evaluated for histopathology.

C. RESULTS:

One animal was found dead on the morning after dosing. Decreased motor activity was reported four hours after dosing in the animal that died. All other animals were reported to be normal during the 2 week observation period. Mean body weight gains were normal during the observation period after treatment (average of 53 grams increase). The small intestine of the animal that died was reported as containing red, gelatinous material but no additional gross necropsy findings were found in any other rats.

007972

D. CONCLUSIONS:

RE-47719 resulted in the death of 1/5 young female rats when administered at 1.4 gm/kg b.wt. Therefore, the LD50 of RE-47719 is > 1.4 gm/kg and the material is placed in Acute Toxicity Category III.

REVIEWED BY: James N. Rowe, Ph.D. *James N. Rowe 5/24/90*
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *J.M.I. 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal (81-2) CHEM. NO. 721F

MRID NO.: 409745-10

TEST MATERIAL: Clethodim Technical

007972

SYNONYMS: RE-45601

STUDY NUMBER: S-2780

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, California 94804-0054

TITLE OF REPORT: The Acute Dermal Toxicity of RE-45601 Technical
(SX-1688) in Adult Male and Female Rabbits

AUTHORS: G.L. Korenaga, B.S.

REPORT ISSUED: January 7, 1987

CONCLUSIONS:

The acute dermal LD50 of neat RE-45601 in male and female rabbits is > 5.0 g/kg. This places this compound in Acute Toxicity Category IV.

CLASSIFICATION: Core Supplementary. Information on the percent body surface area exposed to RE-45601 must be submitted.

A. MATERIALS:

007972

1. TEST COMPOUND: Clethodim Technical
DESCRIPTION: Dark amber liquid
BATCH #: SX-1688
PURITY: 83.3% (from acute oral studies)
2. TEST ANIMALS:
SPECIES: Rabbits
STRAIN: New Zealand White
AGE: 14-16 or 15-17 weeks old when dosed
WEIGHT: Males, 2.78-3/75 kg; females, 2.60-3.20 kg (pre-dosing wt.)
SOURCE: L.I.T. Rabbitry, Whitehall, Montana

B. STUDY DESIGN:

Rabbits used in the 5.0 g/kg screen were conditioned for 6 weeks before dosing and the rabbits used in the 2.0 g/kg screen were conditioned for 7 weeks prior to dosing. Animals were housed individually in wire bottom cages with temperatures ranging from 21.4-22.7°C and the relative humidity ranging from 34.5-70.7%. Animals were fed ad libitum.

Animals were randomly assigned to treatment groups prior to testing by the VAX 11/750 computer using the Taussky-Todd overflow procedure so that body weight differences were minimized. Each animal was identified by a cage card.

Five animal/dose/sex were exposed to neat Clethodim and groups of 5 were sham treated. The fur on the trunks of each animal was clipped on the day prior to dosing. Test material was applied to the animal (percent of body surface area not indicated) and held in contact with the skin by plastic sheet around the trunk. Paper toweling was wrapped over the plastic sheet to prevent tearing. The wraps were secured with porous adhesive tape. After a 24 hour period, the wrappings were removed and any remaining test material was wiped off with a gauze pad moistened with distilled water. Collars were placed on treated animals for 24 hours to prevent oral ingestion of residual test material and on controls for the same time period.

Animals were observed on the day of dosing and at least once each weekday morning and late afternoon for 13 days after treatment. On weekends, they were observed once daily. On day 14, they were observed prior to sacrifice. The application site was scored for irritation at 1, 7 and 14 days using the modified scoring system of Draize et al. (1944). Body weights were weighed immediately before dosing, and at 2, 7 and 14 days after treatment. Mean body weights were compared to controls using Student's t test.

All animals were necropsied. Sections of the skin from each

007972

animal and any abnormal tissues were preserved in formalin for histopathological examination.

Signed and dated statements of adherence to GLPs and quality assurance were included.

C. RESULTS:

In the 5.0 g/kg males (4.5-5.0 g/kg due to rounding error) 1/5 animal died on day 6 postdosing. No males died at a 2.0 g/kg dermal exposure. No females (0/5) died from topical exposure to RE-45601 neat (5.0 g/kg). No deaths were observed in sham-treated controls of either sex.

Signs of toxicity were primarily skin changes including reddened skin, swollen skin, dry/flaky skin and scabbed skin seen in both treated and control animals, although generally of greater incidence in treated groups. Some treated males also had skin abrasion, skin thickening, skin blackened/darkened and crusty skin. Treated females had some skin thickening also.

No summary table of dermal irritation scores were presented. The authors stated (p.10) that at 24 hours postdosing, "...treated and control animals showed comparable slight to severe erythema; erythema was accompanied by moderate to severe edema in treated animals and no to well-defined edema in controls. By Day 7, controls showed no erythema or edema while treated animals showed no to severe erythema and no to well-defined edema. Erythema persisted to Day 14 in one female dosed with 5.0 g/kg."

Other signs of toxicity included decreased motor activity, decreased food intake, unkempt appearance, no feces, collapse, diarrhea and decreased body temperature in the 5.0 g/kg male that died. Ocular discharge was observed in 4 2.0 g/kg males. Two females in the 2.0 g/kg group exhibited reduced food intake as compared to 1/5 female controls.

There were no statistically significant changes in mean body weight between treated and control animals of either sex at any day of observation.

Microscopic examination of the skin indicated that 3/5 males in the 2.0 g/kg group had trace or mild hyperkeratosis (2/5) or severe ulceration of the skin (1/5). In the 5.0 g/kg female group 1/5 animals had moderate acanthosis, chronic dermatitis, trace hyperkeratosis, severe necrosis and ulceration. No control animals in the respective controls had any microscopic changes of the skin.

D. CONCLUSIONS:

The acute dermal LD50 of neat RE-45601 in male and female

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rabbits is > 5.0 g/kg.

007972

REVIEWED BY: James N. Rowe, Ph.D.
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90
M.I. 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal (81-2)

CHEM. NO. 721F

MRID NO.: 409745-11

TEST MATERIAL: SELECT 2.0 EC

007972

SYNONYMS: (CC-14900)

STUDY NUMBER: S-2853

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, California 94804-0054

TITLE OF REPORT: The Acute Dermal Toxicity of SELECT 2.0 EC (SX-1721) in Adult Male and Female Rabbits

AUTHORS: J.H. Hedgecock, B.S.

REPORT ISSUED: October 28, 1986

CONCLUSIONS:

The acute dermal LD50 of SELECT 2.0 EC applied neat in male and female rabbits is > 5.0 g/kg. This places this compound in Acute Toxicity Category IV.

CLASSIFICATION: Core Supplementary. Information on the percent body surface area exposed to SELECT 2.0 EC must be submitted.

007972

A. MATERIALS:

1. TEST COMPOUND: SELECT 2.0 EC
DESCRIPTION: Amber liquid
BATCH #: SX-1721
PURITY: 26.0% RE-45601; 67% HiSol
2. TEST ANIMALS:
SPECIES: Rabbits
STRAIN: New Zealand White
AGE: males, 16-18 weeks; females, 16-18 weeks (when dosed)
WEIGHT: Males, 2.84-3.71 kg; females, 3.06-3.69 kg (pre-dosing wt.)
SOURCE: L.I.T. Rabbitry, Whitehall, Montana

B. STUDY DESIGN:

Rabbits were conditioned for 8 weeks before dosing. Animals were housed individually in wire bottom cages with temperatures ranging from 21.4-21.7°C and the relative humidity ranging from 34.5-60.5%. Animals were fed ad libitum.

Animals were randomly assigned to treatment groups prior to testing by the VAX 11/750 computer using the Taussky-Todd overflow procedure so that body weight differences were minimized. Each animal was identified by a cage card.

Five animal/dose/sex were exposed to neat SELECT 2.0 EC (5.0 g/kg) and groups of 5/sex were sham treated. The fur on the trunks of each animal was clipped on the day prior to dosing. Test material was applied to the animal (percent of body surface area not indicated) and held in contact with the skin by plastic sheet around the trunk. Paper toweling was wrapped over the plastic sheet to prevent tearing. The wraps were secured with porous adhesive tape. After a 24 hour period, the wrappings were removed and any remaining test material was wiped off with a gauze pad moistened with distilled water. Collars were placed on treated animals for 24 hours to prevent oral ingestion of residual test material and on controls for the same time period.

Animals were observed on the day of dosing and at least once each weekday morning and late afternoon for 13 days after treatment. On weekends, they were observed once daily. On day 14, they were observed prior to sacrifice. The application site was scored for irritation at 1, 7 and 14 days using the modified scoring system of Draize et al. (1944). Body weights were weighed immediately before dosing, and at 2, 7 and 14 days after treatment. Mean body weights were compared to controls using Student's t test.

All animals were necropsied. Sections of the skin from each animal and any abnormal tissues were preserved in formalin for histopathological examination.

Signed and dated statements of adherence to GLPs and quality assurance were included.

C. RESULTS:

No mortality was reported.

Signs of toxicity were primarily skin changes including reddened skin and swollen skin in both treated and control animals. Some treated males and females also had skin cracking, skin thickening, skin dry, flaky and skin cracking or scabbed, indicative of more severe treatment related effects. The more severe effects were generally seen by day 6 to 7 postdosing.

No summary table of dermal irritation scores were presented. The authors stated (p.10) that at 24 hours postdosing, "Well-defined to moderate erythema with slight to moderate edema was observed in treated animals...Control males and females showed slight to moderate erythema with no to well-defined edema 24 hours after treatment; this irritation may be attributable to the wrapping procedure as only slight erythema remained in one female at Day 7. Severe erythema was observed in one treated male and two treated females on Day 7, persisting in one treated female through Day 14. Other animals showed slight to moderate erythema with slight to well-defined edema on Day 7 and slight to moderate erythema with no to well-defined edema on Day 14."

Other signs of toxicity found in both treated and/or control groups (both sexes) included decreased food intake, ocular or nasal discharge, reduced feces, conjunctival redness and swollen eyes. These findings were observed from day 1 up to day 14 post dosing.

There were no statistically significant changes in mean body weight between treated and control males at any day of observation. Treated female rabbits had statistically significant depressions in mean body weight at 2, 7 and 14 days ($p < 0.05$).

All treated animals had abnormally appearing skin in the treatment area. Microscopic examination revealed findings of trace to severe acanthosis (3/5 animals, each sex), trace to mild chronic dermatitis (2/5, males; 3/5/females), trace to moderate escharotic exudate (5/5, males; 3/5, females), trace to severe hyperkeratosis (5/5, both sexes) and moderate ulcer (1/5, females).

D. CONCLUSIONS:

The acute dermal LD50 of SELECT 2.0 EC applied neat in male and female rabbits is > 5.0 g/kg.

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SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *J.M.I. 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation (81-3)

CHEM.NO. 721F

MRID NO.: 409745-12

TEST MATERIAL: Clethodim Technical

007972

SYNONYMS: RE-45601

STUDY NUMBER: S-2783

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804-0054

TITLE OF REPORT: The Acute Inhalation Toxicity of RE-45601
Technical (SX-1688)
in Rats

AUTHORS: E.D. Bruce, B.S.

REPORT ISSUED: November 26, 1986

CONCLUSIONS:

Exposure of male and female rats to RE-45601 aerosolized with acetone at average concentrations of 3.9 mg/L of air for 4 hours did not result in any mortality. This places this compound in Acute Toxicity Category III.

CLASSIFICATION: Core Minimum.

A. MATERIALS:

1. TEST COMPOUND: Clethodim Technical
DESCRIPTION: Dark, amber liquid
BATCH #: SX-1688
PURITY: 83.3% by weight
 2. TEST ANIMALS:
SPECIES: Rats
STRAIN: Sprague-Dawley
AGE: RE-45601, 96 days(both sexes) at exposure
acetone controls, 108 days (both sexes) at exposure
WEIGHT: RE-45601: males, 457-483 g; females, 293-310 g
acetone controls, males, 414-454 g; females, 256-293 g
- g SOURCE: Bantin and Kingman, Inc.

B. STUDY DESIGN:

Animals were housed individually in a stainless steel ventilated cage system, and were acclimated for a period of 55 days (RE-45601) or 67 days (acetone vapor controls). Temperature ranges were 20.5-20.8°C, and the relative humidity ranged from 71-72%. The animals were on a 12 hour light/dark cycle and had free access to food and water except during exposure. The animals were randomly assigned to treatment groups, prior to testing, by the VAX 750-11 computer system using the Tausky-Todd overflow procedure. Each animal was individually identified by a numbered ear tag and a cage card.

Five rats per sex were exposed for 4 hours to an aerosol of diluted test material. The generator solution was made by diluting 540 mls of test material to 600 mls with acetone (Mallinckrodt, nanograde). Dilution of the test material was necessary in order to reduce the viscosity and facilitate aerosolization. An aerosol was produced with two pneumatic nebulizers. Liquid metering pumps were used to deliver the generator solution to each nebulizer at a controlled rate of approximately 5 ml/min. The average air flow in the two nebulizers was 6.2 and 6.3 L/min (at 40 psi). Acetone was periodically added to the test material to maintain a relatively stable acetone concentration in the generator reservoir and in the chamber.

The concentration of acetone vapor in the chamber was monitored continuously with a Miran®-IA Infrared gas analyzer (absorbance at 8.3 um). For RE-45601 analysis, samples of the test chamber atmosphere were drawn through preweighed 25 ml Whatman GF/A filters in open face holders. The samples were taken from approximately 25 centimeters into the chamber just above the breathing zone of the rats. Samples were collected at 2.0 L/min. for 3 minutes at least every 30 minutes, beginning 20 minutes after the start of exposure. The amount of total aerosol in the chamber was estimated gravimetrically. The filter samples were placed in 10 ml of acetonitrile and chemically analyzed. A nominal

concentration of acetone plus RE-45601 was calculated by dividing the total weight loss of both reservoirs plus the weight of acetone added by the total air flow through the chamber. Five additional rats/sex were placed in an identical chamber and were exposed to acetone vapor for the same duration at a slightly higher vapor concentration than the treated groups.

Test and control rats were placed in 10 of 16 individual cages on one level within the respective chambers. Stainless steel 27-inch cubical chambers with pyramidal tops and bottoms were used (approximate volume of 0.42 m³). Chamber pressure was maintained negatively relative to the laboratory, and the total flows through the test and control chambers were 83 and 80 L/min, resp. Temperature and relative humidity in the test and control chambers were recorded initially and then every 30 minutes [1) RE-45601, 22.5°C mean with range of 21.5-23.0°C; 57% mean with range of 56-60%, 2) acetone control, 22.7°C mean with range of 21.5-23.0°C; 54% mean with range of 63-66%, respectively]. Particle size of the aerosol was determined by drawing chamber atmosphere through a multi-jet cascade impactor. Impactor samples were obtained at 20.0 L/min for 1.0 minute 90 and 180 minutes after the start of exposure. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated using the cutoff diameters for each stage supplied by the manufacture and assuming a log normal size distribution.

All animals were weighed before exposure and 2, 7 and 14 days following exposure. Due to the different start dates of the exposures and animal ages, no statistical comparisons were performed on the mean body weights of the animals. The animals were observed for clinical signs frequently during the exposure and then twice daily following exposure, except on weekends when they were observed once daily. All animals were examined for gross necropsy at study termination. Skulls, kidneys and a portion of the liver were preserved for possible future histopathological examination. The lungs were infused via the trachea and preserved. The lungs and tracheas were submitted for histopathological evaluation.

Signed and dated statements regarding quality assurance and GLPs were included.

C. RESULTS:

The mean concentration of RE-45601 during the 4-hr exposure period was 3.9 (0.09/S.D.) mg/L (range of 3.7 to 4.0 mg/L) with an average concentration of acetone of 7.4 mg/L. The mean mass median aerodynamic diameter (MMAD) was 2.8 um and the geometric standard deviation (GSD) was 1.89 (90 and 180 minutes). For the acetone controls, an average concentration of 9.0 (1.1/S.D.) mg/L was determined.

Mean body weights (both sexes) in both the treated and control

groups were equal or somewhat lower at day 2 than the pre-exposure values but consistently increased by 10-20 gms over the 14 day observation period.

Clinical signs of toxicity were reported as follows, "During the RE-45601 exposure, salivation was observed in three of ten animals (two males and one females) and all animals showed squinted or closed eyes. Immediately following the exposure, all animals were salivating and five of ten animals (four males and one female) had a colorless eye discharge. Additional signs of toxicity observed following exposure included red nasal discharge, abnormal respiratory sounds, decreased feces, unkempt appearance, and a yellow/red anogenital discharge. No deaths occurred...and all exposed animals appeared normal within eight days of exposure.

During the first hour of the acetone vapor exposure, one male was salivating. Immediately following exposure and throughout the 14-day observation period, all vehicle control animals appeared normal."

Gross necropsy did not reveal any apparent pathological findings in the treated lungs, trachea, kidney or urinary bladder. One of 5 treated females had red patches in the thymus compared to 0/5 in the acetone controls. Microscopic examination of the lungs suggested a comparative increase in the presence of trace chronic interstitial pneumonia in RE-45601 males as compared to controls (5/5 vs 2/5). In the trachea, a comparative increase in trace or moderate chronic tracheitis in treated males as compared to control males was reported (2/5 vs 0/5). The pathologist stated that these lesions were spontaneous in nature and were not unusual for rats of this strain and age.

D. CONCLUSIONS:

Exposure of male and female rats to RE-45601 aerosolized with acetone at average concentrations of 3.9 mg/L of air for 4 hours did not result in any mortality. This places this compound in Acute Toxicity Category III.

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SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *M. Ioannou 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation (81-3)

CHEM.NO. 721F

MRID NO.: 409745-13

TEST MATERIAL: SELECT 2.0 EC

SYNONYMS: CC-14900

STUDY NUMBER: S-2856

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804-0054

TITLE OF REPORT: The Acute Inhalation Toxicity of SELECT 2.0 EC
(SX-1721) in Rats

AUTHORS: D.C. Gilley

REPORT ISSUED: December 10, 1986

CONCLUSIONS:

Exposure of groups of 5 rats/sex to an average aerosol concentration of 5.4 (0.5/S.D.) mg/L (range of 4.4 to 5.8 mg/L) (1.8% SELECT 2.0 EC v/v in distilled water) for 4 hours did not result in any mortality during the following 14 day observation period. This places this mixture (1.8% v/v of SELECT 2.0 EC in water) in Acute Toxicity Category III.

CLASSIFICATION: Core Minimum.

A. MATERIALS:

1. **TEST COMPOUND:** SELECT 2.0 EC
DESCRIPTION: Clear amber liquid
BATCH #: SX-1721
PURITY: 26.1% RE-45601
2. **TEST ANIMALS:**
SPECIES: Rats
STRAIN: Sprague-Dawley
AGE: 55 days (both sexes) at time of exposure
WEIGHT: males, 264-278 g; females, 174-195 g
SOURCE: Bantin and Kingman, Inc.

B. STUDY DESIGN:

Animals were housed individually in a stainless steel ventilated cage system, and were acclimated for a period of 14 days. Temperature ranges were 20.0-21.0°C, and the relative humidity ranged from 71-72%. The animals were on a 12 hour light/dark cycle and had free access to food and water except during exposure. The animals were randomly assigned to treatment groups, prior to testing, by the VAX 750-11 computer system using the Taussky-Todd overflow procedure. Each animal was individually identified by a numbered ear tag and a cage card.

Five rats per sex were exposed for 4 hours to an aerosol of diluted test material. The generator solution was made by diluting 36 mls of test material to 2.0 liters with distilled water. An aerosol was produced with one pneumatic nebulizer. The nebulizer pulled generator solution from a continuously stirred reservoir. Two liters of fresh generator solution were added to the reservoir approximately every 30 minutes. The nebulizer operated at 50 psi for the first 39 minutes and at 60 psi for the remaining 210 minutes. The average air flow through the nebulizer during the exposure was 16.1 L/min.

For RE-45601 analysis, samples of the test chamber atmosphere were drawn through preweighed 25 ml Whatman GF/A filters in open face holders. The samples were taken from approximately 25 centimeters into the chamber just above the breathing zone of the rats. Samples were collected at 2.0 L/min. for 3 minutes at least every 30 minutes, beginning 20 minutes after the start of exposure. The amount of total aerosol in the chamber was estimated gravimetrically. The filter samples were placed in 10 ml of acetonitrile and chemically analyzed. A nominal concentration of SELECT 2.0 EC was calculated by dividing the total weight loss from the reservoir by the total air flow through the chamber. Five additional rats/sex were placed in an identical chamber and were exposed to filtered air for the same duration.

Test and control rats were placed in 10 of 16 individual cages on one level within the respective chambers. Stainless steel 27-inch cubical chambers with pyramidal tops and bottoms were used

(approximate volume of 0.42 m³). Chamber pressure was maintained negatively relative to the laboratory, and the total flows through the test and control chambers was 84 L/min. Temperature and relative humidity in the test and control chambers were recorded initially and then every 30 minutes [1) SELECT 2.0 EC, 21.3°C mean with range of 21.0-22.0°C; 91% mean with range of 70-95%, 2) air control, 23.0°C mean (no reported range); 66% mean with range of 65-70%, respectively]. Particle size of the aerosol was determined by drawing chamber atmosphere through a multijet cascade impactor. Impactor samples were obtained at 20.0 L/min for 1.0 minute 90 and 180 minutes after the start of exposure. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated using the cutoff diameters for each stage supplied by the manufacture and assuming a log normal size distribution.

All animals were weighed before exposure and 2, 7 and 14 days following exposure. Body weights at 2 days following exposure were required in the protocol, but were inadvertently omitted. Mean body weights of the animals for exposed and control animals were compared using a Student's t test. The animals were observed for clinical signs frequently during the exposure and then twice daily following exposure, except on weekends when they were observed once daily. All animals were examined for gross necropsy at study termination. Skulls, kidneys and a portion of the liver were preserved for possible future histopathological examination. The lungs were infused via the trachea and preserved. The lungs and tracheas were submitted for histopathological evaluation.

Signed and dated statements regarding quality assurance and GLPs were included.

C. RESULTS:

The mean concentration of RE-45601 during the 4-hr exposure period was 0.033 (0.001/S.D.) mg/L (range of 0.032 to 0.034 mg/L) with an average aerosol concentration of 5.4 (0.5/S.D.) mg/L (range of 4.4 to 5.8 mg/L) (1.8% SELECT 2.0 EC v/v in distilled water). The mean mass median aerodynamic diameter (MMAD) was 2.74 and 4.12 μ m and the geometric standard deviation (GSD) was 3.15 and 5.72 (90 and 180 minutes, respectively).

No deaths were reported during the study. Squinted or closed eyes were reported during the exposure period in all treated animals. Following exposure, salivation was reported in 9/10 animals and wet fur (due apparently to test material deposition) was observed in 8/10 animals. All animals appeared normal one day after exposure. Controls were unaffected.

Mean body weights of both male and female rats exposed to the test material were not depressed at 7 or 14 days in comparison with the respective controls.

There were no apparent compound-related changes observed during gross necropsy or microscopy of the lungs and trachea.

D. CONCLUSIONS:

Exposure of groups of 5 rats/sex to an average aerosol concentration of 5.4 (0.5/S.D.) mg/L (range of 4.4 to 5.8 mg/L) (1.8% SELECT 2.0 EC v/v in distilled water) for 4 hours did not result in any mortality during the following 14 day observation period. This places this mixture (1.8% v/v of SELECT 2.0 EC in water) in Acute Toxicity Category III.

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SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *J.M.F. 5/30/80*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute eye irritation (81-4) CHEM. NO.: 721F

MRID NO.: 409745-14

TEST MATERIAL: Clethodim Technical

007972

SYNONYMS: RE-45601

STUDY NUMBER: S-2781

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: The Acute Eye Irritation Potential of RE-45601
Technical (SX-1688)

AUTHORS: J.D. Thompson, B.S.

REPORT ISSUED: October 1, 1986

CONCLUSIONS:

Clearing of conjunctival irritation after instillation of RE-45601 in 6 male rabbit eyes occurred after 72 hours. This is Acute Toxicity Category III.

CLASSIFICATIONS: Core Guideline.

007972

A. MATERIALS:

1. **TEST COMPOUND:** Clethodim Technical (RE-45601)
DESCRIPTION: Dark amber liquid
BATCH #: SX-1688
PURITY: 83.3%
2. **TEST ANIMAL:**
SPECIES: Rabbits (males)
STRAIN: New Zealand White
AGE: 11-15 weeks (time of dosing)
WEIGHT: Not given
SOURCE: L.I.T. Rabbitry, Whitehall, Montana

B. STUDY DESIGN:

Young adult rabbits were conditioned for 20-35 days and examined for ocular defects prior to dosing. Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 22.3-22.8°C and the relative humidity ranged from 56.2-71.0%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Each animal was individually identified by a cage card. The test material was delivered neat. Six animals (3 rinsed, 3 unrinsed with water) were initially tested by placing 0.1 ml in the conjunctival sac of one eye of each rabbit, the untreated eye serving as the control. The control and treated eyes of 3 animals were rinsed for one minute at a rate of 250 ml/minute with distilled water after a 30-second exposure. Since the irritation was not extreme, 3 additional rabbits were dosed with test material (one eye/animal) without rinsing. All eyes were examined and graded for ocular reaction at 1 hour after treatment and at 1, 2 and 3 days, using the method of Draize et al. (1944).

All animals were observed at least once each morning and late afternoon for any clinical signs of toxicity, except on weekends, holidays, and day of sacrifice when observations were made once daily. Animals were sacrificed at study end but were not necropsied.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

No irritation was observed in any control eyes. Neither the cornea or lids of any treated (rinsed, unrinsed) animals were affected by RE-45601. In the 3 animals in which rinsing was performed, slight to moderate redness, chemosis and discharge were observed in the conjunctivae of animals at 1 hour and only slight redness (2/3) remained at 24 hours. In the conjunctivae of all animals whose eyes were left unrinsed, moderate to severe redness, slight to moderate chemosis and slight to severe discharge were

007972

observed. By 48 hours, the conjunctivae showed only slight (4/6) or moderate (1/6) redness, no chemosis and one animal had slight discharge. No eye irritation was seen at 72 hours in any animal. A photocopy of the unrinsed eye irritation scores is attached.

D. CONCLUSIONS:

Clearing of conjunctival irritation after instillation of RE-45601 in rabbit eye occurred after 72 hours. This is Acute Toxicity Category III.

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REVIEWED BY: James N. Rowe, Ph.D.
SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90
J.M.L. 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Acute eye irritation (81-4)

CHEM. NO.: 721F

MRID NO.: 409745-15

007972

TEST MATERIAL: SELECT 2.0 EC

SYNONYMS: CC-14900

STUDY NUMBER: S-2854

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: The Acute Eye Irritation Potential of SELECT 2.0
EC
(CC-14900) (SX-1721)

AUTHORS: J.D. Thompson, B.S.

REPORT ISSUED: November 5, 1986

CONCLUSIONS:

At varying times the cornea, iris and conjunctivae were significantly affected by the test material. All areas of the eye had returned to a normal appearance by 14 days.

This places SELECT 2.0 EC in Acute Eye Irritation Category II.

CLASSIFICATIONS: Core Minimum

A. MATERIALS:

007972

1. TEST COMPOUND: SELECT 2.0 EC
DESCRIPTION: Clear amber liquid
BATCH #: SX-1721
PURITY: 26.1% SELECT (RE-45601) and 67.2% Hi Sol
2. TEST ANIMAL:
SPECIES: Rabbits (females)
STRAIN: New Zealand White
AGE: 11-15 weeks (time of dosing)
WEIGHT: Not given
SOURCE: L.I.T. Rabbitry, Whitehall, Montana

B. STUDY DESIGN:

Young adult rabbits were conditioned for 34 days and examined for ocular defects prior to dosing. Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 22.4-22.7°C and the relative humidity ranged from 58.8-77.1%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Each animal was individually identified by a cage card. The test material was delivered neat. Nine animals (3 rinsed, 6 unrinsed with water) were initially tested by placing 0.1 ml in the conjunctival sac of one eye of each rabbit, the untreated eye serving as the control. The control and treated eyes of 3 animals were rinsed for one minute at a rate of 250 ml/minute with distilled water after a 30-second exposure. All eyes were examined and graded for ocular reaction at 1 hour after treatment and at 1, 2, 3, 4, 7, 10 and 14 days, using the method of Draize et al. (1944).

All animals were observed at least once each morning and late afternoon for any clinical signs of toxicity, except on weekends, holidays, and day of sacrifice when observations were made once daily. Animals were sacrificed at study end; animals were not necropsied.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

A photocopy of irritation scores (Table 2) of unrinsed eyes is attached. At varying times the cornea, iris and conjunctivae were significantly affected by the test material. At 1 hour, all treated irides were mildly affected and this continued in some animals through the 72 hour reading. In addition the two rabbits affected at 72 hours had pannus. The cornea had moderate opacity readings in 5/6 animals at 24 hours which were no longer evident at 10 days. The area of involvement of corneal opacity generally ranged from one-quarter or less to less than one-half. One animal

had three-quarters to the entire corneal surface involved at 48 and 72 hours.

The conjunctival response was severe in all animals at 1 hour with regard to redness, chemosis and discharge. By 96 hours the readings were mostly slight to moderate, and the conjunctivae were normal by 14 days.

Rinsing after instillation of test material (Table 3) had a very beneficial effect upon the recovery of the eye from irritation. Response appeared less severe at 1 hour in the conjunctivae (moderate to severe). The conjunctivae were normal in appearance at 7 days. Two of three animals had only slight corneal opacity at 24 hours with mild iritis. By 48 hours all eyes had a normal appearance.

One of three rinsed control eyes had slight redness at 1 hour only. These controls had normally appearing eyes at all other time periods. Information on the non-rinsed eye controls were not submitted.

D. CONCLUSIONS:

At varying times the cornea, iris and conjunctivae were significantly affected by the test material. All areas of the eye had returned to a normal appearance by 14 days.

This places this compound in Acute Eye Irritation Category II.

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SECONDARY REVIEWER: Mike Ioannou, Ph.D. *JML 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Skin irritation (81-5)

CHEM. NO.: 721F

MRID NO.: 409745-16

TEST MATERIAL: Clethodim Technical

007972

SYNONYMS: RE-45601

STUDY NUMBER: S-2782

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: The Four Hour Skin Irritation Potential of RE-45601 Technical (SX-1688)

AUTHORS: G.L. Korenaga, B.S.

REPORT ISSUED: December 9, 1986

CONCLUSIONS:

Slight to severe erythema and slight to moderate edema were observed at 72 hours post-dosing in 3/6 animals treated with RE-45601 dermally for 4 hours. In a second study, the results were less severe with only 2/6 animals exhibiting slight erythema and no edema. These differences may be the result of inadequate removal of the test material from the skin in the first group or to the use of animals from different suppliers. This compound is placed in Acute Skin Irritation Category II due to the presence of severe erythema observed at 72 hours.

CLASSIFICATIONS: Core Minimum.

A. MATERIALS:

007972

1. TEST COMPOUND: Clethodim Technical (RE-45601)
DESCRIPTION: Dark amber liquid
BATCH #: SX-1688
PURITY: 83.2%
2. TEST ANIMAL:
SPECIES: Rabbits (males)
STRAIN: New Zealand White
AGE: 21-23 weeks; 10-12 weeks (time of dosing)
WEIGHT: Not given
SOURCE: L.I.T. Rabbitry, Whitehall, Montana; R&R Rabbitry, Stanwood, WA

B. STUDY DESIGN:

Six young adult rabbits (2 separate groups) were conditioned for 1) approximately 12 weeks (first group) or 2) 2 weeks (second group). Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 22.3-22.7°C and the relative humidity ranged from 46.7-78.3%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Each animal was individually identified by a cage card. The test material was delivered neat. The fur on the backs of the rabbits was clipped the day before testing. One-half milliliter of test material was applied to each of two intact and two abraded sites on the back of each rabbit. Epidermal abrasions (cross hatching) were made with a hypodermic needle penetrating the stratum corneum over areas of approximately one square inch. After application, each treated site was covered with a gauze patch secured with porous tape. The trunk of each animal was loosely wrapped in a plastic sheet. Paper toweling was wrapped around the plastic to prevent tearing and secured non-occlusively with porous tape. Each animal was collared to protect the wrappings during the four-hour exposure period.

After the exposure period, the skin was wiped with gauze pads moistened with mineral oil until there was no apparent test material residue on the skin. Irritation was scored 1, 24 and 72 hours after removal of the test material and at 7 and 14 days post-dosing using the modified method of Draize et al. (1944). The author reported that due to the unusual pattern of irritation the study was repeated with an additional group of animals.

All animals were observed at least once each day for any clinical signs of toxicity. Animals were sacrificed at study end but were not necropsied. Skin sections were taken from one animal for histopathological evaluation.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

Abrading the skin should be avoided in testing for skin irritation. Therefore, this review will only consider the results from the unabraded skin.

No mention is made of control sites for skin in this study but it is assumed that unexposed skin did not have any significant dermal response to the wrapping procedure which would affect the interpretation of responses in the exposed animals.

Photocopies of Tables 2 and 3 with individual skin irritation scores are attached. Only unabraded skin results will be considered in the discussion below.

In the first six animals tested, 2/6 had slight erythema and 1/6 had slight edema at one hour after treatment. The scores appeared to increase in severity with time, although not necessarily in the same animals. Two of six had well defined to moderate redness and 1/6 had moderate edema. At 48 hours, 3/6 had slight to well-defined erythema and edema (same animals). Curiously, at 72 hours, 3/6 animals (same animals at 48 hours) had slight to severe erythema and slight to moderate edema. By day 14 only 1/6 animals had a slight erythema and edema.

Because of the unusual pattern of irritation seen, a second study was performed (see Table 3). At 1 hour after exposure, 1/6 animals exhibited a slight edema of the skin. At 24 hours, 1/6 animals had slight erythema. At 48 hours, 2/6 rabbits exhibited slight to well-defined erythema only. At 72 hours, 2/6 animals (same as 48 hours) had slight erythema. No skin irritation was seen by 14 days after exposure.

A rabbit (No. 3129) of the first group studied was evaluated microscopically. The intact sites were normal histologically.

D. CONCLUSIONS:

Slight to severe erythema and slight to moderate edema were observed at 72 hours post-dosing in 3/6 animals treated with RE-45601 dermally for 4 hours. In a second study, the results were less severe with only 2/6 animals exhibiting slight erythema and no edema. These differences may be the result of inadequate removal of the test material from the skin in the first group or the use of animals from different suppliers.

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SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D. *J.M.I. 5/30/90*
HEAD SECTION I, TOX. BR. II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Skin irritation (81-5)

CHEM. NO.: 721F

MRID NO.: 409745-17

TEST MATERIAL: SELECT 2.0 EC

007972

SYNONYMS: CC-14900

STUDY NUMBER: S-2855

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: The Four Hour Skin Irritation Potential of SELECT
2.0 EC (CC-14900) (SX-1721)

AUTHORS: J.H. Hedgecock, B.S.

REPORT ISSUED: October 28, 1986

CONCLUSIONS:

Based upon the presence of slight to well-defined erythema and edema at 72 hours, in rabbits exposed to 0.5 ml of SELECT 2.0 EC, this compound is classified as a Category III Skin Irritant.

CLASSIFICATIONS: Core Minimum.

A. MATERIALS:

1. TEST COMPOUND: SELECT 2.0 EC (CC-14900)
DESCRIPTION: Amber liquid
BATCH #: SX-1721
PURITY: 26.0% RE-45601 and 67% HiSol
2. TEST ANIMAL:
SPECIES: Rabbits (males)
STRAIN: New Zealand White
AGE: 11-13 weeks (time of dosing)
WEIGHT: Not given
SOURCE: L.I.T. Rabbitry, Whitehall, Montana

007972

B. STUDY DESIGN:

Six young adult rabbits were conditioned for three weeks. Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 22.4-22.8°C and the relative humidity ranged from 56.2-67.6%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Each animal was individually identified by a cage card. The test material was delivered neat. The fur on the backs of the rabbits was clipped the day before testing. One-half milliliter of test material was applied to each of two intact and two abraded sites on the back of each rabbit. Epidermal abrasions (cross hatching) were made with a hypodermic needle penetrating the stratum corneum over areas of approximately one square inch. After application, each treated site was covered with a gauze patch secured with porous tape. The trunk of each animal was loosely wrapped in a plastic sheet. Paper toweling was wrapped around the plastic to prevent tearing and secured non-occlusively with porous tape. Each animal was collared to protect the wrappings during the four-hour exposure period.

After the exposure period, the skin was wiped with sterile gauze pads until there was no apparent test material residue on the skin. Irritation was scored 1, 24 and 72 hours after removal of the test material and at 7, 13 (animal no. 3174 only) and 14 days post-dosing using the modified method of Draize et al. (1944).

All animals were observed at least once each day for any clinical signs of toxicity. Animals were sacrificed at study end but were not necropsied except for one rabbit (no. 3174) which was killed on day 13 because it developed swollen, fluid-filled eyes with corneal opacity and iritis. Limited histopathological evaluation was performed on this animal.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

Abrading the skin should be avoided in testing for skin irritation. Therefore, this review will only consider the results from the unabraded skin. No mention is made of control sites for skin in this study but it is assumed that unexposed skin did not have any significant dermal response to the wrapping procedure which would affect the interpretation of responses in the exposed animals.

A photocopy of Table 2 with individual skin irritation scores is attached. Only unabraded skin results will be considered in the discussion below.

Topical application of SELECT 2.0 EC produced slight to moderate erythema and edema in 6/6 rabbits at one hour post-dosing. At 24 hours, 5/6 animals still had slight to moderate erythema but only 3/6 had slight to well-defined edema. By 72 hours, 5/6 animals had slight to well-defined erythema and 2/6 animals had slight or well-defined edema. On day 7, 4/6 animals were reported with dry and flaky skin, no edema and 3/6 with slight to well-defined erythema. One animal had a well-defined erythema on day 13 when sacrificed with corneal opacity and iritis. By day 14, no animals (5/6) had any reported skin abnormalities.

Microscopic examination did not reveal any histological changes in the skin of the animal sacrificed prematurely. Tan spots of the liver, reddened lungs with yellow foci and swollen, fluid-filled eyes with opaque, non-rounded eye were reported macroscopically. Microscopically, focal necrosis, congestion and edema of the lungs along with chronic inflammation of the conjunctival sac of the eyes were noted.

D. CONCLUSIONS:

Based upon the presence of slight to well-defined erythema and edema at 72 hours, in rabbits exposed to 0.5 ml of SELECT 2.0 EC, this compound is classified as a Category III Skin Irritant.

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James N. Rowe 5/24/90
JML 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Skin sensitization (81-6)

CHEM. NO.: 721F

MRID NO.: 409745-18

007972

TEST MATERIAL: Clethodim Technical

SYNONYMS: RE-45601

STUDY NUMBER: S-2784

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: Modified Buehler Test For The Skin Sensitization
Potential of RE-45601 Technical (SX-1688)

AUTHORS: P.N. Cooper

REPORT ISSUED: December 17, 1986

CONCLUSIONS:

Repeated dermal application (10x) of 5.0% and 0.5% solutions of RE-45601 to the rabbit skin (15 males/dose group) over a 3 week period, followed by a challenge exposure two weeks post-dosing, did not induce any dermal sensitization. DNCB, the positive control, produced significant dermal sensitization, indicating the responsiveness of the test system to a known sensitizer.

CLASSIFICATIONS: Core Guideline.

A. MATERIALS:

1. **TEST COMPOUND:** Clethodim Technical (RE-45601)
DESCRIPTION: Dark amber liquid
BATCH #: SX-1688
PURITY: 83.4%
2. **TEST ANIMAL:**
SPECIES: Guinea pigs (males)
STRAIN: Hartley
AGE: Not given
WEIGHT: 315-379 grams at time of randomization
SOURCE: Charles River Breeding Laboratory, Stoneridge Division, Wilmington, MA

B. STUDY DESIGN:

The guinea pigs were placed in quarantine for 1 week followed by two weeks of conditioning. Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 20.5-21.6°C and the relative humidity ranged from 40.8-76.4%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Animals were randomly assigned to treatment groups by the VAX 11/750 computer using the Taussky-Fodd overflow procedure so that no significant differences existed between mean body weights of the treatment groups. Each animal was individually identified by an ear tag and a cage card.

In addition to RE-45601, 1-chloro-2,4-dinitrobenzene (DNCB), ethanol and nanograde acetone were used in the dermal sensitization test. RE-45601 was diluted to 0.5% and 5.0% weight/weight (w/w) in 80% ethanol or acetone each dosing day. Ethanol was diluted to 80% (v/v) in distilled water each dosing day. DNCB was dissolved at 0.1% w/w in 80% ethanol or acetone each dosing day.

Body weights were measured weekly and statistically analyzed using one-way analysis of variance. The skin sensitization potential was assessed using a modification of the Buehler test (1965). Pre-test screens using the challenge procedures were conducted to determine the dose level causing no to minimal acute irritation (0.5%) and minimal to slight acute irritation (5.0%).

During induction, over a 22-day period, 10 occluded epicutaneous applications were administered on alternate days (Monday, Wednesday, Friday) starting on Wednesday. The right flank of each animal was clipped the day before the first induction application and weekly thereafter for the remainder of the study. For the first induction application, a Hill Top Chamber containing 0.3 ml of the correct dosing solution was applied to the right flank of each animal. The chamber was held in place by a PEG® bandage wrapped around each animal's trunk. After 6 hours, the wraps were removed and the application sites were wiped free of

residual material using water-moistened gauze pads. For the remaining induction applications, 0.4 ml of the dosing solution was applied to the right flank with a one-inch square of Webril® gauze. The gauze was occluded with a two-inch square of polyethylene and wrapped for 6 hours as described above. Blind skin irritation readings were made 24 and 48 hours after the first induction application and at 24 hours after the 5th and 10th induction applications to determine skin irritation from repeated exposures.

Animals were challenged 14 days after the tenth induction application. The upper left flank of each animal was clipped on the day before dosing. A Hill Top Chamber® containing 0.3 ml of the various test materials was applied to the upper left flank and wrapped as previously described. Approximately 24 hours after the start of the exposure, depilatory was applied to the test sites for no more than 30 minutes followed by thorough washing with lukewarm water, drying and return of animals to their cages. After a minimum of 2 hours, the test sites were scored for erythema and edema. The animals were also scored at 48 and 72 hours.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

A photocopy of the summary of incidence of sensitization and mean irritation scores following induction and challenge applications is attached (Table 1).

INDUCTION PHASE:

During induction, 1/15 animals showed a slight erythema at 48 hours with the first induction application of 5.0% RE-45601. After the fifth induction application, 2/15 animals showed slight to well-defined erythema and one of these two rabbits had a slight edema of the skin. After the tenth application, 1/15 rabbits had a severe redness of the skin with a slight edema.

During induction with 0.5% RE-45601, no skin irritation was observed at the first application. After the fifth application, 1/15 rabbits exhibited a severe erythema with a well-defined edema. During the final application, one rabbit had a slight redness at the site of application.

The RE-45601 control, 80% ethanol (v/v in distilled water) and challenge treatment with 0.5% test material, exhibited no skin irritation during the first and fifth induction application. After the 10th application 1/10 animals had a slight redness. One rabbit was also found dead following the eighth induction application (# 119).

The DNCB positive control, did not show any skin irritation after the first application. By the fifth induction application, all animals (10/10) exhibited slight to severe erythema and/or

slight to well-defined edema. After the tenth application, 10/10 animals had moderate to severe erythema and well-defined to moderate edema.

The DNCB control, 80% ethanol (v/v in distilled water) and challenge treatment with 0.1% DNCB, had no skin irritation after the first or fifth induction application but 1/10 animals had severe erythema with well-defined edema after the final induction application.

CHALLENGE:

The positive control showed the rabbits were responsive to a dermal sensitizer, DNCB, with mean scores of 5.9, 5.2 and 5.1 at 24, 48 and 72 hours after challenge treatment. The high dose RE-45601, 5.0% RE-45601, had 1/15 animals with slight erythema at 24 hours after challenge but no skin responses at 48 or 72 hours. No dermal response was seen with the low dose, 0.5% RE-45601, or the irritation control at any time after the challenge exposure. The DNCB irritation control had 1/10 rabbits with slight edema at 24 hours only.

No statistically significant differences between the treated and respective control groups were noted.

D. CONCLUSIONS:

Repeated dermal application (10x) of 5.0% and 0.5% solutions of RE-45601 to the rabbit skin (15 males/dose group) over a 3 week period, followed by a challenge exposure two weeks post-dosing, did not induce any dermal sensitization. DNCB, the positive control, produced significant dermal sensitization, indicating the responsiveness of the test system to a known sensitizer.

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SECTION I, TOX. BR. II (H7509C)
SECONDARY REVIEWER: Mike Ioannou, Ph.D.
HEAD SECTION I, TOX. BR. II (H7509C)

James N. Rowe 5/24/90
J.M.f 5/30/90

DATA EVALUATION REPORT

STUDY TYPE: Skin sensitization (81-6)

CHEM. NO.: 721F

MRID NO.: 409745-19

007972

TEST MATERIAL: SELECT 2.0 EC

SYNONYMS: CC-14900

STUDY NUMBER: S-2857

SPONSOR: Chevron Chemical Company
Ortho Agricultural Chemicals Division
15049 San Pablo Avenue
Richmond, CA 94804-0010

TESTING FACILITY: Chevron Environmental Health Center, Inc.
15299 San Pablo Avenue
Richmond, CA 94804

TITLE OF REPORT: Modified Buehler Test For The Skin Sensitization
Potential of CC-14900 (SELECT 2.0 EC) (SX-1721)

AUTHORS: N. S. Hirose, B.S.

REPORT ISSUED: December 19, 1986

CONCLUSIONS:

Repeated dermal application (10x) of 10.0% and 1.0% solutions of CC-14900 to the rabbit skin (15 males/dose group) over a 3 week period, followed by a challenge exposure (1.0%) two weeks post-dosing, did not induce any dermal sensitization. DNCB, the positive control, produced significant dermal sensitization, indicating the responsiveness of the test system to a known sensitizer. However, the severity and persistence of the skin irritation response observed at the fifth and tenth induction applications strongly suggest a higher challenge concentration of CC-14900 may have produced evidence of dermal sensitivity.

CLASSIFICATIONS: Core Supplementary. A repeat study is requested using a higher challenge concentration (10%) of CC-14900 in order to rule out a potential dermal sensitization response at elevated skin exposures.

A. MATERIALS:

1. TEST COMPOUND: SELECT 2.0 EC
DESCRIPTION: amber liquid
BATCH #: SX-1721
PURITY: 26.1% RE-45601
2. TEST ANIMAL:
SPECIES: Guinea pigs (males)
STRAIN: Hartley
AGE: Not given
WEIGHT: 324-451 grams at time of randomization
SOURCE: Charles River Breeding Laboratory, Stoneridge
Division, Wilmington, MA

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B. STUDY DESIGN:

The guinea pigs were placed in quarantine for 1 week followed by two weeks of conditioning. Animals were housed individually in wire-bottom cages in an air-conditioned room. The temperature ranged from 19.1-20.1°C and the relative humidity ranged from 62.4-80.4%. Animals were kept on a 12-hour light/dark cycle and were watered and fed ad libitum.

Animals were randomly assigned to treatment groups by the VAX 11/750 computer using the Taussky-Todd overflow procedure so that no significant differences existed between mean body weights of the treatment groups. Each animal was individually identified by an ear tag and a cage card.

In addition to CC-14900, 1-chloro-2,4-dinitrobenzene (DNCB), ethanol and nanograde acetone were used in the dermal sensitization test. CC-14900 was diluted to 1.0% and 10.0% weight/weight (w/w) in distilled water each dosing day. Ethanol was diluted to 80% (v/v) in distilled water each dosing day. DNCB was dissolved at 0.1% w/w in 80% ethanol or acetone each dosing day.

Body weights were measured weekly and statistically analyzed using one-way analysis of variance. The skin sensitization potential was assessed using a modification of the Buehler test (1965). Pre-test screens using the challenge procedures were conducted to determine the dose level causing no to minimal acute irritation (1.0%) and minimal to slight acute irritation (10.0%).

During induction, over a 22-day period, 10 occluded epicutaneous applications were administered on alternate days (Monday, Wednesday, Friday). The right flank of each animal was clipped the day before the first induction application and weekly thereafter for the remainder of the study. For the first induction application, a Hill Top Chamber containing 0.3 ml of the correct dosing solution was applied to the right flank of each animal. The chamber was held in place by a PEG® bandage wrapped around each animal's trunk. After 6 hours, the wraps were removed and the application sites were wiped free of residual material using water-

moistened gauze pads. For the remaining induction applications, 0.4 ml of the dosing solution was applied to the right flank with a one-inch square of Webril® gauze. The gauze was occluded with a two-inch square of polyethylene and wrapped for 6 hours as described above. Blind skin irritation readings were made 24 and 48 hours after the first induction application and at 24 hours after the 5th and 10th induction applications to determine skin irritation from repeated exposures.

Animals were challenged 14 days after the tenth induction application. The upper left flank of each animal was clipped on the day before dosing. A Hill Top Chamber® containing 0.3 ml of the various test materials was applied to the upper left flank and wrapped as previously described. Approximately 24 hours after the start of the exposure, depilatory was applied to the test sites for no more than 15 minutes followed by thorough washing with lukewarm water, drying and return of animals to their cages. After a minimum of 2 hours, the test sites were scored for erythema and edema. The animals were also scored at 48 and 72 hours.

Signed and dated statements for quality assurance and GLPs were included.

C. RESULTS:

A photocopy of the summary incidence and mean irritation scores following induction and challenge applications (Table 1) is attached. Photocopies of Tables 2 and 7 are also attached.

INDUCTION PHASE:

The high dose CC-14900 (10%) induced slight erythema or edema in 3/15 rabbits at 48 hours after the first induction application. During the fifth application well-defined to severe erythema and slight to moderate edema were seen in 15/15 animals. Fifteen of fifteen rabbits had slight to severe (increased number with this response as well as scabbing) erythema and slight to moderate edema after the tenth induction application. This suggests a dermal sensitization response at this concentration.

The low dose CC-14900 (1%) did not induce any skin irritation during the entire induction phase. The CC-14900 irritation controls had no evidence of skin irritation during the induction phase.

The DNCB positive control group did not induce any skin irritation after the first application. All animals had well-defined to severe (8/10) erythema and edema after the fifth induction application. All animals had severe redness with many having scabbing and well-defined to severe edema after the tenth application. The DNCB irritation control reported only 1/10 animals after the fifth induction application with severe erythema and slight edema.

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CHALLENGE:

No skin irritation was observed in the high dose, low dose or irritation control groups for CC-14900 at any observation period after the challenge application. The DNCB positive control had mean irritation scores of 4.2, 4.5 and 4.7 at 24, 48 and 72 hours, respectively, after dermal challenge. The DNCB irritation control reported only 1/10 animals with a slight erythema at 72 hours after challenge.

No statistically significant differences between the treated and respective control group mean body weights were noted.

D. CONCLUSIONS:

Repeated dermal application (10x) of 10.0% and 1.0% solutions of CC-14900 to the rabbit skin (15 males/dose group) over a 3 week period, followed by a challenge exposure (1.0%) two weeks post-dosing, did not induce any dermal sensitization. DNCB, the positive control, produced significant dermal sensitization, indicating the responsiveness of the test system to a known sensitizer. However, the severity and persistence of the skin irritation response observed at the fifth and tenth induction application strongly suggest a higher challenge concentration of CC-14900 may have produced evidence of dermal sensitivity. This may be related to a greater penetration of CC-14900 through the skin at the higher concentration level enabling a hypersensitivity response to be initiated.

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EPA No.: 68D80056
DYNAMAC No.: 245-N
TASK No.: 2-45N
April 25, 1990

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DATA EVALUATION RECORD

RE-45601 TECHNICAL

Four-Week Subchronic Oral Toxicity Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

Robert J. Weir

4/24/90

EPA No.: 68D80056
DYNAMAC No.: 2-45N
TASK No.: 245-N
April 25, 1990

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DATA EVALUATION RECORD

RE-45601 TECHNICAL

Four-Week Subchronic Oral Toxicity Study in Mice

REVIEWED BY:

Roman J. Pienta, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Roman J. Pienta
Date: April 25, 1990

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 25, 1990

APPROVED BY:

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Signature: Robert J. Weir
Date: 4/25/90

James Rowe, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: James N. Rowe
Date: 5/24/90

Mike Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 5/30/90

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DATA EVALUATION RECORD

STUDY TYPE: Four-Week Subchronic Oral Toxicity Study in Mice.

MRID NUMBER: 410301-03.

TEST MATERIAL: RE-45601 Technical.

SYNONYMS: Clethodim, (E,E)-(+/)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.

STUDY NUMBER: Laboratory Project Identification: S-2733.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA.

TITLE OF REPORT: Four-Week Subchronic Oral Toxicity Study in Mice Chevron RE-45601 Technical.

AUTHOR: R. H. Cox.

REPORT ISSUED: July 21, 1986.

CONCLUSIONS: RE-45601 technical was administered to male and female CD-1 mice at dietary levels of 0, 100, 250, 625, 1500, and 4000 ppm for 4 weeks. There were no overt signs of toxicity or dose-related effects on mortality, clinical observations, body weights, or food consumption. Hematology examination revealed a dose-related decrease in red cell mass as manifested by decreased erythrocytes, hemoglobin, and hematocrit values. Increased liver weights and increased hypertrophy of centrilobular hepatocytes in male and female mice exposed to 4000 ppm of RE-45601 technical were observed. Increased liver weights were also observed in male mice administered 1500 ppm in the diet.

Classification: CORE Supplementary.

A. MATERIALS:

1. Test Compound: RE-45601 technical; description: amber liquid; batch No.: SX-1688; purity: 83.3%.
2. Test Animals: Species: mouse; strain: CD-1; age: 4 weeks; weight: males--25.6 to 33.5 g, females--17.4 to 24.5 g at study initiation; source: Charles River Breeding Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Following 20 days of acclimation, animals were assigned by a weight randomization program to the following test groups:

Test Group	Dose Level (ppm)	Number of Animals	
		Males	Females
1 Control	0	10	10
2 Low (LDT)	150	10	10
3 Mid-1 (MDT)	250	10	10
4 Mid-2 (MDT)	625	10	10
5 Mid-3 (MDT)	1500	10	10
6 High (HDT)	4000	10	10

2. Diet Preparation: Frozen liquid test material was allowed to reach room temperature. For each dose level, an appropriate amount of test material was weighed into an amber glass vial and dissolved in a total of 15 mL pesticide-grade acetone. The test material was then added to feed to prepare a 2-kg premix and mixed in a Hobart mixer for 5 minutes. The remaining amount of required feed was added and further mixed an additional 15 minutes. There was no weight adjustment to correct for purity. Control diet was prepared by mixing acetone (15 mL/kg feed) with an appropriate amount of feed and mixing for 15 minutes.

Test diets were prepared weekly and provided to the animals immediately after preparation and on Day 4 of each week. During the interim, unused test diet was stored in the dark at -20°C. Test diet samples were analyzed for homogeneity prior to initiation of the study and were analyzed for test material concentration on Day 0, Day 4, and Day 8 of the study.

Results: Samples analyzed for homogeneity indicated a homogeneous mix within acceptable limits at all dose levels. The homogeneity study showed that test material concentrations ranged from 95.2 to 96.5% of theoretical concentration, and coefficients of variation ranged from ± 0.9 to $\pm 2.6\%$. Table 1 summarizes results of stability studies. Concentrations of freshly prepared diets were found to be 87.0, 88.5, 94.5, 96.8, and 95.2% of normal concentrations for 100, 250, 625, 1500, and 4000 ppm, respectively. Stability of the test compound ranged from 84.2 to 94.9% for samples stored frozen for 3 days, and from 72.6 to 94.9% for samples stored frozen for 3 days followed by 4 days in the hopper at room temperature. Coefficients of variation ranged from ± 0.0 to 8.7% for freshly prepared diets, ± 2.0 to 8.1% for diets stored frozen for 3 days, and ± 1.5 to $\pm 10\%$ for diets stored frozen for 3 days followed by 4 days at room temperature. Because the relative instability of low concentrations of the test material at room temperature, the diet was replaced twice weekly.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and tap water ad libitum.
4. Statistics: The following procedures were utilized in analyzing the numerical data. Means and standard deviations were calculated for body weights and body weight gains, food consumption, clinical hematology values, absolute organ weights, and organ-to-terminal body weight ratios. Data from the control group were compared statistically to data from the same sex of treated groups.

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TABLE 1. Stability of RE-45601 Technical

Dose Level (ppm)	Sample Type ^a	Average ppm Found	Percent of Theoretical Concentration ^b	Coefficient of Variation
100	0	72.8	87.0	± 3.2% (n = 4)
	4	71.2	85.1	± 2.0% (n = 4)
	8	60.8	72.6	± 10.0% (n = 3)
250	0	185	88.5	± 8.7% (n = 4)
	4	176	84.2	± 8.1% (n = 4)
	8	164	78.5	± 5.9% (n = 3)
625	0	494	94.5	± 4.5% (n = 4)
	4	469	89.7	± 5.7% (n = 4)
	8	454	86.8	± 4.5% (n = 4)
1500	0	1220	96.8	± 0.0% (n = 4)
	4	1170	92.9	± 3.2% (n = 4)
	8	1120	88.9	± 5.1% (n = 4)
4000	0	3190	95.2	± 2.7% (n = 4)
	4	3180	94.9	± 4.0% (n = 4)
	8	3180	94.9	± 1.5% (n = 4)

^a0 = Freshly prepared diet.

4 = 3 days frozen.

8 = 3 days frozen + 4 days in hopper.

^bBased on theoretical concentrations of 83.7, 209, 523, 1260, and 3350 ppm active ingredient.174
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Levine's test of homogeneity of variances was used. When variances of untransformed data were heterogeneous, analyses were performed on transformed ($\log_{10}$, square, square root, reciprocal, and arcsine) or rank transformed data. Analyses of variance was performed, and significance between controls and each dose group was determined with Dunnett's t-test.

5. Quality Assurance: A quality assurance statement was signed and dated on June 4, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity. Animals were examined daily for signs of overt toxicity.

Results: No mortality occurred during the study. The author reported no abnormal observations regarding clinical signs.

2. Body Weight: Animals were weighed at initiation of the study and weekly thereafter.

Results: Representative data on mean body weights and mean body weight gains are summarized on Table 2. Mean body weights and body weight gains were similar in dosed and control male and female mice throughout the dosing period.

3. Food Consumption and Compound Intake: Food consumption was determined weekly.

Results: All food consumption values were similar between compound-treated and control groups. Compound consumption was not calculated.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus at termination of the study for hematology analysis from all animals. The CHECKED (X) parameters were examined:

TABLE 2. Representative Mean Body Weights ($g \pm S.D.$)
for Mice Fed RE-45601 Technical for 4 Weeks^a

Dose Level (ppm)	<u>Mean Body Weight Gains at Weeks:</u>			Body Weight Gains (g) 0-4 Weeks
	0	2	4	
<u>Males</u>				
0	29.5 ± 1.07	33.1 ± 1.30	34.8 ± 1.41	5.3 ± 1.21
100	29.8 ± 1.94	33.0 ± 1.44	35.3 ± 1.75	5.5 ± 0.76
250	29.7 ± 1.93	32.6 ± 1.82	34.4 ± 2.11	4.6 ± 0.94
625	29.2 ± 1.90	32.3 ± 2.43	34.1 ± 2.68	4.9 ± 1.27
1500	29.0 ± 1.10	32.3 ± 1.46	34.3 ± 1.66	5.3 ± 1.02
4000	29.9 ± 1.40	32.6 ± 2.00	34.9 ± 2.57	5.1 ± 1.85
<u>Females</u>				
0	21.9 ± 1.36	24.6 ± 1.52	27.0 ± 1.76	5.1 ± 0.93
100	21.8 ± 1.19	24.3 ± 1.22	26.9 ± 1.61	5.1 ± 1.10
250	21.5 ± 0.78	24.2 ± 1.04	26.2 ± 1.15	4.7 ± 0.77
625	21.9 ± 1.01	25.5 ± 1.59	27.5 ± 1.45	5.6 ± 0.78
1500	21.0 ± 1.90	24.4 ± 2.05	26.6 ± 2.07	5.6 ± 2.02
4000	21.1 ± 1.90	23.2 ± 1.61	25.4 ± 1.72	4.4 ± 2.52

^aData were extracted from CBI document, Table 1.

a. Hematology:

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	X Coagulation: thromboplastin time (PT)
X Reticulocyte count (RETIC) ^a	
Red cell morphology	

Results: Representative selected clinical hematology values are presented in Table 3. Clinical hematology parameters were similar in control and compound-treated groups. A dose-related decrease in erythrocyte, hemoglobin, and hematocrit parameters was observed in male mice. A significant ($p < 0.05$) decrease was observed in erythrocyte and hemoglobin values in 1500-ppm and 4000-ppm males and 1500-ppm females. Hematocrit values also were significantly ($p < 0.05$) decreased in 4000-ppm males. Although not statistically significant, a dose-related increase in reticulocyte counts was exhibited in animals measured, which coincided with the decreases in erythrocyte, hemoglobin, and hematocrit values. The author stated that the decreases appeared to be treatment-related in both sexes.

b. Clinical Chemistry: Blood samples were not collected for clinical chemistry.

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected. The kidneys, lungs, livers, and all gross lesions were examined histologically. In addition, the (XX) organs were weighed:

^aReticulocyte counts were performed on all control animals and on any animal showing signs of anemia.

TABLE 3. Representative Hematology Results ($\pm$ SD) in Mice Fed RE-45601 Technical For 4 Weeks^a

Parameter	Dose Level (ppm)					
	0	100	250	625	1500	4000
<u>Males</u>						
Erythrocyte Count (10^6 /mL)	9.89 $\pm$ 0.25	9.89 $\pm$ 0.48	9.68 $\pm$ 0.48	9.54 $\pm$ 0.38	9.46 $\pm$ 0.29*	9.05 $\pm$ 0.55*
Hemoglobin (g/dL)	16.6 $\pm$ 0.25	16.5 $\pm$ 0.96	16.2 $\pm$ 0.79	15.9 $\pm$ 0.68*	15.9 $\pm$ 0.60*	15.3 $\pm$ 0.71*
Hematocrit (%)	50.1 $\pm$ 1.90	52.3 $\pm$ 3.29	42.9 $\pm$ 2.93	48.6 $\pm$ 3.60	48.7 $\pm$ 3.11	46.2 $\pm$ 3.54*
Reticulocyte Count (%)	2.1 $\pm$ 0.58	0	0	2.4 $\pm$ 0.79	2.6 $\pm$ 0.98	2.9 $\pm$ 1.34
<u>Females</u>						
Erythrocyte Count (10^6 /mL)	9.30 $\pm$ 0.43	9.59 $\pm$ 0.53	9.83 $\pm$ 0.25	9.59 $\pm$ 0.30	9.21 $\pm$ 0.42*	9.09 $\pm$ 0.45
Hemoglobin (g/dL)	16.5 $\pm$ 0.88	16.2 $\pm$ 0.90	16.7 $\pm$ 0.52	16.2 $\pm$ 0.54	15.6 $\pm$ 0.54*	15.9 $\pm$ 0.61
Hematocrit (%)	50.5 $\pm$ 3.76	49.4 $\pm$ 4.31	51.9 $\pm$ 2.16	49.2 $\pm$ 2.11	48.7 $\pm$ 3.10	48.5 $\pm$ 1.91
Reticulocyte Count (%)	2.4 $\pm$ 0.81	0	0	0	2.4 $\pm$ 0.81	2.8 $\pm$ 0.90

^aData were extracted from CBI document, Table 3.*Significantly different from the control value, $p < 0.05$.

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<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
X Tongue	X Aorta	XX Brain with stem
X Salivary glands	X Heart	X Peripheral nerve
X Esophagus	X Bone marrow	(sciatic nerve)
X Stomach	X Lymph nodes	X Spinal cord
X Duodenum	(mesenteric)	(3 levels)
X Jejunum	X Spleen	X Pituitary
X Ileum	X Thymus	X Eyes
X Cecum		(optic nerve)
X Colon		
X Rectum		
XX Liver	<u>Urogenital</u>	<u>Glandular</u>
XX Gallbladder	XX Kidneys	XX Adrenals
X Pancreas	X Urinary bladder	Lacrimal gland
	XX Testes	X Mammary gland
<u>Respiratory</u>	XX Epididymides	(left inguinal)
X Trachea	X Prostate	X Parathyroids
XX Lung	X Seminal vesicle	X Thyroids
	XX Ovaries	Harderian glands
	XX Uterus	
		<u>Other</u>
		Bone (sternum and femur)
		X Skeletal muscle
		X Skin
		X All gross lesions and masses
		X Femur

Results:

- a. Organ Weights: Representative results of terminal absolute and relative liver (with gallbladder) weights are presented in Table 4. The absolute liver weight was significantly ($p < 0.05$) greater than the control value for 1500- and 4000-ppm males and 4000-ppm females. For the same groups of animals, the relative (to body weight) liver weights were also significantly greater than the respective controls. All other absolute and relative organ weights were similar between control and dosed groups of both sexes of mice.
- b. Gross Pathology: There were no compound-related changes in macroscopic pathology in dosed animals sacrificed after 4 weeks. Gross pathology lesions included one mottled lung, two dark livers, three pale livers, one kidney cyst, one unequally sized kidney, one irregularly shaped kidney, one ileum with lumen material, three uteri with lumen fluid, one distended uterus, one uterus with a thickened wall, one externally opaque eye, and one internally opaque eye.

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TABLE 4. Mean Liver Weights (g $\pm$ S.D.) and Liver-to-Body Weight Ratios in Mice Fed RE-45601 Technical for 4 Weeks^a

Dose Level (ppm)	Absolute Weight ^b (g)	Relative Weight (%)
<u>Males</u>		
0	1.29 $\pm$ 0.06	4.339 $\pm$ 0.33
100	1.34 $\pm$ 0.10	4.463 $\pm$ 0.23
250	1.30 $\pm$ 0.09	4.463 $\pm$ 0.44
625	1.34 $\pm$ 0.10	4.580 $\pm$ 0.27
1500	1.46 $\pm$ 0.20*	4.931 $\pm$ 0.49*
4000	1.83 $\pm$ 0.18*	6.161 $\pm$ 0.42*
<u>Females</u>		
0	1.00 $\pm$ 0.09	4.634 $\pm$ 0.34
100	1.01 $\pm$ 0.10	4.451 $\pm$ 0.22
250	0.98 $\pm$ 0.08	4.517 $\pm$ 0.30
625	1.10 $\pm$ 0.08	4.802 $\pm$ 0.29
1500	1.12 $\pm$ 0.18	4.928 $\pm$ 0.41
4000	1.22 $\pm$ 0.13*	5.694 $\pm$ 0.43*

^aData were extracted from CBI document Tables 4 and 5.^bBased on 10 animals/sex/dose.*Significantly different from control values, $p < 0.05$.

- c. Microscopic Pathology: Table 5 summarizes histopathologic findings. Increased hypertrophy of centrilobular hepatocytes was observed in all male mice (minimal to moderate) and 8 of 10 female mice (minimal to slight) fed 4000 ppm RE-45601. The author considered the liver alterations to be compound-related in both sexes. No other treatment-related histologic changes were observed. A variety of other spontaneous findings were considered to be incidental.
- D. STUDY AUTHOR'S CONCLUSIONS: The study author concluded that the dietary administration of RE-45601 technical to mice at the level of 4000 ppm for 4 weeks resulted in toxicity as evidenced by decreased red cell mass, increased liver weights, and increased hypertrophy of hepatocytes. Administration of 1500 ppm also resulted in slight decreases in red cell mass in both sexes and increased liver weights in male mice.
- E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was acceptable. However, since this is a 4-week oral toxicity study, it does not conform to Guideline No. 82-1 which sets criteria for a subchronic 90-day oral toxicity study. We agree with the author's assessment that liver was the apparent organ site for toxicity in that increased liver weights were observed in male and female mice exposed to 4000 ppm of test compound, liver weights were also increased in male mice exposed to 1500 ppm, and increased hypertrophy of centrilobular hepatocytes occurred in mice exposed to 4000 ppm of test material. An apparent dose-related decrease occurred in red cell mass as evidenced by slight decreases in RBC, hemoglobin, and hematocrit values. All other parameters measured were similar in treated groups when compared with respective controls. The study has a number of limitations: a 4-week exposure may not be sufficient to establish a no-effect level; no clinical biochemistry determinations were made; and dietary analysis data showed that some of the dose concentrations were not within acceptable limits, indicating that dose levels may have been lower than nominal values.

TABLE 5. Nonneoplastic Histopathologic Lesions in Mice Fed Mf-45601 Technical for 4 Weeks^a

Organ/Finding	Dose level (ppm)											
	Males						Females					
	0	100	250	625	1500	4000	0	100	250	625	1500	4000
<u>Liver</u>	(10) ^b	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Focal congestive necrosis	1	1	1	1	2	4	2	1	0	0	0	0
Hypertrophy, centrilobular	4	0	3	2	4	10	0	0	0	0	1	8
Focal endonuclear infiltration	0	2	1	0	0	1	2	1	2	3	3	2
Hepatic vacuolization	0	0	0	0	0	0	0	1	1	0	0	0
<u>Kidney</u>	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Focal mononuclear infiltration	7	9	8	8	5	5	5	8	4	6	8	6
Regenerative epithelium	1	1	0	0	0	1	1	3	3	0	1	1
Pelvic dilatation	0	0	0	0	0	1	0	0	0	0	0	0
Hypoplasia, unilateral	0	0	0	0	0	0	0	0	0	1	0	0
<u>Stomach</u>	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(2)	(1)
Hyperkeratosis	0	0	0	0	0	1	0	0	0	0	0	0
Acanthosis	0	0	0	0	0	1	0	0	0	0	0	0
Erosion/ulceration	0	0	0	0	0	0	0	0	0	0	1	0
<u>Uterus</u>	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Distention of bursa	0	0	0	0	0	0	0	0	0	0	0	1
<u>Intestine</u>	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Congestion	0	0	0	0	0	0	0	0	0	0	1	0
<u>Uterus</u>	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(2)	(2)	(0)	(2)	(0)
Dilatation	0	0	0	0	0	0	0	0	0	0	1	0
Endometrial hyperplasia	0	0	0	0	0	0	0	2	2	0	1	0

^aData were extracted from CBI document, Table 7.

^bNumbers in parentheses refer to number of animals for which tissues were examined histologically.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-L
TASK No.: 2-45L
April 23, 1990

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DATA EVALUATION RECORD
CLETHODIM IMINE SULFONE
5-Week Oral Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: *Roman J. Penta for*
Date: *April 25, 1990*

EPA No.: 68D80056
DYNAMAC No.: 245-L
TASK No.: 2-45L
April 25, 1990

007972

DATA EVALUATION RECORD

CLETHODIM IMINE SULFONE

5-Week Oral Toxicity Study in Rats

REVIEWED BY:

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Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 25, 1990

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Date: 5/30/90

DATA EVALUATION RECORD

007972

GUIDELINE §82-1

STUDY TYPE: Subchronic oral toxicity study in rats.

MRID NUMBER: 410301-04.

TEST MATERIAL: RE-47719; clethodim imine sulfone.

SYNONYM(S): N/A.

STUDY NUMBER: S-3158.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc., Richmond, CA.

TITLE OF REPORT: Five-Week Oral Toxicity Study in Rats with RE-47719 (SX-1800).

AUTHORS: Bagos, A. C., Beatty, P. W.

REPORT ISSUED: November 15, 1988.

CONCLUSIONS: RE-47719 was fed to male and female Sprague-Dawley rats at calculated mean concentrations of 0, 80.8, 871, or 7,820 ppm for 5 weeks (nominal doses of 100, 1,000, or 8,000 ppm). After correction for test compound instability in the diet in both the frozen state and when in food hoppers under in-use conditions, the respective mean compound intakes were 0, 6.66, 70.9, or 604 mg/kg/day for males and 0, 7.82, 83.7, or 723 mg/kg/day for females. No compound-related effects were observed on mortality, clinical signs, ophthalmology, hematology parameters, or gross lesions in males or females, or on food consumption in females. Compound-related effects in males and females of the high-dose group included significant increases in serum cholesterol, absolute liver weight, and relative (to brain and body) liver weight; food consumption was significantly decreased in high-dose males but not females. Effects in high-dose males and females that may have been compound related included kidney hydronephrosis (incidences not significant), significantly lower mean body weights, and significantly decreased mean body weight gains. No compound-related effects were noted in animals of the low- or mid-dose groups. On the basis of these results, the 5-week oral LOEL of RE-47719 is 7,820 ppm (604 and 723 mg/kg/day in males and females, respectively) and the NOEL is 871 ppm (70.9 and 83.7 mg/kg/day in males and females, respectively).

Classification: This study cannot be CORE classified, since there are no available EPA Pesticide Guidelines for a 5-week feeding study.

A. MATERIALS:

1. Test Compound: RE-47719; description: white solid; batch No.: SX-1800; purity: 99.3%.
2. Test Animals: Species: rat; strain: Sprague-Dawley; age: 44 days; weight: males--203 to 230 g, females--141 to 169 g; source: Charles River Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: After a quarantine period of 9 days, and an acclimation period of 5 days, rats were assigned to the following test groups using a computerized randomization procedure:

Test Group	Nominal Dose in Diet ^a (ppm)	Measured Initial Concentrations (ppm)	Main Study (5 weeks)	
			Males	Females
1 Control	0	0	10	10
2 Low (LDT)	100	80.8	10	10
3 Mid (MDT)	1,000	871	10	10
4 High (HDT)	8,000	7,820	10	10

^aNo correction was made for purity of the test substance.

Each animal was identified by a metal ear tag and color-coded cage card and housed individually in a room with a 12-hour light/dark cycle, temperature of 20-23°C, and humidity of 55-78%.

2. Diet Preparation: Diets were prepared weekly to contain target concentrations of 0, 100, 1,000, or 8,000 ppm of the test material. For preparation of the 8,000-ppm diet, a weighed amount of the neat test material was added directly to Purina Certified Rodent Chow #5002 and mixed for a total of 25 minutes. Diets containing 1,000 and 100 ppm of the test material were prepared by mixing weighed amounts of the 8,000-ppm diet with rodent chow for 15 minutes. Diets were frozen until use. Samples for determination of test compound in the diet were collected and frozen at the beginning of study weeks 1, 3, and 5. Samples for analysis of homogeneity of the test substance in the diets were taken from the initial batch of each diet immediately after mixing from three different locations in the mixing bowl; similar triplicate samples were collected after each of two additional 15-second mixing intervals; all samples were frozen. Samples for analysis of compound stability in the diet were taken immediately before use from diets mixed for use during weeks 1, 3, and 5; each sample was immediately frozen, then assayed after 1, 3, and 6 days of storage in the freezer. For diets prepared for week 1, diet samples that had been stored for 1, 3, or 6 days in the freezer were also analyzed after they had remained for 2-3 days in the animals' food hoppers. For diets prepared for weeks 2, 3, and 5, diet samples that had been stored frozen for 2-7 days were also analyzed after they had remained for 1 day in the animals' food hoppers. An average concentration

of test material in the diet for weeks 1, 3, and 5 and over the 5-week study duration was calculated from an average of the frozen and hopper samples.

Results: The diets were homogeneous; coefficients of variation for 9 samples were 4.8%, 2.7%, and 3.2% for the 100-, 1,000-, and 8,000-ppm diets, respectively. After preparation and storage for 1 day in the freezer, test compound concentrations in the diets were within 6% of nominal (analyses for diets prepared for use in weeks 1, 3, and 5); 94.1%, 97.0%, and 96.4% of the nominal concentrations were found in the 100-, 1,000-, and 8,000-ppm diets, respectively. The test compound was somewhat unstable in the frozen 100- and 1,000-ppm diets; after 6 days of frozen storage of diets (prepared for use during weeks 1, 3, and 5), 91.6%, 95.4%, and 101.2% of the nominal concentrations were found in the 100-, 1,000-, and 8,000-ppm diets, respectively. The test compound was unacceptably unstable under in-use conditions in the diets that had been present in food hoppers for 1-3 days. After 3 days of frozen storage and 3 days in food hoppers (diets prepared for use in week 1), 63.5%, 79.9%, and 93.8% of the nominal concentrations were found in the 100-, 1,000-, and 8,000-ppm diets, respectively; after 2 days of frozen storage and 1 day in food hoppers (diets prepared for use in week 3), 74.7%, 82.0%, and 100.0% of the nominal concentrations were found in the 100-, 1,000-, and 8,000-ppm diets, respectively. Because of the instability of the diets in the food hoppers, diets were changed daily beginning on the 13th day of the study (previous to this, diets were changed every 2-3 days). The untreated control diets were found to be free of test material. The calculated mean concentration of test material in the diet over the 5-week study duration was 80.8, 871, and 7,820 ppm for the 100-, 1,000-, and 8,000-ppm dose groups, respectively.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.
4. Statistics: Body weight, body weight gain, food consumption, relative food consumption, hematology (except differential leukocyte and reticulocyte counts and erythrocyte morphology), clinical chemistry, and absolute and relative organ weights were analyzed using Bartlett's test for homogeneity followed by analysis of variance. Statistically significant differences between groups were evaluated by a two-sided Dunnett's Test. Reticulocyte counts and any data found to have nonhomogeneous variances by Bartlett's Test were analyzed using a nonparametric Kruskal-Wallis analysis of variance followed by a Mann-Whitney U-test when the analysis of variance indicated significant differences.

5. Quality Assurance: A quality assurance statement was signed and dated November 15, 1988. A GLP statement was signed and dated November 15, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of mortality. Observations for signs of toxicity were conducted daily.

Results: No compound-related deaths or clinical signs were reported. One male of the 100-ppm group was prematurely sacrificed because of clinical findings related to a break across the bridge of the nose.

2. Body Weight: Body weights were determined on the first day of the study and every 7 days thereafter. Terminal body weights were recorded on the day of sacrifice.

Results: Mean body weights and representative body weight gains are presented in Table 1. Mean body weights of males of the 8,000-ppm group were significantly lower than control at days 7 ($p < 0.01$), 14 ($p < 0.01$), and 21 ($p < 0.05$) and remained below control at days 28 and 35 (not significant, N.S.). The mean body weight gain of males of the 8,000-ppm group was significantly ($p < 0.01$) lower than control for the week ending day 7, marginally lower than control for the weeks ending days 14, 21, and 28 (data not shown, N.S.), and for the entire period of compound administration (days 0-35, N.S.).

In females of the 8,000-ppm group, lower mean body weights and lower body weight gains were seen at the same times as males of the 8,000-ppm group, but none of the differences from control were significant.

No clear compound-related effects on body weight or body weight gain were noted in rats of the 1,000- or 100-ppm groups.

3. Food Consumption and Compound Intake: Food consumption was determined, and mean daily diet consumption was calculated weekly. Food consumption was measured on every second day from day 0 to day 12. Starting on day 13, food consumption was determined daily. Compound intake was calculated for each test group for each week that the test material concentration was measured in the diet. Compound intake was calculated from the mean relative food consumption and mean concentration in the diet.

Results: Mean food consumption and compound intake data are presented in Table 2. Mean food consumption of males

TABLE 1. Mean Body Weights and Representative Mean Body Weight Gains (g $\pm$ S.D.) of Rats Fed RE-477:9 for 5 weeks¹

Dose Level (ppm)	Mean Body Weights on Day:						Mean Body Weight Gain on Days:	
	0	7	14	21	28	35	0-7	0-35
<u>Males</u>								
0	217 $\pm$ 6	264 $\pm$ 7	310 $\pm$ 9	346 $\pm$ 12	377 $\pm$ 14	397 $\pm$ 17	47.4 $\pm$ 3.5	180 $\pm$ 15
100	216 $\pm$ 6	263 $\pm$ 11	311 $\pm$ 15	348 $\pm$ 20	381 $\pm$ 25	403 $\pm$ 26	46.8 $\pm$ 6.0	186 $\pm$ 22
1,000	217 $\pm$ 8	263 $\pm$ 11	311 $\pm$ 12	348 $\pm$ 14	378 $\pm$ 15	398 $\pm$ 18	45.6 $\pm$ 3.2	181 $\pm$ 14
8,000	214 $\pm$ 7	249 $\pm$ 8**	293 $\pm$ 11**	328 $\pm$ 14*	358 $\pm$ 16	379 $\pm$ 21	34.6 $\pm$ 4.0**	165 $\pm$ 17
<u>Females</u>								
0	155 $\pm$ 7	174 $\pm$ 11	194 $\pm$ 13	215 $\pm$ 19	227 $\pm$ 15	236 $\pm$ 17	19.1 $\pm$ 6.1	81 $\pm$ 13
100	151 $\pm$ 7	169 $\pm$ 7	187 $\pm$ 9	204 $\pm$ 12	217 $\pm$ 13	228 $\pm$ 14	18.0 $\pm$ 5.8	77 $\pm$ 10
1,000	153 $\pm$ 8	173 $\pm$ 7	192 $\pm$ 10	210 $\pm$ 12	225 $\pm$ 13	237 $\pm$ 16	19.9 $\pm$ 4.3	84 $\pm$ 11
8,000	155 $\pm$ 8	171 $\pm$ 7	192 $\pm$ 11	208 $\pm$ 12	220 $\pm$ 14	231 $\pm$ 14	16.4 $\pm$ 1.9	75 $\pm$ 10

¹N=10, except for males of the 100-ppm group after day 21 (N=9).²Data was extracted from tables 4 to 7 of the study report.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

TABLE 2. Mean Food Consumption (g/rat/day $\pm$ S.D.) and Compound Intake of Rats Fed RE-47719 for 5 Weeks^{a,b}

Dose Level (ppm)	Mean Food Consumption for Week Ending Day:					Mean Compound Intake ^c
	7	14	21	28	35	
<u>Males</u>						
0	24.3 ± 1.5	24.7 ± 1.3	25.2 ± 1.6	25.9 ± 1.5	24.9 ± 1.3	
100	24.5 ± 1.9	25.3 ± 2.3	25.8 ± 2.5	27.0 ± 2.9	26.5 ± 2.5	6.66
1,000	24.1 ± 1.7	24.4 ± 1.3	25.2 ± 1.5	25.8 ± 1.7	26.0 ± 1.8	70.9
8,000	20.0 ± 1.6**	23.5 ± 1.1	24.0 ± 1.3	25.1 ± 1.6	25.5 ± 1.6	604.0
<u>Females</u>						
0	18.5 ± 2.3	18.6 ± 1.8	19.2 ± 2.6	20.0 ± 2.2	19.4 ± 1.9	
100	18.2 ± 1.8	17.8 ± 2.3	18.2 ± 2.1	18.9 ± 2.5	18.3 ± 2.0	7.82
1,000	17.9 ± 1.0	17.9 ± 1.0	18.5 ± 0.9	19.6 ± 1.2	19.9 ± 1.4	83.7
8,000	17.1 ± 1.3	18.0 ± 0.9	17.9 ± 1.2	18.6 ± 1.0	18.3 ± 1.2	723.0

^aN=10 except for males of the 100-ppm group after day 21 and males of the control and 8,000-ppm groups after day 28 (N=9).

^bData was extracted from tables 8 and 9, and 12 and 13 of the study report.

^cmg RE-47719/kg body weight/day calculated for weeks 1, 3, and 5.

**Significantly different from controls (p < 0.01).

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of the 8,000-ppm group was significantly lower than controls ($p < 0.01$) in the week ending on study day 7. No clear compound-related effects on mean food consumption were noted in females of the 8,000-ppm group or in males or females of the 1,000- or 100-ppm groups.

Mean calculated compound intake, which took into account compound degradation in the diets while they were frozen or were present in the food hoppers, was 6.66, 70.9, and 604 mg/kg/day for males and 7.82, 83.7, and 723 mg/kg/day for females of the 100-, 1,000-, and 8,000-ppm groups, respectively.

4. Ophthalmology: Ophthalmological examinations were performed prior to start of the study (Day -7) and prior to terminal sacrifice (Day 35).

Results: No compound-related ophthalmologic effects were noted.

5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta at terminal sacrifice for hematology and clinical analysis from 10 animals/sex/group. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	Coagulation:thromboplastin
X Reticulocyte count (RETIC)	time (PT)
X Red cell morphology	

Results: Reticulocyte counts (in percent) were significantly elevated in males but not females of the 8,000-ppm group; values were 0.5 ± 0.3 , 0.8 ± 0.5 , 0.9 ± 0.6 , and 1.6 ± 1.0 ($p < 0.002$) in males and 1.0 ± 0.5 , 1.8 ± 1.4 , 1.0 ± 0.6 , and 1.0 ± 0.5 in females of the 0-, 100-, 1,000-, and 8,000-ppm groups, respectively. The reviewers agree with the study author that the significant increase in reticulocyte count in high-dose males may not be compound-related because it was accounted for by spuriously high values in only two of the nine animals. The reviewers also note that similarly elevated values were seen in 3 of 10 females of the 100-ppm group (mean not significantly different from control), thus suggesting that the elevated values may be representative of the normal range of variation in this laboratory. However, the study authors did not provide historical control values to examine this possibility.

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b. Clinical Chemistry:

<u>Electrolytes</u>		<u>Other</u>	
X	Calcium	X	Albumin
X	Chloride	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine
X	Phosphorus	X	Blood urea nitrogen
X	Potassium	X	Cholesterol
X	Sodium	X	Globulins
		X	Glucose
<u>Enzymes</u>		X	Total bilirubin
X	Alkaline phosphatase (ALP)	X	Direct bilirubin
	Cholinesterase	X	Total protein
X	Creatine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase	X	Indirect bilirubin
X	Serum alanine aminotransferase (SGPT)	X	Uric acid
X	Serum aspartate aminotransferase (SGOT)	X	BUN/creatinine ratio
	Gamma glutamyltransferase (GGT)	X	Creatinine

Results: Selected clinical chemistry data are presented in Table 3. Serum cholesterol was significantly elevated ($p < 0.01$) in males and females of the 8,000-ppm group. The reviewers agree with the study authors that this increase may be compound-related but that the toxicological significance is unclear because of a lack of correlation with other serum chemistry or histopathology findings. However, the reviewers note that the increases in serum cholesterol do correlate with the increased absolute and relative weights of the liver in males and females of the 8,000-ppm group (see below).

In rats of the 8,000-ppm group, the activity of alkaline phosphatase was significantly decreased ($p < 0.05$) in females, and the activity of alanine aminotransferase was significantly decreased ($p < 0.01$) in males. The reviewers do not consider these changes to be toxicologically important because the changes were minimal (also noted by study authors), there was no consistency between sexes, there was no correlative histopathology (also noted by study authors), and toxic effects are normally indicated by increases rather than decreases in the activities of these enzymes. In addition, the study authors indicated that the activity values were within the clinically normal ranges, but did not provide historical control data to verify this assertion.

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TABLE 3. Selected Mean Clinical Chemistry Data from Rats Fed RE-47719 for 5 Weeks^a

Parameter :	Dose Level (ppm)						
	0 ^b	Males			Females		
		100 ^c	1,000 ^b	8,000 ^c	0 ^c	100 ^b	1,000 ^c
ALK ^d (IU/L)	93 ± 18	107 ± 27	105 ± 16	83 ± 14	90 ± 25	103 ± 21	61 ± 15 ^a
ALT (IU/L)	28 ± 6	25 ± 4	24 ± 4	19 ± 3 ^{**}	27 ± 8	23 ± 6	24 ± 5
CHOL (MG/DL)	58 ± 8	60 ± 14	63 ± 12	91 ± 15 ^{**}	60 ± 10	72 ± 15	82 ± 11 ^{**}
ALB (GM/DL)	4.0 ± 0.2	4.2 ± 0.2	4.1 ± 0.2	4.4 ± 0.1 ^{**}	4.3 ± 0.2	4.3 ± 0.2	4.4 ± 0.3
GLOB (GM/DL)	1.8 ± 0.2	1.5 ± 0.2 [*]	1.5 ± 0.1 ^{**}	1.5 ± 0.2 ^{**}	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.1
A:G Ratio	2.3 ± 0.3	2.6 ± 0.2 ^{**}	2.7 ± 0.3 [*]	3.0 ± 0.4 ^{**}	3.1 ± 0.4	3.3 ± 0.5	3.2 ± 0.4
CA (MG/DL)	9.5 ± 0.4	9.7 ± 0.4	9.7 ± 0.3	10.0 ± 0.3 ^{**}	9.6 ± 0.2	9.8 ± 0.4	9.8 ± 0.4

^aData was extracted from tables 20 and 21 of the study report.^bN=10.^cN=9.^dAbbreviations:

ALK - alkaline phosphatase
 ALT - alanine aminotransferase
 CHOL - cholesterol
 ALB - albumin
 GLOB - globulin
 A:G Ratio - albumin/globulin ratio
 CA - calcium

^{*}Significantly different from controls (p < 0.05).^{**}Significantly different from controls (p < 0.01).

In male rats of the 8,000-ppm group, serum albumin, the albumin:globulin ratio, and serum calcium were significantly increased ($p < 0.01$); serum globulin was significantly decreased ($p < 0.01$). Significant decreases in serum globulin were also noted in males of the 1,000-ppm group ($p < 0.01$) and 100-ppm group ($p < 0.05$) and significant increases in the albumin:globulin ratio were seen in males of the 1,000-ppm group ($p < 0.05$) and 100-ppm group ($p < 0.01$). The reviewers do not consider these changes to be compound-related because they are minimal (also noted by the study authors), inconsistent between sexes, and (except for calcium) no dose-response relationship was evident (also noted by study authors). Although the study authors indicated that all these values were within clinically normal ranges, they did not provide historical control data to verify this assertion.

6. Urinalysis: Urinalysis was not performed.
7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta	XX Brain
X Salivary glands	X Heart	X Peripheral nerve (sciatic nerve)
X Esophagus	X Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes (mesenteric)	X Pituitary
X Duodenum	X Spleen	X Eyes (optic nerve)
X Jejunum	X Thymus	
X Ileum		
X Cecum		
X Colon		
X Rectum		
XX Liver	<u>Urogenital</u>	<u>Glandular</u>
Gallbladder	XX Kidneys	XX Adrenals
X Pancreas	X Urinary bladder	Lacrimal gland
	XX Testes	X Mammary gland
	Epididymides	X Thyroids
<u>Respiratory</u>	Prostate	X Parathyroids
X Trachea	Seminal vesicle	X Harderian glands
X Lung	XX Ovaries	
	X Uterus	
		<u>Other</u>
		X Bone (femur)
		X Skeletal muscle
		Skin
		X All gross lesions and masses

Microscopic evaluations were conducted for all preserved tissues from the 0- and 8,000-ppm groups and all animals that were killed in extremis, and for livers and all tissues with gross lesions for all animals from the 100- and 1,000-ppm groups.

Results:

- a. Organ Weights: Absolute and relative weights of the liver are presented in Table 4. The reviewers agree with the study authors that the significant increases in absolute and relative (to body and brain) weight of the liver in males ($p < 0.01$) and females ($p < 0.05$) of the 8,000-ppm group are compound-related, although no correlative histopathological changes were observed. The reviewers also agree with the study authors that significant ($p < 0.05$) increases in absolute and relative (to brain) adrenal weight in males of the 100-ppm group are not compound-related because of the absence of significant changes at higher doses.
- b. Gross Pathology: There were no compound-related macroscopic findings.
- c. Microscopic Pathology: Representative histopathology findings are presented in Table 5. The study authors considered the histopathological lesions that were found to be spontaneous in nature and not compound-related. However, the reviewers note that the kidney hydronephrosis observed in 3/10 males and 2/10 females of the 8,000-ppm group was not present in controls and may be treatment-related (note, however, that an incidence of 4/10 would be required for statistical significance at the 0.05 level). The study authors did not provide data to determine whether or not the incidences of hydronephrosis in high-concentration animals fell within the historical control range.

D. STUDY AUTHORS' CONCLUSIONS:

Groups of 10 adult Sprague-Dawley rats of each sex were fed a control diet or diets containing target concentrations of 100, 1,000, or 8,000 ppm RE-47719 (99.3% pure) for 5 weeks. By analysis, the estimated overall mean concentrations in diets for 1-6 days were 80.8, 871, and 7,820 ppm for the low-, mid-, and high-concentration diets, respectively. The test material was observed to be less stable in the diets at the lower concentrations. Increases in absolute and relative liver weights and serum cholesterol levels were observed in both male and female rats of the high-concentration group. These changes

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TABLE 4. Mean Liver Weights and Mean Liver-to-Body and Liver-to-Brain Weight Ratios of Rats Fed RE-47719 for 5 Weeks^{a,b}

Dose Level (ppm)	Liver Weight (g)	Liver-to-Body Weight (%)	Liver-to-Brain Weight (g/g)
<u>Males</u>			
0	11.99 ± 0.68	3.20 ± 0.18	5.80 ± 0.27
100	11.82 ± 1.31	3.10 ± 0.19	5.84 ± 0.60
1,000	12.00 ± 1.31	3.18 ± 0.30	5.92 ± 0.67
8,000	13.62 ± 1.04**	3.81 ± 0.18**	6.63 ± 0.47**
<u>Females</u>			
0	7.21 ± 1.04	3.31 ± 0.45	3.71 ± 0.51
100	6.60 ± 0.38	3.13 ± 0.20	3.44 ± 0.20
1,000	6.89 ± 0.84	3.11 ± 0.31	3.62 ± 0.46
8,000	8.28 ± 1.30*	3.86 ± 0.56*	4.26 ± 0.56*

^aN=10, except for males of the 100-ppm group (N=9).^bData was extracted from tables 22 and 23 of the study report.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

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TABLE 5. Selected Histopathological Findings in Rats Fed RE-47719 for 5 Weeks^a

Tissue/Lesion	Dose Level (ppm):							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
<u>Kidney</u>	(10) ^b	(1)	(0)	(10)	(10)	(1)	(1)	(10)
Hydronephrosis	0	0	0	3	0	1	1	2
<u>Liver</u>	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration, clear	0	0	0	0	0	1	1	0
Extramedullary hematopoiesis	4	1	3	2	2	0	0	0
Chronic inflammation	1	0	1	1	1	0	0	2
<u>Mandibular lymph node</u>	(3)	(4)	(3)	(2)	(2)	(1)	(1)	(1)
Congestion	3	4	3	2	2	1	1	1

^aData was extracted from Appendix 0 of the study report.^bNumbers in parentheses equal total number of animals examined.

are believed to be compound-related; however, their toxicological significance is unclear since no correlative histopathologic findings were noted in the liver or other tissues. No mortality, clinical signs of toxicity, changes in hematology parameters, or macroscopic or microscopic changes were observed that could be attributed to the test material. Decreases in body weights, body weight gains, and food consumption noted in high-concentration males early in the study are attributable to the poor palatability of the diet. No compound-related effects were observed in animals from the low- and mid-concentration groups. The no-observed-effect level in this study for dietary exposure to RE-47719 is approximately 871 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The reviewers noted the following deficiencies: (1) The study authors reported that this study was submitted as supplemental information in support of subchronic testing intended to satisfy Pesticide Guideline No. 82-1, 90-day subchronic oral toxicity study. The test substance was administered for 5 weeks rather than 90 days. (2) The stability, homogeneity, and concentration of the test substance in the diet were not determined prior to the start of the study. (3) When aliquots of the test diets were present in the food hoppers under in-use conditions, the test compound was unacceptably unstable at the lower two concentrations (100 or 1,000 ppm nominal concentrations) regardless of whether diets were changed every 2-3 days (1st 12 days of study) or every day (day 13 through termination). (4) The study authors did not provide historical control data to support their assertions that certain parameter changes in treated animals were within clinically normal ranges.

The reviewers agree with the study authors that the significant increases in serum cholesterol, absolute liver weight, and relative (to brain and body) liver weight in males and females of the high-concentration group are compound-related, although their toxicological significance is unclear.

The study authors indicated that there were no compound-related microscopic changes. However, the reviewers note that the kidney hydronephrosis seen in 3/10 males and 2/10 females of the high-concentration group, but in none of the control animals, may be compound-related; the study authors did not provide data to determine whether or not the incidences of hydronephrosis in high-concentration animals fell within the historical control range.

The study authors considered the lower body weights, decreased body weight gains, and decreased food consumption in high-dose males to be attributable to the poor palatability of the diet.

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However, the reviewers note that lower body weights and decreased body weight gains, although not significantly different from controls, occurred in high-dose females without an observable effect on food consumption. Thus, the lower body weights and decreased body weight gains in high-dose animals may be compound-related effects that are, at least in part, independent of diet palatability.

The reviewers agree with the study authors that no compound-related effects were seen on mortality, clinical signs, ophthalmology, hematology parameters, or gross lesions in males and females, or on food consumption in females.

Based upon the above results, the 5-week oral LOEL for RE-47719 is approximately 7,820 ppm (604 and 723 mg/kg/day for males and females, respectively) and the NOEL is approximately 871 ppm (70.9 and 83.7 mg/kg/day for males and females, respectively).

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-M
TASK No.: 2-45M
April 26, 1990

007972

DATA EVALUATION RECORD

CLETHODIM METABOLITE 5-HYDROXY SULFONE

5-Week Subchronic Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

Lawrence J. Prentice for

Date:

April 26, 1990

EPA No.: 68D80056
DYNAMAC No.: 245-M
TASK No.: 2-45M
April 26, 1990

007972

DATA EVALUATION RECORD

CLETHODIM METABOLITE 5-HYDROXY SULFONE

5-Week Subchronic Feeding Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
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Date: April 26, 1990

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Signature: James N. Rowe
Date: 5/24/90

Mike Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 5/30/90

DATA EVALUATION RECORD

007972

STUDY TYPE: Five-week subchronic feeding study in rats.

MRID NUMBER: 4-0301-05.

TEST MATERIAL: RE-51228; 5-Hydroxy Sulfone.

SYNONYMS: Not applicable.

STUDY NUMBER: S-3162.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc.,
Richmond, CA.

TITLE OF REPORT: Five-Week Oral Toxicity Study in Rats with RE-
51228 (SX-1803).

AUTHORS: Bagos, A. C.; Beatty, P. W.

REPORT ISSUED: November 15, 1988.

CONCLUSIONS: RE-51228 was fed to male and female Sprague-Dawley rats at calculated mean concentrations of 73.2, 856, and 7290 ppm (nominal concentrations of 100, 1000, and 8000 ppm) for 5 weeks. Compound intakes were 0, 5.94, 67.7, and 588 mg/kg/day for males and 0, 6.43, 75.5, and 663 mg/kg/day for females after correcting for test compound instability. No compound-related effects were exhibited for mortality, clinical observations, body weights, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, or gross or microscopic pathology. The reviewers consider the dose levels to be ineffective; the animals should have been able to tolerate higher dose levels of RE-51228.

Classification: This study cannot be CORE classified, since there are no available EPA Pesticide Guidelines for a 5-week feeding study.

A. MATERIALS:

1. **Test Compound:** RE-51228; description: off-white solid; batch No.: SX-1803; purity: 94.8%.
2. **Test Animals:** Species: rat; strain: Sprague-Dawley Crl: CD(SD)BR; age: 44 days; weight: males--203 to 266 g, females--146 to 180 g; source: Charles River Laboratories, Inc., Portage, Michigan.

B. STUDY DESIGN:

1. **Animal Assignment:** Following a quarantine period of 13 days and an acclimation period of 8 days, animals were assigned to the following test groups using a computerized randomization procedure:

Test Group	Dietary Dose ^a (ppm)	Calculated Mean Concentrations (ppm)	Main Study (5 weeks)	
			Males	Females
1 Control	0	0	10	10
2 Low (LDT)	100	73.2	10	10
3 Mid (MDT)	1000	856	10	10
4 High (HDT)	8000	7290	10	10

^aThe target concentrations of the active ingredient were corrected for purity (94.8, 948, and 7584 ppm) for 100-, 1000-, and 8000-ppm diets, respectively.

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Rats were housed individually in a room with temperature and humidity controls set at 20 to 24°C and 55 to 74%, respectively, with a 12-hour light/dark cycle.

2. Diet Preparation: Diets were prepared weekly and stored frozen until use. A concentrated premix of the 8000-ppm diet was prepared by mixing the appropriate amount of test material directly with the rodent chow for a minimum of 25 minutes. The test diets were prepared by diluting the premix with the appropriate amount of untreated diet to give the required concentrations, and mixed for 15 minutes. Untreated diet was provided for the control animals. Diets were thawed and offered to the animals three times/week from days 0 to 12 and daily from days 13 to 34 owing to poor stability of the test material in the diet.

Nine samples of the initial test diet were analyzed for homogeneity. Samples for analysis of compound stability in the diet were taken immediately before use from diets mixed during weeks 1, 3, and 5; samples were frozen and assayed after 1, 3, and 6 days of storage in the freezer. For diets prepared for week 1, diet samples that had been stored for 1, 3, or 6 days in the freezer were also analyzed following 2-3 days in the food hoppers. For diets prepared for weeks 3 and 5, diet samples that had been stored frozen for 2 to 7 days were also analyzed following 1 day in the food hoppers. In addition, the high-dose diet prepared for week 2 was analyzed after 1 day of freezer storage. The average test material concentrations for weeks 1, 3, and 5 and for the study duration were calculated by the study authors from an average of frozen and hopper samples.

Results: The diets were considered to be homogeneous; coefficients of variation for nine samples were 7.1%, 4.3%, and 3.8% for the 100-, 1000-, and 8000-ppm diets, respectively. The untreated control diet was found to be free of test material. After preparation and storage for 1 day in the freezer, test compound concentrations in the diets were within 16% of nominal (analyses for diets prepared for use in weeks 1, 3, and 5); 84.7%, 95.9%, and 96.2% of the nominal concentrations found in the 100-, 1000-, and 8000-ppm diets, respectively. The 100-ppm diet was similarly unstable after 1 and 6 days of frozen storage. After 6 days of frozen storage of diets (prepared for use during weeks 1, 3, and 5), 84.1%, 96.6%, and 93.2% of the nominal concentrations were found in the 100-, 1000-, and 8000-ppm diets, respectively. The test compound was unacceptably unstable under in-use conditions in the diets remaining in the food hoppers for 1 to 3 days. After 3 days of frozen storage and 3 days in food hoppers (diets prepared for use in week 1), 65.7%, 78.8%, and 101% of the

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nominal concentrations were found in the 100-, 1000-, and 8000-ppm diets, respectively; after 2 days of frozen storage and 1 day in food hoppers (diets prepared for use in week 3), 74.6%, 82.7%, and 94.2% of the nominal concentrations were found in the 100-, 1000-, and 8000-ppm diets, respectively. As a result of the instability of the diets in the food hoppers, diets were changed daily beginning on study day 13. The mean test material concentration determined by the study author for the 5-week study duration was 73.2, 856, and 7290 ppm for the 100-, 1000-, and 8000-ppm test diets, respectively.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.
4. Statistics: The following procedures were utilized in analyzing the numerical data: body weight, food consumption, hematology (with exceptions of differential leukocyte and reticulocyte counts and erythrocyte morphology), and clinical chemistry; and organ weights were analyzed using Bartlett's test for homogeneity followed by analysis of variance. Significant differences between groups were evaluated using Dunnett's test. Data with nonhomogeneous variances (e.g., reticulocyte counts) were analyzed using a nonparametric Kruskal-Wallis analysis of variance; significant differences were evaluated using the Mann-Whitney U-Test.
5. Quality Assurance: A quality assurance statement was signed and dated November 15, 1988. A GLP Statement was signed and dated November 15, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of mortality and morbidity with the exception of study day 25 when animals were inadvertently inspected only once. Observations for signs of toxicity were conducted once daily.

Results: No deaths occurred during the study. Clinical findings (open sores, scabs, thinning of fur, malocclusion, and ocular or nasal discharge) were found sporadically in control and treated animals and were not considered to be related to dosing.

2. Body Weights: Body weights were recorded at study initiation and every 7 days, thereafter. Terminal body weights were recorded on day of sacrifice.

Results: Representative data on mean body weights and body weight gains are summarized in Table 1. Body weights and

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TABLE 1. Mean Body Weights of Rats Fed RE-51228 for 5 Weeks^{a,b}

Dose Level (ppm)	Mean Body Weights (g $\pm$ S.D.) at Days:				Total Body Weight Gains (g) Days 0-37
	0	7	21	37	
<u>Males</u>					
0	232 $\pm$ 15	284 $\pm$ 20	366 $\pm$ 29	416 $\pm$ 39	184 $\pm$ 25
100	232 $\pm$ 19	281 $\pm$ 22	360 $\pm$ 33	408 $\pm$ 40	177 $\pm$ 26
1000	233 $\pm$ 18	284 $\pm$ 21	361 $\pm$ 27	413 $\pm$ 31	181 $\pm$ 18
8000	230 $\pm$ 16	278 $\pm$ 19	356 $\pm$ 26	404 $\pm$ 29	174 $\pm$ 16
<u>Females</u>					
0	166 $\pm$ 7	184 $\pm$ 11	218 $\pm$ 12	235 $\pm$ 11	69 $\pm$ 7
100	167 $\pm$ 9	185 $\pm$ 9	217 $\pm$ 16	236 $\pm$ 17	69 $\pm$ 14
1000	163 $\pm$ 7	181 $\pm$ 10	216 $\pm$ 10	230 $\pm$ 12	67 $\pm$ 9
8000	163 $\pm$ 10	185 $\pm$ 9	212 $\pm$ 15	231 $\pm$ 19	69 $\pm$ 5

^aBased on 10 rats/sex/group.^bData extracted from tables 4 to 7 of the study report.

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body weight gains of dosed animals were similar to those of concurrent controls.

3. Food Consumption and Compound Intake: Food consumption was determined and mean daily diet consumption was calculated three times weekly from study initiation to study day 12 and daily beginning on study day 14. Compound intake was calculated from the consumption and body weight gain data.

Results: Mean food consumption and relative food consumption (g/100 g body weight/day) of dosed males and females were similar to concurrent controls. The mean compound intakes for 5 weeks of the study were 5.94, 67.7, and 533 mg/kg/day for males and 6.43, 75.5, and 563 mg/kg/day for females fed 100, 1000, and 8000 ppm RE-51223. Calculated compound intakes accounted for compound degradation in the frozen and unfrozen diets.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on all animals prior to study initiation and study termination.

Results: Ophthalmological findings were considered to be spontaneous in nature and not related to dosing.

5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta at terminal sacrifice for hematology and clinical analysis from 10 animals/sex/group. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	X Coagulation:thromboplastin time (PT)
X Reticulocyte count (RETIC)	
X Red cell morphology	

Results: Selected hematology results are presented in Table 2. There were no effects of biological importance on hematology parameters. Hemoglobin and hematocrit concentrations and erythrocyte counts were depressed in mid- and high-dose males, and reticulocyte counts were

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TABLE 2. Selected Mean Hematology Data From Rats Fed RE-51228 for 5 Weeks^{a,b}

Dose Level (ppm)	Hematological parameters:			
	HCT (%)	HGB (g/dL)	RBC (10 ⁶ /mm ³)	Reticulocytes (%)
<u>Males</u>				
0	43.5 ± 0.9	15.2 ± 0.3	7.88 ± 0.46	1.9 ± 1.0
100	42.1 ± 2.1	14.9 ± 0.9	7.68 ± 0.45	2.5 ± 1.3
1000	41.3 ± 1.3 ^c (39.8 ± 5.1) ^{***}	14.7 ± 0.4 ^c (14.1 ± 1.9) ^{**}	7.50 ± 0.28 ^c (7.19 ± 1.01)	1.9 ± 0.9 ^c (2.1 ± 1.0)
8000	42.2 ± 1.2 ^d (40.8 ± 3.1) ^{**}	14.9 ± 0.4 ^d (14.4 ± 1.1) [*]	7.68 ± 0.28 ^d (7.43 ± 0.61)	2.7 ± 1.0 ^d (3.1 ± 1.3)
<u>Females</u>				
0	38.0 ± 1.6	13.8 ± 0.6	6.92 ± 0.35	2.3 ± 1.2
100	38.9 ± 1.2	14.0 ± 0.4	7.09 ± 0.21	2.8 ± 0.6
1000	39.4 ± 1.1	14.3 ± 0.3	7.22 ± 0.27	2.5 ± 0.9
8000	38.8 ± 1.8	14.0 ± 0.7	7.03 ± 0.35	2.4 ± 0.8

^aBased on 10 rats/sex/group.^bData extracted from tables 14 and 15 of the study report.^cValues presented in parentheses are those reported by the study author; values without parentheses are those recalculated by the reviewers without one outlier (animal No. G48).^dValues presented in parentheses are those reported by the study author; values without parentheses are those recalculated by the reviewers without two outliers (animal Nos. H62 and H67).

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.02).

***Significantly different from controls (p < 0.002).

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increased in these same animals; however, these changes in mean values were a result of the abnormally low values (hemoglobin, hematocrit, erythrocyte counts) of one outlier (animal No. G48) in mid-dose males and two outliers (animal Nos. H62 and H67) in high-dose males. When these means were recalculated by the reviewers without the outliers, the hematocrit, hemoglobin, erythrocyte, and reticulocyte counts of mid- and high-dose males were similar to concurrent controls. There were no changes in the hematology of dosed females.

b. Clinical Chemistry:

<u>Electrolytes</u>		<u>Other</u>	
X	Calcium	X	Albumin
X	Chloride	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine
X	Phosphorus	X	Blood urea nitrogen
X	Potassium	X	Bun/creatinine ratio
X	Sodium	X	Cholesterol
		X	Globulins
		X	Glucose
		X	Total bilirubin
		X	Direct/indirect bilirubin
		X	Total protein
		X	Triglycerides
		X	Uric acid
<u>Enzymes</u>			
X	Alkaline phosphatase (ALP)		
	Cholinesterase		
X	Creatinine phosphokinase		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)		
X	Serum aspartate aminotransferase (SGOT)		
	Gamma glutamyltransferase (GGT)		

Results: There were no effects of biological importance on clinical chemistry parameters in rats fed RE-51228. The calcium and phosphorus levels of mid-dose males were slightly but significantly ($p < 0.05$) decreased when compared to concurrent controls, but were within the range of that of historical laboratory controls. In addition, the calcium and phosphorus levels of low- and high-dose males and all dosed females were similar to those of concurrent controls. Therefore, these depressed electrolyte levels are not considered to be of biological importance. Normal animal variability was found in many indices.

¹Hazleton Laboratories 1984. Representative Historical Control Data.

6. Urinalysis: Urinalyses were not performed.
7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta	XX Brain
X Salivary glands	X Heart	X Peripheral nerve (sciatic nerve)
X Esophagus	X Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes (mesenteric)	X Pituitary
X Duodenum	X Spleen	X Eyes (optic nerve)
X Jejunum	X Thymus	
X Ileum		
X Cecum		
X Colon		
X Rectum		
XX Liver	<u>Urogenital</u>	<u>Glandular</u>
Gallbladder	XX Kidneys	XX Adrenals
X Pancreas	X Urinary bladder	Lacrimal gland
	XX Testes	X Mammary gland
	Epididymides	X Thyroids
<u>Respiratory</u>	Prostate	X Parathyroids
X Trachea	Seminal vesicle	X Harderian glands
X Lung	XX Ovaries	
	X Uterus	
		<u>Other</u>
		X Bone (sternum and femur)
		X Skeletal muscle
		Skin
		X All gross lesions and masses

All tissues were examined histologically in control and high-dose animals. Only liver and abnormal tissues were examined in low- and mid-dose groups.

Results:

- a. Organ Weights: There were no effects of biological importance in organ weights.
- b. Gross Pathology: There were no macroscopic pathological changes that were considered to be compound related by the study authors. The adequacy of the gross examination could not be verified, since the

summary tabulation included only the kidney, mandibular lymph node, salivary gland, and stomach.

- c. Microscopic Pathology: There were no histological findings that were considered by the study authors to be related to dosing. The few lesions exhibited by the dosed animals (Table 3) were considered by the study author to be normal age- and strain-related changes.

D. STUDY AUTHORS' CONCLUSIONS:

The 5-week dietary administration of RE-51228 to male and female rats resulted in no deaths and no signs of toxicity that could be attributed to dosing. The NOEL for RE-51228 is 7290 ppm, the calculated concentration of RE-51228 at the highest dose tested.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct of the study was acceptable; however, the study authors reported that this study was intended as supplemental information submitted in support of subchronic testing for Pesticide Guideline No. 82-1, 90-Day Subchronic Oral Toxicity Study. Since the length of this study was 5 weeks, the criteria of these guidelines (test substance administered for a period of at least 90 days) were not satisfied. In addition, the stability, homogeneity, and concentration of the test material in the diet were not determined prior to study initiation. When samples of the test diet were analyzed for stability under in-use conditions, the test material was unacceptably unstable at 100- and 1000-ppm nominal concentrations regardless of whether the diets were changed every 2 to 3 days (study days 1-12) or every day (study day 13 until study termination). The 100-ppm nominal concentration was also found to be unstable after freezer storage for as little as 1 day. The study report diet analyses indicated that diets were changed every day after study day 7, whereas the text of the study report indicated that diets were changed every day after study day 12 owing to instability; this discrepancy should be clarified. Adequacy of the macroscopic examination could not be verified since summary tabulation included only four tissues.

The reviewers agree with the study authors that no effects of biological importance were found in animals dosed with 100, 1000, or 8000 ppm RE-51228. The reviewers consider these dose levels to be ineffective; the animals should have been able to tolerate higher dose levels of RE-51228; higher dose levels would also have tolerated instability in the test diet to a greater extent.

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TABLE 3. Selected Nonneoplastic Histopathology Findings for Rats Fed RE-51228 for 5 Weeks^a

Organ/Findings	Dose Level (ppm):							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
<u>Kidney</u>	(10) ^b	(0)	(2)	(10)	(10)	(2)	(0)	(10)
Degeneration, tubular	0	0	0	2	0	0	0	0
Hydronephrosis	0	0	0	1	0	0	0	0
<u>Liver</u>	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoiesis, extramedullary	4	3	0	2	1	0	0	1
<u>Lung</u>	(10)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
Infiltration, lymphocytic	0	0	0	3	0	0	0	0
<u>Lymph Node, Mandibular</u>	(0)	(2)	(0)	(3)	(2)	(1)	(0)	(1)
Congestion	0	2	0	3	2	1	0	1

^aData extracted from Appendix N of the study report.^bNumbers in parentheses equal number of animals examined.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12045)

EPA No.: 68D80056
DYNAMAC No.: 245-J
TASK No.: 2-45J
April 25, 1990

007972

DATA EVALUATION RECORD

RE-45601 TECHNICAL

Ninety-Day Subchronic Oral Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: *Robert J. Weir*

Date: 4/24/90

EPA No.: 68D80056
DYNAMAC No.: 2-45J
TASK No.: 245-J
April 25, 1990

007972

DATA EVALUATION RECORD

RE-45601 TECHNICAL

Ninety-Day Subchronic Oral Toxicity Study in Dogs

REVIEWED BY:

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Date: April 25, 1990

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Date: April 25, 1990

APPROVED BY:

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Signature: *M. Ioannou*

Date: 5/30/90

007972

DATA EVALUATION RECORD

GUIDELINE §82-1

STUDY TYPE: Ninety-Day Subchronic Oral Toxicity Study in Dogs.

MRID NUMBER: 410301-06.

TEST MATERIAL: RE-45601 Technical.

SYNONYMS: Clethodim; (E,E)-(+/)-2-[1-[[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one.

STUDY NUMBER: Laboratory Project Identification: S-2759.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Bio/Dynamics, Inc., East Millstone, NJ.

TITLE OF REPORT: A Ninety-Day Subchronic Oral Toxicity Study in Dogs with Chevron RE-45601 Technical.

AUTHOR: I.W. Daly.

REPORT ISSUED: February 24, 1987.

CONCLUSIONS: RE-45601 was fed to male and female dogs at dietary levels of 0, 1, 25, 75, or 125 mg/kg/day for 90 days. Absolute liver weight was significantly increased by 34% and 30% in males and females receiving 125 mg/kg/day, respectively. Relative liver weights were slightly but nonsignificantly increased by 15-16% in males and females receiving 75 and 125 mg/kg/day. Liver lesions, consisting of cytoplasmic vesiculation and vacuolation, were most severe in the high-dose males and females, and were considered to be treatment-related at the high dose. Mean serum cholesterol values were significantly increased in females receiving 125 mg/kg/day. The activity of alkaline phosphatase was significantly increased in females following oral treatment with 125 mg/kg/day. An increased mean globulin value and a decreased albumin/globulin ratio were noted in the high-dose males after 3 months of treatment. There was no effect of dosing on mortality, body weight, food consumption, hematology parameters, ophthalmology, clinical signs or gross pathology.

The LOEL is 75 mg/kg/day based on increased relative liver weights in males and females. The NOEL for systemic toxicity is 25 mg/kg/day.

Classification: CORE Supplementary pending submission of data on the stability and concentration of test material in the capsules.

A. MATERIALS:

1. Test Compound: RE-45601 technical; description: dark liquid; batch No.: SX-1688; purity: 83.3% (supplied in two shipments).
2. Test Animals: Species: dog; strain: beagle; age: 5.5-6 months at initiation of treatment; weight: males--8.4-10.6 kg, females--7.2-9.6 kg; source: Marshall Research Farms, North Rose, NY.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 24-51 days. Animals were ranked by body weight and distributed into five blocks of four animals per sex so that body weight means for each group were comparable. The animals were randomly assigned to the following groups:

Test Group	Dose in Diet (mg/kg/day)	<u>Number of Animals</u>	
		Males	Females
1 Control	0	4	4
2 Low (LDT)	1	4	4
3 Mid-1 (MDT)	25	4	4
4 Mid-2 (MDT)	75	4	4
5 High (HDT)	125	4	4

Dogs were housed individually in elevated metal grid cages. A 12-hour light/dark cycle was maintained; temperature and humidity were monitored and recorded twice daily. The dogs had been vaccinated for distemper, hepatitis, leptospirosis, parvovirus, bordetella-parainfluenza, papilloma virus, and adenovirus by the supplier. Vaccinations for immunization against canine distemper, adenovirus type 2, hepatitis, parainfluenza, parvovirus, *Leptospira canicola*, *Leptospira interrogans*, and rabies were performed by the study laboratory during the acclimation period.

2. Diet Preparation: Dogs were administered the test substance orally via gelatin capsules on a daily basis (7 days per week). Appropriate amounts of the test substance were weighed and placed into gelatin capsules 1-6 days prior to administration with the exception of the capsules for Test Day 1, which were prepared on Test Day 1. Capsules were refrigerated until administration. Individual doses were adjusted by most recent weekly body weight. Control animals received empty gelatin capsules.

Results: Data on analyzed concentrations and stability of test material in the capsules were not provided.

3. Food and Water Consumption: Animals received 400 g Purina Certified Canine Diet No. 5007 fresh daily for 4 hours immediately postdose, and water ad libitum.
4. Statistics: All parameters were first tested for equality of variances using Bartlett's test. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one-way analysis of variance (ANOVA) using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated, a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case (equal variance), standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

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5. Quality Assurance: A quality assurance statement was signed and dated September 18, 1986.

C. METHODS AND RESULTS:

1. Observations: Dogs received detailed examinations at pretest and weekly thereafter. Dogs were also observed twice daily for mortality and signs of toxicity.

Results: No compound-related signs of toxicity were seen. All control and treated animals survived the 3-month duration of the study.

2. Body Weight: Body weights were recorded at pretest, weekly during treatment, and at terminal sacrifice.

Results: Table 1 summarizes mean body weight data for selected intervals. No treatment-related effects on body weight or body weight gain were noted. One control female exhibited a weight loss of 0.7 kg during the treatment period, but body weight gain data for the treated animals were unremarkable.

3. Food Consumption and Compound Intake: Food consumption was measured at pretest and weekly thereafter (measured and recorded daily, 5 days/week), for up to 14 weeks. Diet wastage was estimated, and corrections were made. Compound intake was not calculated.

Results: The mean food consumption of all control and treated groups was variable. However, there were no patterns evident that suggested any treatment-related effects. The food consumption data for the control females, which experienced some weight loss during the study, were unremarkable.

4. Ophthalmological Examinations: Ophthalmologic examinations were performed on the animals at pretest and at termination. The lids and conjunctiva of the eyes were examined grossly. The cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disk were examined by indirect ophthalmoscopy. Tropicamide was used to induce mydriasis.

Results: There were no ocular abnormalities noted, following 3 months of treatment, which were attributed to the administration of test substance.

TABLE 1. Representative Results of Mean Body Weights (kg $\pm$ S.D.) and Mean Body Weight Gain of Dogs Dosed Orally With RE-45601 Technical for 90 Days

Dose Group (mg/kg/day)	Mean Body Weight (kg) at Week:				Mean Body Weight Gain (kg) 0-13 Weeks
	0	3	7	13	
<u>Males</u>					
0	9.8 ± 1.1	10.4 ± 1.0	10.8 ± 0.8	11.3 ± 0.9	1.5
1	9.8 ± 0.7	10.4 ± 0.9	11.4 ± 0.9	12.0 ± 1.0	2.2
25	9.7 ± 0.7	10.5 ± 0.6	11.2 ± 0.7	11.7 ± 1.3	2.0
75	9.7 ± 0.8	10.5 ± 0.9	11.3 ± 1.5	11.9 ± 1.9	2.2
125	9.8 ± 0.8	10.5 ± 0.6	11.4 ± 1.5	12.2 ± 1.3	2.4
<u>Females</u>					
0	8.3 ± 0.5	8.7 ± 0.8	9.2 ± 1.2	9.6 ± 1.7	1.3
1	8.3 ± 0.6	8.7 ± 0.4	8.9 ± 0.4	9.6 ± 0.4	1.3
25	8.3 ± 0.7	8.9 ± 0.7	9.5 ± 0.6	10.1 ± 0.8	1.8
75	8.3 ± 0.4	8.9 ± 0.4	9.7 ± 0.4	10.3 ± 1.0	2.0
125	8.3 ± 1.0	9.3 ± 1.0	9.6 ± 0.7	10.0 ± 1.2	1.7

^aBased on 4 animals/sex/group. Data extracted from Table T1-1 of the study report.

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5. Hematology and Clinical Chemistry: Blood was collected from unanesthetized dogs via jugular venipuncture at days 8, 35, 55, and 91 for hematology and clinical analyses from all males and females of each dose group. Dogs were fasted overnight prior to blood collections. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count [†]
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB
X Platelet count [†]	X Erythrocyte count (RBC) [†]
X Red cell morphology	X Mean corpuscular volume (MCV)
	X Coagulation:thromboplastin time (PT)

Results: Mean total leukocyte counts were significantly increased in the males receiving 75 (61% increase) and 125 (53% increase) mg/kg/day at day 35, and remained significantly (but not statistically) elevated at days 55 and 91. The increases in mean leukocyte counts in males may be compound-related; however, the study authors did not consider this finding to be of toxicological significance, since they occurred in only one sex and were not statistically different from controls at days 55 and 91. Moreover, the increases in leukocyte counts were within the normal range. Mean activated partial thromboplastin time values were significantly reduced in females receiving 75 and 125 mg/kg/day at day 35 only.

b. Clinical Chemistry

<u>Electrolytes</u>	<u>Other</u>
X Calcium [†]	X Albumin [†]
X Chloride [†]	X Albumin/globulin ratio (calculated)
X Magnesium	X Blood creatinine [†]
X Phosphorus [†]	X Blood urea nitrogen [†]
X Potassium [†]	X Cholesterol
X Sodium [†]	X Globulins (calculated)
	X Glucose [†]
	X Total bilirubin [†]
	X Uric acid

Recommended by Subdivision F (October 1982) Guidelines for subchronic toxicity studies.

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Enzymes

- X Alkaline phosphatase (ALP)
- X Creatinine phosphokinase
- X Lactic acid dehydrogenase
- X Serum alanine aminotransferase (SGPT)[†]
- X Serum aspartate aminotransferase (SGOT)[†]
- X Gamma glutamyltransferase (GGT)

Results: Table 2 summarizes data on selected clinical chemistry parameters. Alkaline phosphatase activity decreased in controls with aging. However, the activity of this enzyme increased with time in males and females receiving 125 mg/kg/day, and the increase reached statistical significance ($p \leq 0.05$) only in females. Mean cholesterol levels were slightly increased compared to controls in males receiving 75 and 125 mg/kg/day. While the differences were statistically significant ($p \leq 0.05$) in the females treated with the 75-mg/kg/day dose at days 35 and 55, the levels were not markedly different from pretest values, and for this reason, the increases were not considered treatment related by the study authors. However, the increases noted in the mean cholesterol values of the high-dose females were statistically significant ($p \leq 0.05$) at days 35 and 55 and were considered to be treatment related. An increased mean globulin value and a decreased albumin/globulin ratio were noted in the high-dose males after 3 months of treatment.

6. Urinalysis: Urine was collected from all dogs at pretest (days 13, 14, and 15) and on days 32, 33, 56, 57, 89, and 90. Animals were not deprived of food or water prior to urine collections. The CHECKED (X) parameters were examined:

X Appearance	X Glucose
X Volume	X Ketones
X Specific gravity	X Bilirubin
X pH	X Blood
X Sediment (microscopic)	Nitrate
X Protein	X Urobilinogen

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TABLE 2. Selected Clinical Chemistry Parameters (Mean $\pm$ S.D.) of Dogs Dosed Orally With RE-45601 Technical for 90 Days^a

parameter/ interval	Dose Level (mg/kg/day)									
	Males					Females				
	0	1	25	75	125	0	1	25	75	125
<u>alkaline phosphatase</u> (IU/L)										
Day 8	93 $\pm$ 21	99 $\pm$ 26	92 $\pm$ 27	83 $\pm$ 20	95 $\pm$ 5	86 $\pm$ 16	88 $\pm$ 13	87 $\pm$ 39	102 $\pm$ 26	95 $\pm$ 31
Day 35	95 $\pm$ 21	95 $\pm$ 16	106 $\pm$ 15	94 $\pm$ 24	120 $\pm$ 24	79 $\pm$ 5	71 $\pm$ 14	80 $\pm$ 31	100 $\pm$ 18	134 $\pm$ 41
Day 55	88 $\pm$ 17	83 $\pm$ 21	95 $\pm$ 16	84 $\pm$ 19	126 $\pm$ 37	80 $\pm$ 11	63 $\pm$ 15	72 $\pm$ 21	98 $\pm$ 14	132 $\pm$ 50
Day 91	79 $\pm$ 21	68 $\pm$ 13	83 $\pm$ 19	85 $\pm$ 30	132 $\pm$ 45	75 $\pm$ 23	56 $\pm$ 15	54 $\pm$ 11	93 $\pm$ 19	141 $\pm$ 57
<u>cholesterol</u> (mg/dL)										
Day 8	156 $\pm$ 10	181 $\pm$ 37	172 $\pm$ 12	181 $\pm$ 34	140 $\pm$ 16	138 $\pm$ 30	161 $\pm$ 22	152 $\pm$ 28	171 $\pm$ 14	174 $\pm$ 12
Day 35	130 $\pm$ 18	140 $\pm$ 36	147 $\pm$ 13	173 $\pm$ 37	138 $\pm$ 20	120 $\pm$ 18	147 $\pm$ 15	150 $\pm$ 43	170 $\pm$ 19	190 $\pm$ 23
Day 55	137 $\pm$ 26	149 $\pm$ 21	160 $\pm$ 13	172 $\pm$ 52	149 $\pm$ 19	129 $\pm$ 18	147 $\pm$ 15	157 $\pm$ 38	179 $\pm$ 16	181 $\pm$ 26
Day 91	144 $\pm$ 21	159 $\pm$ 30	168 $\pm$ 20	183 $\pm$ 21	181 $\pm$ 24	136 $\pm$ 35	174 $\pm$ 51	174 $\pm$ 42	179 $\pm$ 24	214 $\pm$ 27
<u>albumin/globulin ratio</u> (g/dL)										
Day 8	1.8 $\pm$ 0.2	1.9 $\pm$ 0.1	2.1 $\pm$ 0.1	2.0 $\pm$ 0.2	1.9 $\pm$ 0.4	1.8 $\pm$ 0.3	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2	1.9 $\pm$ 0.4	1.9 $\pm$ 0.2
Day 35	2.2 $\pm$ 0.1	2.3 $\pm$ 0.2	2.6 $\pm$ 0.2	2.3 $\pm$ 0.3	2.6 $\pm$ 0.3	2.1 $\pm$ 0.2	2.2 $\pm$ 0.3	2.3 $\pm$ 0.3	2.5 $\pm$ 0.3	2.5 $\pm$ 0.3
Day 55	2.4 $\pm$ 0.6	2.7 $\pm$ 0.3	2.6 $\pm$ 0.3	2.7 $\pm$ 0.4	2.9 $\pm$ 0.5	2.3 $\pm$ 0.3	2.2 $\pm$ 0.3	2.3 $\pm$ 0.2	2.5 $\pm$ 0.4	2.0 $\pm$ 0.3
Day 91	2.3 $\pm$ 0.1	2.5 $\pm$ 0.1	2.5 $\pm$ 0.2	2.2 $\pm$ 0.1	2.8 $\pm$ 0.4	2.3 $\pm$ 0.2	2.1 $\pm$ 0.3	2.5 $\pm$ 0.3	2.5 $\pm$ 0.3	2.6 $\pm$ 0.3
<u>albumin/globulin ratio</u>										
Day 8	1.8 $\pm$ 0.2	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.2	1.6 $\pm$ 0.3	1.8 $\pm$ 0.2	1.6 $\pm$ 0.2	1.6 $\pm$ 0.2	1.6 $\pm$ 0.3	1.7 $\pm$ 0.2
Day 35	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.1	1.4 $\pm$ 0.1	1.2 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.2	1.5 $\pm$ 0.3	1.3 $\pm$ 0.1	1.3 $\pm$ 0.2
Day 55	1.3 $\pm$ 0.3	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	1.2 $\pm$ 0.1	1.1 $\pm$ 0.2	1.5 $\pm$ 0.2	1.6 $\pm$ 0.2	1.5 $\pm$ 0.1	1.3 $\pm$ 0.2	1.7 $\pm$ 0.2
Day 91	1.4 $\pm$ 0.1	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	1.5 $\pm$ 0.0	1.1 $\pm$ 0.1	1.4 $\pm$ 0.2	1.6 $\pm$ 0.2	1.4 $\pm$ 0.2	1.3 $\pm$ 0.1	1.3 $\pm$ 0.2

^aBased on four animals/sex/group. Data extracted from Table 14-1 of the study report.

*Significantly different from controls at $p \leq 0.05$.

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Results: No effects of treatment with RE-45601 on urinalysis parameters were seen.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta†	XX Brain† with stem
X Salivary glands†	XX Heart†	X Peripheral nerve
X Esophagus†	X Bone marrow†	(sciatic nerve)†
X Stomach†	X Lymph nodes†	X Spinal cord
X Duodenum†	(mesenteric)	(3 levels)
X Jejunum†	X Spleen†	XX Pituitary†
X Ileum†	X Thymus†	X Eyes
X Cecum†		(optic nerve)
X Colon†		
X Rectum†		
XX Liver†	<u>Urogenital</u>	<u>Glandular</u>
X Gallbladder†	XX Kidneys†	XX Adrenals†
X Pancreas†	X Urinary bladder†	Lacrimal gland
	XX Testes†	X Mammary gland
<u>Respiratory</u>	XX Epididymides	(left inguinal)
X Trachea†	X Prostate	XX Parathyroids†
XX Lung†	Seminal vesicle	XX Thyroids†
	XX Ovaries	Harderian glands
	X Uterus†	
		<u>Other</u>
		X Bone (sternum and femur)
		X Skeletal muscle
		X Skin
		X All gross lesions and masses†

Recommended by Subdivision F (October 1982) Guidelines for subchronic toxicity studies.

Results:

- a. Organ Weights: Table 3 presents mean liver weight data. Absolute liver weight and liver-to-body weight ratios of males and female dogs receiving 75 mg/kg/day were slightly, but nonsignificantly, increased. The absolute liver weights in male and females receiving 125 mg/kg/day were significantly ($p \leq 0.01$) increased by 34% and 30%, respectively. Relative liver weights were also increased (by 15-16%) in the high-dose males and females, but the increase did not reach statistical significance. The increases in absolute and relative liver weights in the males and females receiving 75 and 125 mg/kg/day were considered by the study authors to be due to administration of the test substance. No other alterations were noted in organ weights.
- b. Gross Pathology: Gross pathological findings noted at gross necropsy either occurred with similar incidence and severity in the treated and control animals or occurred sporadically. The findings were not considered to be related to the test substance.
- c. Microscopic Pathology: Table 4 summarizes data on representative histopathological findings in dogs treated with RE-45601 technical in the diet for 90 days. Cytoplasmic vesiculation and vacuolation of the central lobular hepatocytes were observed in all control and treated males, in three of four control females, and in all treated females. This finding was most severe in the males and females in the high-dose group and was considered to be treatment-related. The lesions were similar in severity in animals receiving 0, 1, 25, and 75 mg/kg/day. Other nonneoplastic findings either occurred with similar incidence and severity in the treated and control animals or occurred sporadically and were not considered treatment related.

D. STUDY AUTHORS' CONCLUSIONS:

RE-45601 was administered orally, via gelatin capsules, to 32 beagle dogs (four/sex/group) at dose levels of 1, 25, 75, and 125 mg/kg/day for a period of at least 90 days. Control animals (four/sex) received empty gelatin capsules. Mean total leukocyte counts were significantly increased in the males receiving 75 and 125 mg/kg/day at 1 month, and mean activated partial thromboplastin time values were significantly reduced in females treated with 75 and 125 mg/kg/day at 1 month. The presence of these findings at the two highest dose levels was not considered to be of toxicological significance. Although

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TABLE 3. Mean Liver Weights (g $\pm$ S.D.) and Liver-to-Body Weight Ratios (Mean $\pm$ S.D.) of Dogs Dosed Orally With RE-45601 Technical for 90 Days^a

Dose Level (mg/kg/day)	Liver Weight	Liver/Body Weight Ratio
<u>Males</u>		
0	293.6 $\pm$ 26.7	2.67 $\pm$ 0.27
1	287.3 $\pm$ 11.3	2.56 $\pm$ 0.28
25	304.3 $\pm$ 34.1	2.84 $\pm$ 0.67
75	339.8 $\pm$ 47.7	3.00 $\pm$ 0.24
125	393.3 $\pm$ 58.0**	3.38 $\pm$ 0.31
<u>Females</u>		
0	254.5 $\pm$ 17.2	2.83 $\pm$ 0.41
1	239.8 $\pm$ 28.6	2.62 $\pm$ 0.22
25	269.8 $\pm$ 41.7	2.86 $\pm$ 0.43
75	293.0 $\pm$ 20.8	2.99 $\pm$ 0.14
125	330.7 $\pm$ 25.5**	3.36 $\pm$ 0.20

^aData extracted from Table T5-1 of the study report.

^{**}Significantly different from controls (p $\leq$ 0.01).

TABLE 4. Representative Histopathological Findings in Dogs Dosed Orally With RE-45601 Technical for 90 Days^a

Organ/Finding	Dose level (mg/kg/day)									
	Males					Females				
	0	1	25	75	125	0	1	25	75	125
<u>Lungs</u>										
Subacute/chronic interstitial inflammation	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Alveolar emphysema	2	2	4	4	4	4	2	4	4	0
Congestion	2	1	4	3	2	4	0	3	3	0
	0	3	4	4	4	2	3	2	2	3
<u>Spleen</u>										
Congestion	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
	2	3	0	0	1	2	0	2	0	1
Reticuloendothelial cells: brown pigment	0	0	2	4	0	1	3	1	2	2
<u>Liver</u>										
Central lobular hepatocellular cytoplasm: vacuolated/ vacuolated	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Perivascular/periductal accumulation of mixed inflammatory cells	4	4	4	4	4	3	4	4	4	4
Granulomatous inflammation/ granuloma(s)	4	4	4	3	3	4	4	4	4	4
Extramedullary hematopoiesis	3	3	2	2	1	2	2	0	2	1
	1	1	2	1	1	3	0	0	1	1
<u>Kidneys</u>										
Bilateral glomeruli/hypoplasia	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Bilateral medullae/mineralization	4	4	4	3	4	4	4	4	4	4
	3	4	4	2	4	3	3	3	4	3

^aThe numbers in parentheses indicate the number of animals with tissues examined. Data extracted from Appendix L, Table IV of the study report.

these findings were observed among the mid- and high-dose groups at 1 month, the fact that they occurred in only one sex and were not evident after 2 and 3 months of treatment indicates that they are not of toxicological significance. The high-dose males and females exhibited increased mean alkaline phosphatase levels over time as compared with controls. In the females, these differences were statistically significant. Mean cholesterol levels were slightly increased compared to controls in females receiving 75 and 125 mg/kg/day. The increase noted in the high-dose females only was considered treatment related, since the mid-dose values were not markedly different from pretest values. In addition, an increased mean globulin value and decreased albumin/globulin ratio were noted in the high-dose males after 3 months of treatment.

Mean liver weights and liver-to-body weight ratios were slightly to statistically significantly increased in males and females receiving 75 and 125 mg/kg/day. Cytoplasmic vesiculation/vacuolation of the centrilobular hepatocytes was observed in both control and treated males and females; however, the finding was most prominent in the males and females in the high-dose group and was therefore considered to be test-substance related. Evaluation of mortality, physical observations, ophthalmology, body weights, food consumption, urinalyses, and gross postmortem examinations did not reveal any effects considered attributable to the administration of RE-45601.

In conclusion, under the conditions of this study, the effects noted in the males and females receiving 75 and 125 mg/kg/day with regard to organ weight data, clinical laboratory data, and microscopic pathology were considered treatment related. The NOEL for RE-45601 is 25 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was acceptable. However, data on analyzed concentrations and stability of test material in the capsules were not provided.

Dose-related effects were observed in the liver, with increases in absolute and relative weights in males and females at 75 and 125 mg/kg/day, and increases in alkaline phosphatase activity in males and females at 125 mg/kg/day (which reached statistical significance only in females). Although cytoplasmic vesiculation and vacuolation of the central lobular hepatocytes was observed in all control and treated males, three-fourths of the control females, and in all treated females, the severity of this lesion was most prominent in the high-dose animals. Mean cholesterol levels were significantly increased

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in females receiving 125 mg/kg/day. In addition, an increased mean globulin value and decreased albumin/globulin ratio were noted in the high-dose males after 3 months of treatment.

Increases in mean total leukocyte counts observed in males receiving 75 and 125 mg/kg/day were possibly dose related; these were not considered by the study authors to be of toxicological significance. In addition, there was no effect of dosing on mortality, body weight, or food consumption. ←

We agree with the study author's assessment of a NOEL of 25 mg/kg/day for systemic toxicity.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 33063)

EPA No.: 68D80056
DYNAMAC No.: 245-I
TASK No.: 2-45I
April 25, 1990

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DATA EVALUATION RECORD

CLETHODIM

Subchronic Oral Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

Date:

Roman J. Penta for
April 25, 1990

EPA No.: 68D80056
DYNAMAC No.: 245-I
TASK No.: 2-45I
April 25, 1990

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DATA EVALUATION RECORD

CLETHODIM

Subchronic Oral Toxicity Study in Rats

REVIEWED BY:

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Date: April 25, 1990

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Date: 5/30/90

DATA EVALUATION RECORD

GUIDELINE §82-1

STUDY TYPE: Subchronic Oral Toxicity Study in Rats.

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MRID NUMBER: 410301-07.

TEST MATERIAL: RE-45601 Technical.

SYNONYMS: Clethodim; Select; (E,E)-(+/)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[-2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.

STUDY NUMBER: SOCAL 2501.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health, Inc., Richmond, CA.

TITLE OF REPORT: 13-Week Oral Toxicity Study in Rats With RE-45601 Technical (SX-1688).

AUTHOR: K. K. Dougherty.

REPORT ISSUED: July 16, 1986.

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CONCLUSIONS:

When RE-45601 Technical was fed to male and female Sprague-Dawley rats at 0, 50, 500, 2500, or 5000 ppm for 13 weeks followed by a 6-week recovery, there were no overt signs of toxicity or dose-related effects on mortality, hematology, urinalyses, ophthalmology, or gross pathology. Body weights and body weight gains of males and females fed 2500 and 5000 ppm were depressed following dosing, and body weights of females fed 5000 ppm remained depressed throughout the recovery period. Food consumption of males and females fed 5000 ppm (and sporadically males fed 500 and 2500 ppm) was reduced during the dosing period. Cholesterol, total protein, and globulin levels of males fed 5000 ppm were increased following dosing. Absolute and relative liver weights of males and females fed 2500 and 5000 ppm were also increased at this time. These findings were considered to be related to the centrilobular hypertrophy of the liver exhibited histologically in males and females fed 2500 and 5000 ppm. Liver weights of females remained increased following recovery. Increased relative brain and kidney weights observed in males and females fed 5000 ppm were considered to be a reflection of the decreased body weights of these animals. The LOEL of RE-45601 is 2500 ppm and the NOEL is 500 ppm.

Classification: Core Minimum.

A. MATERIALS:

1. Test Compound: RE-45601 Technical; description: viscous, clear amber liquid; batch No.: SX-1688; purity: 83.4%.
2. Test Animals: Species: rat; strain: Sprague-Dawley [Cr1:CD[®](SD)BR]; age: 45 days; weight: males--152 to 240 g, females--127-154 g; source: Charles River Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Following a 7-day acclimation period, animals were assigned to the following test groups using a computerized randomization procedure:

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Test Group	Dose in Diet (ppm)	Main study (13 weeks)		Recovery (6 weeks)	
		Males	Females	Males	Females
1 Control	0	12	12	12	12
2 Low (LDT)	50	12	12	-- ^a	--
3 Mid A (MDT)	500	12	12	--	--
4 Mid B (MDT)	2500	12	12	12	12
5 High (HDT)	5000	11	12	12	12

^aRecovery studies were not conducted on animals fed 50 and 500 ppm.

Rats were housed individually in a room with temperature and humidity controls set at 21.1 to 24.2°C and 40.4 to 78.0%, respectively, with a 12-hour light/dark cycle.

2. Diet Preparation: The test material was analyzed prior to the study, five times during the study, and at study termination. The average purity (83.4%) was used to calculate the target concentrations. A correction factor was not reported to have been incorporated. The test diets were prepared on a weekly basis. The high-dose test concentration (5000 ppm) was dissolved in 7 mL of acetone, diluted with the appropriate amount of basal diet to give the required concentration, and mixed for approximately 20 minutes. The 50-, 500-, and 2500-ppm diets were prepared by mixing appropriate amounts of the 5000-ppm diet with basal diet and were mixed for 15 minutes. The control diet was prepared by mixing 10 mL of acetone/kg of diet. Diets were frozen until use. They were dispensed twice each week for the control, 2500-, and 5000-ppm diets for the 13-week dosing period and twice each week for 50- and 500-ppm diets for 9 weeks, and then were dispensed three times/week for 4 weeks. Homogeneity was analyzed on samples taken from the initial batch of test diet. Stability and concentration were analyzed at study initiation and periodically throughout the study.

Results: Homogeneity was acceptable; the coefficient of variation for nine samples was 3.9, 2.6, 1.6, and 2.5% for the 50-, 500-, 2500-, and 5000-ppm diets, respectively. The mean percentage of nominal concentrations at the beginning of the feeding interval (3 to 7 days frozen) was 87.1, 90.5, 91.6, and 93.8% for the 50-, 500-, 2500- and 5000-ppm samples, respectively. Following the feeding interval (up to 7 days frozen, 4 days unfrozen), the mean

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percentage of nominal was 72.1, 80.5, 87.7, and 92.6% for the 50-, 500-, 2500-, and 5000-ppm samples, respectively.

3. Food and Water Consumption: Animals received Purina Certified Rodent Chow Meal #5002 and water ad libitum.
4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, body weight gains, food consumption, organ weights, hematology, serum chemistry, and urinalyses were evaluated using analysis of variance. Significant differences among groups were evaluated using Dunnett's test. Differential leukocyte counts were evaluated using the method of Kruskal-Wallis and the Mann-Whitney U-test.
5. Quality Assurance: A quality assurance statement was signed and dated February 19, 1987. A GLP statement was signed and dated February 24, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least once daily for signs of toxicity, morbidity, and mortality. Animals were examined for clinical signs weekly.

Results: There was no effect of dosing on survival. One high-dose (5000 ppm) male died during study week 11 as a result of human error, and one low-dose (50 ppm) female was sacrificed during study week 7 because she was pregnant. There were no clinical findings that were considered to be compound related. Incidental observations (red eye discharge, red nasal discharge, broken teeth, scabs, and open sores) were found in control and dosed animals.

2. Body Weight: Rats were weighed at study initiation and weekly thereafter.

Results: Representative mean body weights and body weight gains during dosing and recovery are summarized in Tables 1 and 2. Mean body weights of high-dose males (9 to 11% reduction) and females (9 to 12% reduction) were significantly ($p < 0.01$) depressed throughout the dosing period. Body weight gains were depressed by 18% in males and 24% in females of this group when compared with concurrent controls at dosing termination. Body weights of males fed 2500 ppm were slightly (6% reduction) but significantly ($p < 0.05$) depressed from study weeks 3 to 13; body weight gains were depressed by 10% in animals of this group when compared to concurrent controls at dosing termination. Body weights and body weight gains of females fed 2500 ppm and males and females fed 50 and 500 ppm were similar to concurrent controls. During the 6-week recovery, body weights of females fed 5000 ppm remained significantly depressed [11% ($p < 0.01$) reduction immediately following dosing; 7% ($p < 0.05$) reduction at

TABLE 1. Representative Results of Mean Body Weights and Mean Body Weight Gains
($\pm$ S.D.) of Rats Fed RE-45601 for 13 Weeks^{a,b}

Dose Level (ppm)	Mean Body Weights (g) on Day:					Body Weight Gain (g) (0-91 days)
	0	14	35	56	77	91
Males						
0	226 $\pm$ 17	329 $\pm$ 26	421 $\pm$ 36	478 $\pm$ 45	515 $\pm$ 54	536 $\pm$ 57
50	224 $\pm$ 14	330 $\pm$ 20	423 $\pm$ 24	481 $\pm$ 30	522 $\pm$ 39	543 $\pm$ 42
500	222 $\pm$ 16	314 $\pm$ 24	399 $\pm$ 31	456 $\pm$ 37	492 $\pm$ 42	511 $\pm$ 44
2500	221 $\pm$ 17	314 $\pm$ 26	397 $\pm$ 35*	448 $\pm$ 42*	483 $\pm$ 50*	499 $\pm$ 51*
5000	222 $\pm$ 15	302 $\pm$ 19**	380 $\pm$ 24**	426 $\pm$ 28**	456 $\pm$ 35**	477 $\pm$ 41**
Females						
0	160 $\pm$ 9	201 $\pm$ 13	240 $\pm$ 15	262 $\pm$ 12	273 $\pm$ 13	280 $\pm$ 14
50	161 $\pm$ 9	201 $\pm$ 14	243 $\pm$ 22	263 $\pm$ 22	278 $\pm$ 28	285 $\pm$ 31
500	163 $\pm$ 7	204 $\pm$ 10	244 $\pm$ 13	264 $\pm$ 18	277 $\pm$ 22	284 $\pm$ 23
2500	159 $\pm$ 9	198 $\pm$ 13	234 $\pm$ 15	255 $\pm$ 17	266 $\pm$ 19	275 $\pm$ 19
5000	158 $\pm$ 8	188 $\pm$ 12**	217 $\pm$ 15**	233 $\pm$ 16**	242 $\pm$ 17**	250 $\pm$ 18**

^aBased on 24 animals/sex for controls, 2500 ppm, and 5000 ppm; and 12 animals/sex for 50 and 500 ppm.

^bThis data was extracted from tables 5 and 6 of the study report.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

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TABLE 2. Representative Results of Mean Body Weights and Mean Body Weight Gains ($\pm$ S.D.) During the 6-Week Recovery Period of Rats Fed RE-45601 Technical for 13 Weeks^{a,b}

Dose Level (ppm)	Mean Body Weights (g) on Day:				Body Weight Gains (91-133 days)
	91	105	119	133	
<u>Males</u>					
0	535 ± 57	549 ± 62	562 ± 67	573 ± 67	39 ± 13
2500	489 ± 45**	512 ± 47	527 ± 49	540 ± 54	51 ± 15
5000	487 ± 33**	516 ± 35	542 ± 39	554 ± 41	67 ± 15**
<u>Females</u>					
0	278 ± 14	281 ± 15	290 ± 19	295 ± 22	17 ± 10
2500	277 ± 22	286 ± 23	299 ± 27	302 ± 25	26 ± 13
5000	248 ± 17**	259 ± 22*	266 ± 21*	273 ± 22*	25 ± 13

^aBased on 12 animals/sex/dose with the exception of high-dose males (11 animals).

^bThis data was extracted from tables 7 and 8 of the study report.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

the end of the recovery period]. Body weights of males fed 2500 and 5000 ppm were slightly but nonsignificantly depressed during the recovery period (6 to 9%, 2500 ppm; 3 to 9%, 5000 ppm). The body weight gain of males fed 5000 ppm was significantly ($p < 0.01$) increased (171% of that of concurrent control weight gain) during the recovery period. Body weight gains of males fed 2500 ppm and females fed 2500 and 5000 ppm were 131, 153, and 147% of those of concurrent controls following recovery. An inexplicable body weight reduction was exhibited in dosed and control females on recovery day 126; the study authors attributed this finding to a possible malfunction in the automatic watering system. Females were reweighed and weights were found to be increased on day 128.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated two to three times weekly during dosing and weekly during recovery. Compound intake was calculated from the consumption and body weight gain data.

Results: A slight but significant (sporadic significance of $p < 0.05$ and $p < 0.01$) reduction in food consumption was seen in males and females fed 5000 ppm throughout the dosing period when compared to concurrent controls. Decreased values ranged from 91 to 97% of control consumption in males and 87 to 97% of control consumption in females. The food consumption of males fed 500 and 2500 ppm was also reduced at sporadic intervals throughout the study. Food consumption was comparable to that of concurrent controls during the 6-week recovery. Relative food consumption measured as grams of food consumed divided by 100 g body weight was slightly but significantly ($p < 0.05$ and $p < 0.01$) increased in males and females fed 5000 ppm from study weeks 8 to 13, which reflected the reduced body weights of these animals. Relative food consumption was significantly increased in males fed 2500 and 5000 ppm during the first 2 weeks of recovery and in females fed this same dose during the 6 weeks of recovery. The mean compound intakes for 13 weeks of the study were 2.3, 25, 134, and 279 mg/kg/day for males and 2.8, 30, 159, and 341 mg/kg/day for females fed 50, 500, 2500, and 5000 ppm, respectively.

4. Ophthalmology: Ocular examinations were performed at the initiation and termination of dosing.

Results: There were no ocular lesions related to dosing with RE-45601. Ocular examinations were not conducted at the end of the recovery period.

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5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta following the 13-week dosing period for hematology and clinical analysis from 12 animals/sex/group. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count [†]
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	X Coagulation:thromboplastin time (PT)
X Reticulocyte count (RETIC)	
Red cell morphology	

Results: There were no effects of biological importance on hematology parameters in rats dosed with clethodim. Consequently, these parameters were not measured at the end of the recovery period.

b. Clinical Chemistry

<u>Electrolytes</u>	<u>Other</u>
X Calcium [†]	X Albumin [†]
X Chloride [†]	X Albumin/globulin ratio
Magnesium	X Blood creatinine [†]
X Phosphorus [†]	~~X Blood urea nitrogen[†]~~
X Potassium [†]	X BUN/creatinine ratio
X Sodium [†]	X Cholesterol
	X Globulins
<u>Enzymes</u>	X Glucose [†]
X Alkaline phosphatase (ALP)	X Total bilirubin [†]
Cholinesterase	X Direct bilirubin
X Creatinine phosphokinase	X Total protein [†]
X Lactic acid dehydrogenase	X Triglycerides
X Serum alanine aminotransferase (SGPT) [†]	X Indirect bilirubin
X Serum aspartate amino-transferase (SGOT) [†]	X Uric acid
Gamma glutamyl-transferase (GGT)	

[†]Recommended by Subdivision F (October 1982) Guidelines for subchronic oral toxicity studies.

Results: Cholesterol, total protein, and globulin levels were slightly but significantly ($p < 0.05$) increased in males fed 5000 ppm following dosing (31, 5, and 9% increase from concurrent controls, respectively) and slightly but nonsignificantly increased in females fed this same dose (Table 3). The study authors considered these increases to be a result of the centrilobular hypertrophy of the liver found in these animals. The BUN/creatinine ratio of females fed 2500 ppm was found to be slightly but significantly ($p < 0.01$) decreased following dosing (Table 3); since this decrease was not found in females dosed with 5000 ppm or in males at any dose level, the study authors considered this finding to be incidental and unrelated to dosing. Clinical chemistry parameters were not measured at the end of the recovery period, since changes at the end of dosing were not considered by the study author to be of biological significance.

6. Urinalysis: Urine was collected from fasted animals at dosing termination. The CHECKED (X) parameters were examined^a:

X Appearance	X Glucose
X Volume	X Ketones
X Specific gravity	X Bilirubin
X pH	X Blood
X Sediment (microscopic)	Nitrate
X Protein	Urobilinogen

Results: There were no toxicologically important effects of dosing on urinary parameters. Consequently, these parameters were not measured at the end of the recovery period.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

^aUrinalyses are not required by Subdivision F (October 1982) Guidelines for subchronic oral toxicity studies.

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TABLE 3. Selected Clinical Chemistry Results ($\pm$ S.D.) in Rats Fed RE-43601 Technical for 13 Weeks^a

Dose Level (ppm)	Parameter			
	Cholesterol (mg/dL)	Total Protein (g/dL)	Globulin (g/dL)	BUN/ Creatinine Ratio
<u>Males</u>				
0	70 $\pm$ 22	6.4 $\pm$ 0.2	3.3 $\pm$ 0.2	24.7 $\pm$ 3.7
50	67 $\pm$ 12	6.4 $\pm$ 0.2	3.3 $\pm$ 0.2	25.8 $\pm$ 3.8
500	65 $\pm$ 17	6.4 $\pm$ 0.2	3.2 $\pm$ 0.2	26.7 $\pm$ 4.5
2500	76 $\pm$ 20	6.6 $\pm$ 0.2	3.4 $\pm$ 0.2	24.9 $\pm$ 4.2
5000	92 $\pm$ 29*	6.7 $\pm$ 0.3*	3.6 $\pm$ 0.3*	24.9 $\pm$ 3.3
<u>Females</u>				
0	32 $\pm$ 20	6.6 $\pm$ 0.3	3.3 $\pm$ 0.3	27.5 $\pm$ 4.7
50	37 $\pm$ 28	6.9 $\pm$ 0.6	3.5 $\pm$ 0.3	24.9 $\pm$ 4.5
500	79 $\pm$ 12	6.5 $\pm$ 0.3	3.2 $\pm$ 0.2	24.4 $\pm$ 4.1
2500	38 $\pm$ 20	6.7 $\pm$ 0.3	3.4 $\pm$ 0.2	21.7 $\pm$ 2.9**
5000	91 $\pm$ 15	6.8 $\pm$ 0.3	3.5 $\pm$ 0.3	24.4 $\pm$ 4.2

^aThis data was extracted from tables 24 and 25 of the study report.*Significantly different from control values at $p < 0.05$.**Significantly different from control values at $p < 0.01$.

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
X Tongue	X Aorta [†]	XX Brain
X Salivary glands [†]	XX Heart [†]	X Peripheral nerve
X Esophagus [†]	Bone marrow [†]	(sciatic nerve) [†]
X Stomach [†]	X Lymph nodes	X Spinal cord
X Duodenum [†]	(mesenteric) [†]	(3 levels)
X Jejunum [†]	XX Spleen [†]	X Pituitary [†]
X Ileum [†]	X Thymus [†]	X Eyes
X Cecum [†]		(optic nerve)
X Colon [†]	<u>Urogenital</u>	
X Rectum	XX Kidneys [†]	<u>Glandular</u>
XX Liver [†]	X Urinary bladder [†]	XX Adrenals [†]
Gallbladder [†]	XX Testes [†]	Lacrimal gland
X Pancreas [†]	X Epididymides	X Mammary gland
	X Prostate	X Thyroids [†]
<u>Respiratory</u>	X Seminal vesicle	X Parathyroids [†]
X Trachea [†]	XX Ovaries [†]	X Harderian glands
X Lung [†]	X Uterus	
		<u>Other</u>
		Bone (sternum and femur)
		X Skeletal muscle
		X Skin
		X All gross lesions and masses [†]
		X Skull

A complete inventory of tissues was examined for all control rats and rats dosed with 5000 ppm, and for all rats in other groups that died or were sacrificed during the study. Lungs, liver, kidneys, and gross lesions were examined for all rats dosed with 50, 500, and 2500 ppm. Liver, kidneys, and gross lesions were examined for all recovery animals.

Recommended by Subdivision F (October 1982) Guidelines for subchronic oral toxicity studies.

Results:

- a. Organ Weights: Tables 4 and 5 summarize organ weight changes. Liver weights and liver-to-body or brain weight ratios were increased in males and females receiving 2500 and 5000 ppm. The absolute weights were significantly increased ($p < 0.01$) in females receiving 5000 ppm (14% compared to controls) but not in males (9% compared to controls). The liver-to-body weight ratios were significantly increased in males (12 and 26% increase, respectively, compared to controls) fed 2500 and 5000 ppm and in females at the same doses (12 and 28% increase, respectively). The liver-to-brain weight ratio was significantly ($p < 0.01$) increased in females fed 5000 ppm (13%) but not in males fed 5000 ppm (8%). Relative kidney and brain (to body) weights of males and females fed 5000 ppm were significantly ($p < 0.01$) increased when compared to concurrent controls following dosing; however, these increased weights were considered by the study authors to be a reflection of the decreased body weights of these animals. Significantly ($p < 0.01$) decreased absolute and relative (to body and to brain) ovarian weights of females fed 50 ppm were not considered to be compound related by the study authors, since these ovarian weight changes were not found at other doses. Following recovery, the effects on liver weights were almost completely reversed in males; however, the absolute liver weights of females fed 2500 ppm and relative (to body) liver weights of females fed 2500 or 5000 ppm remained significantly increased ($p < 0.05$) (Table 5). No differences were found in other organ weights at this time.
- b. Gross Pathology: There were no compound-related macroscopic findings following dosing or recovery. Findings observed were similar in control and dosed animals and were considered common age- and strain-related changes.
- c. Microscopic Pathology:
- Nonneoplastic: Table 6 summarizes nonneoplastic findings in rats fed RE-45601 Technical for 13 weeks. Following dosing, centrilobular hypertrophy of the liver was exhibited in males and females fed 2500 (67% males, 17% females) and 5000 ppm (83% males, 58% females). The hypertrophy was reported to be more severe and its incidence greater in males when compared

TABLE 4. Selected Mean Organ Weights ($\pm$ S.D.) and Organ-to-Body Weight Ratios of Rats Fed RE-45601 Technical for 13 Weeks^{a,b}

Dose Level (ppm)	Liver		Kidneys		Brain	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Males						
0	14.33 $\pm$ 2.30	2.80 $\pm$ 0.26	3.56 $\pm$ 0.36	0.70 $\pm$ 0.05	2.14 $\pm$ 0.08	0.42 $\pm$ 0.05
50	14.76 $\pm$ 1.84	2.87 $\pm$ 0.26	3.61 $\pm$ 0.39	0.70 $\pm$ 0.06	2.18 $\pm$ 0.07	0.43 $\pm$ 0.02
500	13.58 $\pm$ 1.34	2.79 $\pm$ 0.18	3.43 $\pm$ 0.39	0.71 $\pm$ 0.06	2.14 $\pm$ 0.09	0.44 $\pm$ 0.04
2500	15.16 $\pm$ 2.62	3.13 $\pm$ 0.31*	3.52 $\pm$ 0.43	0.73 $\pm$ 0.07	2.13 $\pm$ 0.13	0.45 $\pm$ 0.05
5000	15.61 $\pm$ 2.74	3.52 $\pm$ 0.35**	3.38 $\pm$ 0.36	0.77 $\pm$ 0.07*	2.15 $\pm$ 0.12	0.49 $\pm$ 0.04**
Females						
0	7.04 $\pm$ 0.54	2.68 $\pm$ 0.18	1.98 $\pm$ 0.12	0.75 $\pm$ 0.04	2.01 $\pm$ 0.06	0.77 $\pm$ 0.04
50	7.47 $\pm$ 0.87	2.82 $\pm$ 0.16	2.05 $\pm$ 0.18	0.78 $\pm$ 0.07	2.00 $\pm$ 0.06	0.77 $\pm$ 0.09
500	7.11 $\pm$ 0.53	2.67 $\pm$ 0.16	1.95 $\pm$ 0.13	0.73 $\pm$ 0.06	1.98 $\pm$ 0.06	0.75 $\pm$ 0.06
2500	7.69 $\pm$ 0.73	2.99 $\pm$ 0.19**	2.06 $\pm$ 0.13	0.81 $\pm$ 0.05	2.02 $\pm$ 0.11	0.79 $\pm$ 0.06
5000	8.00 $\pm$ 0.69**	3.42 $\pm$ 0.21**	2.00 $\pm$ 0.19	0.86 $\pm$ 0.08**	2.02 $\pm$ 0.08	0.87 $\pm$ 0.06**

^aBased on 12 animals/sex/dose with the exception of 50-ppm males and females (11 animals).

^bData extracted from tables 32 and 33 of the study report.

*Significantly different from controls at $p < 0.05$.

**Significantly different from controls at $p < 0.01$.

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TABLE 5. Mean Liver Weights, Liver-to-Body, and Liver-to-Brain Ratios of Rats Fed RE-45601 Technical for 13 Weeks Followed by a 6-Week Recovery^a

Dose Level (ppm)	Absolute (g)	Relative	
		Body (%)	Brain (%)
<u>Males</u>			
0	15.89 ± 2.89	2.89 ± 0.26	7.02 ± 1.35
2500	14.94 ± 2.21	2.90 ± 0.22	6.77 ± 1.21
5000	16.14 ± 1.80	3.04 ± 0.21	7.42 ± 0.61
<u>Females</u>			
0	7.59 ± 0.79	2.74 ± 0.17	3.80 ± 0.47
2500	8.58 ± 1.04*	3.03 ± 0.31*	4.26 ± 0.63
5000	7.90 ± 0.82	3.10 ± 0.30**	4.04 ± 0.47

^aData extracted from tables 34 and 35 of the study report.

*Significantly different from controls at p < 0.05.

**Significantly different from controls at p < 0.01.

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TABLE 6. Representative Nonneoplastic Findings in Rats Fed RE-45601
Technical for 13 Weeks^a

Organ/Finding	Dose Level (ppm):									
	Males					Females				
	0	50	500	2500	5000	0	50	500	2500	5000
<u>Liver</u>	(12) ^b	(12)	(12)	(12)	(12)	(12)	(11)	(12)	(12)	(12)
Hypertrophy, centrilobular										
Trace	0	0	0	7	1	0	0	0	2	5
Mild	0	0	0	1	4	0	0	0	0	1
Moderate	0	0	0	0	5	0	0	0	0	1
Infiltration, lymphocytic	3	4	8	6	7	5	5	10	5	7
<u>Kidney</u>	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
Degeneration, tubular	1	1	2	4	1	0	0	0	0	0
Infiltration, lymphocytic	1	1	2	1	1	3	0	2	1	2
Regeneration	2	5	3	7	8	0	1	1	1	1
Microconcretion	0	0	0	0	0	0	1	0	3	0

^aData extracted from Appendix Q of the study report.^bThe numbers in parentheses are the numbers of tissues examined.

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to females. The study authors considered this finding to be an adaptive response associated with enzyme induction. There was no reported incidence of liver hypertrophy in recovery animals. The incidence of lymphocytic infiltration of the liver was slightly increased in all dosed males and females fed 500 and 5000 ppm when compared to concurrent controls. The incidence of lymphocytic infiltration of the liver continued to be slightly increased in dosed males when compared to concurrent controls following recovery. This finding was considered by the study authors to be unrelated to dosing. Slight increases in the incidence of lesions of the kidneys of dosed males were considered by the study authors to be spontaneous and unrelated to dosing. Other incidental findings were considered to be normal age- and strain-related changes.

D. STUDY AUTHOR'S CONCLUSIONS:

The author concluded that dietary administration of RE-45601 technical for 13 weeks to Sprague-Dawley rats at levels of 0, 50, 500, 2500, or 5000 ppm produced depressed body weights and body weight gains in males and females receiving the two highest doses. Body weights of females remained low following a 6-week recovery period. Food consumption indicated non-palatability at 2500 and 5000 ppm. Cholesterol, total protein, and globulin levels of males fed 5000 ppm were increased following dosing and were considered to be related to the centrilobular hypertrophy of the liver found in these animals. Increased absolute and relative liver weights in males and females fed 2500 and 5000 ppm were also considered to be a result of this finding; the hypertrophy was considered to be an adaptive response. Hypertrophy of the liver was not exhibited following recovery. Increased relative brain and kidney weights of males and females fed 5000 ppm were considered to be a reflection of the depressed body weights of these animals. The NOEL was 500 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were acceptable. However, the test diet analysis was not presented in a manner that was easily interpreted, and the stability results appeared to be unacceptable for the 50-, 500-, and 2500-ppm diets following the feeding interval (mean percentage of nominal, 72.1, 80.5, and 87.7%, respectively). The testing laboratory attempted to remedy this instability by offering diets from the

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freezer three times/week beginning at study week 10 for the 50- and 500-ppm dosage levels. In addition, the quality assurance statement was signed and dated 8 months following the date of the final report. The study author did not report the collection of bone marrow for histological examination following dosing, which is recommended by EPA Pesticide Assessment Guidelines, 1982, for subchronic oral toxicity studies.

We agree that the incidence of centrilobular hypertrophy of the liver found in males and females dosed with 2500 and 5000 ppm may have been an adaptive response associated with enzyme induction. This finding appears to be reversible. The LOEL is 2500 ppm, and the NOEL is 500 ppm for RE-45601 Technical.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-K
TASK No.: 2-45K
April 26, 1990

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DATA EVALUATION RECORD
CLETHODIM HIGH PURITY AND PROCESS NEUTRALS
Five-Week Subchronic Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

Roman J. Pereira for

Date:

April 26, 1990

EPA No.: 68D80056
DYNAMAC No.: 245-K
TASK No.: 2-45K
April 26, 1990

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DATA EVALUATION RECORD
CLETHODIM HIGH PURITY AND PROCESS NEUTRALS
Five-Week Subchronic Feeding Study in Rats

REVIEWED BY:

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Signature: J. M. Ioannou
Date: 5/31/90

DATA EVALUATION RECORD

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STUDY TITLE: Five-Week Subchronic Feeding Study in Rats.

MRID NUMBER: 410301-08.

TEST MATERIAL: RE-45601, high purity (SX-1718);
RE-45601, process neutrals (SX-1717).

SYNONYMS: Clethodim; Cloproxydim; High-Purity RE-45601-(E,E)-(+/)-
-2-[1-((3-chloro-2-propenyl)oxyimino)propyl]-5-
[2(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.

STUDY NUMBER: S-2763.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals
Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc.,
Richmond, CA.

TITLE OF REPORT: Five-Week Subchronic Feeding Study of High Purity
RE-45601 (SX-1718) and RE-45601 Process Neutrals (SX-1717) in Rats.

AUTHOR: J. R. Cushman.

REPORT ISSUED: April 6, 1987.

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CONCLUSIONS:

RE-45601 (high purity, 96.2% active ingredient) and the process neutrals (3.3% active ingredient) were fed to male and female Sprague-Dawley rats for 5 weeks at dietary levels of 6800 ppm and 1200 ppm, respectively. There were no compound-related effects on mortality or clinical observations. Body weights and body weight gains were depressed throughout the study in males and females fed the high-purity RE-45601; gains were 33.5% and 42.4% lower than controls for males and females, respectively. Decreased relative food consumption of these animals indicated that the diet was not initially palatable. Body weights and body weight gains of males fed the process neutrals were slightly depressed; gains were 12% lower than controls. Erythrocyte counts, hematocrit, and hemoglobin concentration of males and females fed high-purity RE-45601 were depressed at study termination. There were no changes in hematology parameters of animals fed the process neutrals and only minor changes in clinical chemistry parameters of animals fed either test material. Absolute and relative (to body and to brain) liver weights of males and relative (to body and to brain) liver weights of females fed high-purity RE-45601 were increased and adrenal weights of males and females fed high-purity RE-45601 were decreased. Absolute and relative (to body and to brain) liver weights of females fed the process neutrals were increased. Centrilobular hypertrophy of the liver was exhibited in animals fed high-purity RE-45601 and the process neutrals; the incidence was higher in animals fed high-purity RE-45601 and in males as compared with females. There were no correlating histologic adrenal changes. The high-purity RE-45601 appeared to be of greater toxicity, and the active ingredient contained in the process neutrals may have produced the effects exhibited in these animals.

Classification: This study cannot be CORE classified, since there are no available EPA Pesticide Guidelines for a 5-week feeding study.

A. MATERIALS:

1. Test Compound: RE-45601, high purity--description: clear, amber liquid; batch No. SX-1718; purity: 96.2%. RE-45601, process neutrals--description: dark, amber liquid; batch No.: SX-1717; purity: 3.2% RE-45601 at study initiation, 3.3% RE-45601 at study termination.
2. Test Animals: Species: rat; strain: Sprague-Dawley Crl:CD(SD)BR; age: males--44 days; females--46 days; weight: males--181 to 202 g; females--136 to 160 g; source: Charles River Breeding Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Following 2 weeks of quarantine and 1 week of acclimation, animals were assigned to the following test groups by computer randomization:

Test Group	Dietary Dose (ppm)	Main Study (5 weeks)	
		Males	Females
1 Control	0	10	10
2 Process neutrals	1200	10	10
High-purity RE-45601	6800	10	10

The purpose of this study was to clarify the results of a 5-week feeding study (SOCAL 2457) with RE-45601 technical (SX-1653, 84.3% purity) in which males and females fed 4000 and 8000 ppm exhibited decreased body weight gain, mild anemia, increased liver weights, and mild liver hypertrophy. Males fed 1000 ppm exhibited decreased hemoglobin levels, increased liver weights, and trace to mild centrilobular hypertrophy. No effects were found at 5 or 20 ppm. Dose levels of high-purity RE-45601 and process neutrals were selected to approximate the 8000-ppm dietary level of RE-45601 technical used in the previous 5-week study with technical RE-45601.

Animals were housed individually in a room with temperature and humidity controls set at 21.2 to 23.9°C and 34.2 to 69.9%, respectively, with a 12-hour light/dark cycle. The animals were examined to ensure they were in good health prior to study initiation.

2. Diet Preparation: Diets were prepared weekly (approximately 8 kg of each diet) and offered to the animals biweekly. Prew weighed concentrations of the appropriate test material were dissolved in approximately 7 mL of nanograde acetone/kg of diet mixture. The test mixture/acetone solution plus an acetone rinse of approximately 3 mL/kg of diet mixture was mixed with approximately half of the rodent chow for 5 minutes and remixed for 15 minutes with the addition of the remaining rodent chow. Process neutral diets were based on analysis of the content of RE-45601, since it was the only component of the complex mixture for which an analytical method was developed. Calculations for dietary levels were based on a RE-45601 content of 3.3%.

The control diet was prepared by mixing 10 mL acetone/kg of rodent chow and mixing for 15 minutes. Diets were stored at 5°C. Nine homogeneity samples were taken for analysis from the initial batch of each diet, and three samples were taken for analysis from each subsequent diet mixture. Three frozen and unfrozen samples were taken weekly for stability analysis.

Results: Based on initial sampling (nine samples), the study author reported that the homogeneity results of the process neutrals were high (percent of target concentration, 162%), although consistent throughout the study (coefficient of variation $\pm 2.1\%$), and should therefore be regarded as within acceptable limits. The percent of target concentration for the high-purity RE-45601 was 97.9%, with a coefficient of variation of $\pm 1.2\%$. The mean percent of target concentration for five intervals of analysis of process neutrals was 148.2% for freshly prepared diet, 143.2% for diets frozen 3 days, and 133.6% for diets frozen 3 days and unfrozen 4 days. As with the homogeneity results, the study author reported that these test results were high but consistent throughout the study period and should therefore be regarded as within acceptable limits. The mean percent of target concentration for five intervals of analysis of the high-purity RE-45601 was 96.1% for freshly prepared diet, 95% for diets frozen 3 days, and 98.9% for diets frozen 3 days and unfrozen 4 days.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.
4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, food consumption, hematology, clinical chemistry and organ weight data were analyzed using analysis of variance and Dunnett's test for significant differences among groups. Differential leukocyte counts were analyzed using the Kruskal-Wallis test.
5. Quality Assurance: A quality assurance statement was signed March 30, 1987. A GLP Statement was signed and dated April 6, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of mortality and weekly for signs of toxicity.

Results: No mortalities occurred. No clinical findings suggesting a compound-related effect were observed. Alopecia, open sores, and/or scabs on the skin of the neck

were observed in two controls and in one dosed animal fed each test diet. No explanation was reported by the study author.

2. Body Weight: Rats were weighed at study initiation and weekly thereafter.

Results: Representative data on mean body weights and body weight gains are presented in Table 1. Mean body weights and body weight gains of males and females fed 6800 ppm of high-purity RE-45601 were significantly ($p < 0.01$) depressed from study day 7 to study termination when compared to concurrent controls. Body weights were depressed from 10.4% to 15.3% in males and from 9.3% to 14.6% in females; body weight gains were depressed 33.5% in males and 42.4% in females.

Mean body weights of males fed 1200 ppm of the process neutrals were slightly depressed throughout the study (3 to 5%) and significantly ($p < 0.05$) depressed (6.5%) at study termination. Body weight gains of these animals were depressed by 12% ($p < 0.05$) when compared to concurrent controls. Body weights and body weight gains of females fed 1200 ppm of the process neutrals were similar to concurrent controls.

3. Food Consumption and Compound Intake: Consumption was determined, and mean daily diet consumption was calculated twice weekly. Compound intake was calculated from the consumption and body weight gain data.

Results: Representative data on mean food consumption are presented in Table 2. Food consumption of males fed 6800 ppm of high-purity RE-45601 was significantly depressed ($p < 0.01$) throughout the study (28% depressed on day 3, 11 to 16% depressed from day 7 to day 35 when compared to concurrent controls). The food consumption of females from this same group was significantly ($p < 0.05$) depressed at sporadic intervals (weeks 1, 3, and 5) throughout the study (10 to 17% depressed when compared to concurrent controls). The mean relative food consumption (g/100 g body weight/day) of the males fed high-purity RE-45601 was significantly lower than controls ($p < 0.01$) during study week 1 only, and slightly but nonsignificantly lower than controls for females during this time. The mean relative food consumption of males and females was similar to that of concurrent controls from study week 2 to study termination. The study author considered these findings to indicate that the test diet was not initially palatable to the animals and that this decreased food consumption may have contributed to the slower growth rate of these animals.

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TABLE 1. Mean Body Weights (g $\pm$ S.D.) and Mean Body Weight Gains of Rats Fed High-Purity RE-45601 and Its Process Neutrals for 5 Weeks^{a,b}

Dose Level (ppm)	Mean Body Weight on Days:				Mean Body Weight Gain (g) Day 0-35)
	0	7	21	35	
<u>Males</u>					
0	224 ± 14	281 ± 20	370 ± 29	433 ± 34	209 ± 22
1200 ^c	221 ± 7	273 ± 9	351 ± 15	405 ± 24*	184 ± 23*
6800 ^d	228 ± 9	252 ± 9**	320 ± 10**	367 ± 13**	139 ± 12**
<u>Females</u>					
0	162 ± 7	184 ± 9	219 ± 10	240 ± 13	78 ± 11
1200 ^c	160 ± 7	181 ± 7	218 ± 11	239 ± 9	79 ± 7
6800 ^d	160 ± 10	167 ± 12**	194 ± 11**	205 ± 11	45 ± 7**

^aBased on 10 animals/sex/group.^bData was extracted from tables 3 and 4 of the study report.^cProcess neutrals.^dHigh-purity RE-45601.*Significantly different from controls at $p \leq 0.05$.**Significantly different from controls at $p < 0.01$.

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TABLE 2. Representative Mean Food Consumption of Rats Fed High-Purity RE-45601 and Its Process Neutrals for 5 Weeks^{a,b}

Dose Level (ppm)	Food Consumption on Day:				
	7	14	21	28	35
<u>Males</u>					
0	30.9 ± 3.2	31.3 ± 3.3	33.0 ± 3.0	32.3 ± 3.1	32.6 ± 2.7
1200 ^c	28.4 ± 1.1*	29.2 ± 1.6	29.9 ± 1.5*	29.5 ± 2.1*	28.7 ± 2.1**
6800 ^d	26.6 ± 1.9**	28.0 ± 1.6**	29.0 ± 3.2**	28.1 ± 1.3**	27.4 ± 1.9**
<u>Females</u>					
0	20.8 ± 0.8	22.3 ± 1.3	22.7 ± 1.6	22.4 ± 1.9	22.3 ± 1.5
1200 ^c	20.8 ± 2.1	21.5 ± 1.9	22.5 ± 2.3	24.1 ± 2.8	21.9 ± 2.0
6800 ^d	18.8 ± 1.9*	20.2 ± 3.2	20.1 ± 3.2*	20.7 ± 4.6	18.5 ± 3.5**

^aBased on 10 animals/sex/group.^bData was extracted from tables 5 and 6 of the study report.^cProcess neutrals.^dHigh-purity RE-45601.

*Significantly different from control at p < 0.05.

**Significantly different from control at p < 0.01.

The food consumption of males fed 1200 ppm of the process neutrals was slightly depressed (7 to 12% depressed when compared to concurrent controls) throughout the study period; the depression was significant during weeks 1, 3, 4, and 5. The food consumption of females of this group was similar to that of concurrent controls. Mean relative food consumption of the dosed males was slightly but nonsignificantly lower than concurrent controls throughout the study.

The compound intake of the high-purity RE-45601 and process neutrals of RE-45601 was reported to decrease in males and females throughout the study, reflecting the decrease in food consumption. The mean compound intakes were 597 and 667 mg RE-45601/kg/day for males and females, respectively, fed high-purity RE-45601 and 148 and 175 mg process neutrals/kg/day for males and females, respectively, based on the analyzed concentrations of RE-45601. The mean compound intake of RE-45601 for the latter animals was 4.87 and 5.78 mg/kg/day for males and females, respectively. These results were not presented in the study report; Tables 9 and 10 which were described in the text to contain these data were missing from the report.

4. Ophthalmology: Pupil responses were examined at study termination.

Results: Normal pupil responses were reported for all animals.

5. Hematology and Clinical Chemistry: Blood was collected from the descending aorta at study termination for hematology and clinical analysis from 10 males and 9 females. Significantly different serum chemistry parameters were compared with data from age- and strain-matched laboratory historical controls (studies SOCAL 2241 and SOCAL 2457). The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	Coagulation:thromboplastin
X Reticulocyte count (RETIC)	time (PT)
X Red cell morphology	

Results: Representative hematology results are presented in Table 3. The erythrocyte counts and hemoglobin concentration of males and females fed high-purity RE-45601

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TABLE 3. Mean Hematology Values of Rats Fed High-Purity RE-45601 and Its Process Neutrals for 5 Weeks^{a,b}

Parameters	Dose Level (ppm):					
	Males			Females		
	0	1200 ^c	6800 ^d	0	1200 ^c	6800 ^d
Hemoglobin (g/dL)	15.0 ± 0.6	14.8 ± 0.5	14.1 ± 0.4**	14.6 ± 0.90	14.1 ± 0.7	13.7 ± 0.6*
Hematocrit (%)	40.6 ± 1.8	39.5 ± 1.3	38.0 ± 1.2**	39.1 ± 2.20	38.0 ± 1.7	37.2 ± 1.6
Erythrocyte count (10 ⁶ /mm ³)	7.34 ± 0.38	7.25 ± 0.29	6.9 ± 0.26*	7.14 ± 0.44	6.87 ± 0.35	6.63 ± 0.27*
Reticulocyte count (%)	2.5 ± 1.1	2.3 ± 1.3	3.0 ± 1.4	2.6 ± 0.90	2.4 ± 1.0	3.1 ± 2.1

^aBased on 10 males and 9 females/dose.^bData was extracted from tables 11 and 12 of the study report.^cProcess neutrals.^dHigh-purity RE-45601.

*Significantly different from controls at p < 0.05.

**Significantly different from controls at p < 0.01.

was slightly but significantly ($p < 0.01$ and $p < 0.05$) depressed at study termination (hemoglobin depressed 6% and erythrocyte counts depressed 4 to 6% from concurrent controls). In addition, the hematocrit level of males fed high-purity RE-45601 was significantly ($p < .01$) depressed at this time, while that of females was slightly but nonsignificantly depressed (6.5% reduction in males, 5% in females). Reticulocyte counts were nonsignificantly increased by 20% in males and females of this group when compared to concurrent controls. The study author considered these results to indicate a compound-related anemia. Hematology parameters of males and females fed the process neutrals were considered by the reviewers to be similar to concurrent controls.

b. Clinical Chemistry

<u>Electrolytes</u>		<u>Other</u>	
X	Calcium	X	Albumin
X	Chloride	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine
X	Phosphorus	X	BUN/creatinine ratio
X	Potassium	X	Blood urea nitrogen
X	Sodium	X	Cholesterol
X	Globulins	X	Glucose
		X	Total bilirubin
		X	Direct/indirect bilirubin
<u>Enzymes</u>		X	Total protein
X	Alkaline phosphatase (ALP)	X	Triglycerides
	Cholinesterase	X	Uric acid
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)		
X	Serum aspartate aminotransferase (SGOT)		
	Gamma glutamyltransferase (GGT)		

Results: Total protein and albumin levels of males fed high-purity RE-45601 were slightly but significantly ($p < 0.01$) increased, and alkaline phosphatase levels of females fed the process neutrals were slightly but significantly ($p < 0.05$) decreased when compared to that of concurrent controls; however, these values were within the range of those for corresponding historical controls, were found in one sex only, and were considered by the study author to be unrelated to dosing. No other changes were observed in serum chemistry parameters. Summary tabulation for eight serum chemistry parameters was not included in the study report; however, individual data were provided.

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6. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	Aorta	Brain
Salivary glands	Heart	X Peripheral nerve (sciatic nerve)
Esophagus	Bone marrow	X Spinal cord (3 levels)
Stomach	Lymph nodes	Pituitary
Duodenum	X Spleen	Eyes (optic nerve)
Jejunum	Thymus	
Ileum		
Cecum		
Colon		
Rectum	<u>Urogenital</u>	<u>Glandular</u>
XX Liver	XX Kidneys	XX Adrenals
Gallbladder	X Urinary bladder	Lacrimal gland
Pancreas	XX Testes	Mammary gland
	X Epididymides	Thyroids
	Prostate	Parathyroids
	Seminal vesicle	Harderian glands
<u>Respiratory</u>	XX Ovaries	
Trachea	X Uterus	
X Lung		
		<u>Other</u>
		Bone (sternum and femur)
		Skeletal muscle
		Skin
		X All gross lesions and masses

Results:

- a. Organ Weights: Representative organ weight data of rats fed high-purity RE-45601 and process neutrals of RE-45601 are presented in Table 4. Absolute and relative (to body and to brain) liver weights of males and relative (to body and to brain) liver weights of females fed high-purity RE-45601 were significantly ($p < 0.05$ and $p < 0.01$) increased when compared to concurrent controls (7 to 12%, 24 to 34%, and 8 to 12% increased from concurrent controls for absolute, relative-to-body, and relative-to-brain weights, respectively). Absolute and relative (to body and to brain) liver weights of females fed the process neutrals were also significantly ($p < 0.05$ and $p < 0.01$)

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TABLE 4. Selected Mean Organ Weights, Organ-to-Body Weight, and Organ-to-Brain Weight Ratios in Rats Fed High-Purity RE-45601 and Its Process Neutrals for 5 Weeks^a

Dose level (ppm)	Liver		Adrenals			
	(g)	(% body wt)	(% brain wt)	(g)	(% body wt)	(% brain wt)
<u>Males</u>						
0	12.17 ± 1.18	3.03 ± 0.15	5.93 ± 0.64	0.0618 ± 0.013	0.0155 ± 0.0035	0.0300 ± 0.0063
1200 ^b	12.24 ± 1.61	3.25 ± 0.20*	5.91 ± 0.73	0.0602 ± 0.013	0.0160 ± 0.0034	0.0291 ± 0.0063
6800 ^c	13.66 ± 0.70*	4.06 ± 0.15**	6.65 ± 0.33*	0.0457 ± 0.010*	0.0136 ± 0.0029	0.0272 ± 0.0047*
<u>Females</u>						
0	6.97 ± 0.70	3.18 ± 0.32	3.65 ± 0.28	0.0664 ± 0.0122	0.0303 ± 0.0051	0.0347 ± 0.0061
1200 ^b	7.65 ± 0.44*	3.49 ± 0.20*	4.05 ± 0.20**	0.0687 ± 0.0081	0.0314 ± 0.0046	0.0365 ± 0.0052
6800 ^c	7.48 ± 0.59	3.95 ± 0.15**	3.95 ± 0.31*	0.0550 ± 0.0087*	0.0291 ± 0.0047	0.0291 ± 0.0043*

^aData was extracted from tables 17 to 22 of the study report.^bProcess neutrals.^cHigh-purity RE-45601.

*Significantly different from controls at p < 0.05.

**Significantly different from controls at p < 0.01.

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increased when compared to concurrent controls (10 to 11% increase). The increased relative (to body) liver weight of males of this group was considered to be a reflection of the reduced body weights of these animals.

Absolute and relative (to brain) adrenal weights of males and females fed high-purity RE-45601 were significantly ($p < 0.05$) (17 to 26% from concurrent controls) depressed. In the absence of histological findings, the study author considered these decreased weights to be unrelated to dosing. Similarly, testes to brain weight ratios of males fed the process neutrals were significantly ($p < 0.05$) depressed with no observed histological changes. Increases of the weights of kidney and testes relative to body weights of animals fed high-purity RE-45601 were considered to be a reflection of the decreased body weights of these animals.

- b. Gross Pathology: There were no compound-related macroscopic findings. The lesions exhibited were considered spontaneous age- and strain-related changes.
- c. Microscopic Pathology:

Nonneoplastic: Table 5 summarizes the incidence of frequently occurring nonneoplastic findings. When compared to concurrent controls, the incidence of trace to mild centrilobular hypertrophy was increased in all dosed males and females. The incidence was higher in the animals fed the high-purity RE-45601 and in males as compared with females of both groups (high-purity RE-45601: 10/10 males, 8/10 females; process neutrals: 6/10 males, 3/10 females). The incidence of lymphocytic infiltration of the liver appeared to be high in control and dosed animals. The incidence of kidney regeneration was increased in dosed males and females when compared to concurrent controls; however, the study author reported these findings to be of trace severity and spontaneous. All other histological findings were considered to be unrelated to dosing.

D. STUDY AUTHORS' CONCLUSIONS:

Dietary administration of 6800 ppm high-purity RE-45601 to Sprague-Dawley rats for 5 weeks produced a lower body weight gain, mild anemia, and mild liver hypertrophy characterized by

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TABLE 5. Selected Nonneoplastic Histopathology Findings in Rats Fed High-Purity RE-45601 and Its Process Neutrals for 5 Weeks^a

Organ/Finding	Dose Levels (ppm):					
	Males			Females		
	0	1200 ^b	6800 ^c	0	1200 ^b	6800 ^c
<u>Liver</u>	(10) ^d	(10)	(10)	(10)	(10)	(10)
Hypertrophy, centrilobular	0	6	10	0	3	8
Infiltration, lymphocytic	9	8	6	7	9	9
<u>Kidney</u>	(10)	(10)	(10)	(10)	(10)	(10)
Regeneration	2	4	7	1	3	3

^aData was extracted from Appendix K of the study report.

^bProcess neutrals.

^cHigh-purity RE-45601.

^dNumbers in parentheses equal numbers of tissues examined.

increased liver weights and trace to mild centrilobular hypertrophy. Decreased food consumption indicated that the diet was not initially palatable to the animals. Dietary administration of 1200 ppm RE-45601 process neutrals for 5 weeks produced a lower body weight gain in males, increased liver weights in females, and mild centrilobular hypertrophy in the liver of males and females. These results are consistent with those found when animals were dosed with RE-45601 technical, indicating that the active ingredient caused the changes in body weight gain, erythrocyte parameters, and liver weights, and that the process neutrals appeared to have a minor contribution to these effects.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The purpose of this study was to clarify the results of a 5-week feeding study (SOCAL 2457) with RE-45601 technical (SX-1653, 83.4% purity). The conduct of the study was acceptable for this purpose; however, the study author also reported that this study was intended to satisfy Pesticide Guideline No. 82-1, 90-Day Subchronic Oral Toxicity Study. Since the length of this study was 5 weeks, the criteria of the guidelines (test substance administered for a period of at least 90 days) were not satisfied. In addition, the tissues examined histologically for this study did not follow the list of those recommended for Guideline No. 82-1. The tables indicated in the text (study tables 9 and 10) to contain the data describing test compound intake were missing from the study report as were two pages (third page of study tables 15 and 16) summarizing clinical chemistry data for males and females. In addition, the historical laboratory control values reported for specific clinical chemistry indices (study tables 15 and 16) did not indicate (with one exception) the parameter measured. The histopathology summary data indicated "total examined"; this was assumed by the reviewers to be total tissues examined.

Even though the analyzed dietary concentrations of the process neutrals were determined to be substantially greater than the target concentration, these values were consistent throughout the study and were considered to be acceptable. Absolute and relative (to brain) adrenal weights of males and females fed high-purity RE-45601 were found to be significantly depressed (17 to 26% reduction from concurrent controls). In the absence of histological findings, the study authors considered these findings to be unrelated to dosing. Since adrenal weights and adrenal to brain weight ratios were significantly decreased from 17 to 26% in males and females, the reviewers consider this to be a compound-related finding. The reviewers agree with the study author that the high-purity RE-45601 appears to be of greater toxicity and the active ingredient was the primary toxic agent. The effects exhibited by animals fed 8000 ppm technical RE-45601 in the previous 5-week feeding study (SOCAL 2457) were comparable to those exhibited by animals fed 6800 ppm high-purity RE-45601.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-0
TASK No.: 2-450
April 26, 1990

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DATA EVALUATION RECORD

CLETHODIM

Four-Week Repeated-Dose Dermal Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: *Robert J. Weir*

Date: *April 26, 1990*

EPA No.: 68D80056
DYNAMAC No.: 245-0
TASK No.: 2-450
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DATA EVALUATION RECORD

CLETHODIM

Four-Week Repeated-Dose Dermal Toxicity Study in Rats

REVIEWED BY:

William L. Richards, Ph.D.
Principal Reviewer
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Date: April 26, 1990

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DATA EVALUATION RECORD

GUIDELINE §82-2

STUDY TYPE: Four-week repeated-dose dermal toxicity study in rats.

MRID NUMBER: 410301-09

TEST MATERIAL: FE-45601 Technical.

SYNONYM(S): Clethodim; Select; (E,E)-(+/-)-2-[1-[[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.

STUDY NUMBER(S): S-2848.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc., Richmond, CA.

TITLE OF REPORT: Four-Week Repeated-Dose Dermal Toxicity Study in Rats with RE-45601 Technical (SX-1688).

AUTHOR(S): J.H. Hedgecock.

REPORT ISSUED: December 22, 1987.

CONCLUSIONS:

Over a 4-week period, groups of six male and six female Sprague-Dawley rats were dosed with twenty-one 6-hour dermal applications of 1 mL RE-45601 Technical/kg/day at concentrations of 1%, 10%, or 100% (w/w) in 0.7% carboxymethyl cellulose (CMC) and 1% Tween 80 in distilled water; this corresponds to dose levels of 10, 100, or 1000 mg/kg/day, respectively. Controls received the vehicle alone. There were no treatment-related effects on mortality, body weight, food consumption, hematology, clinical chemistry, or gross or microscopic lesions. The severity of skin irritation at all dose levels increased through day 23 in a dose-related fashion. Incidences of skin irritation were clearly dose-related in both sexes only on day 2; thereafter, incidences in dosed animals were maximal (6/6) in both sexes (except for a 3/6 incidence in low-dose females on day 9). Compound-related anogenital discharge and staining of the anogenital fur occurred in high-dose animals of both sexes. Treatment-related depressions in food efficiency and body weight gain occurred in high-dose males, and treatment-related increases in absolute and relative (to body and brain) liver weight occurred in high-dose females. On the basis of anogenital discharge and staining of the anogenital fur in both sexes, depressions in food efficiency and body weight gain in males, and increases in absolute and relative liver weight in females dosed at 1000 mg/kg/day, the systemic repeated-dose dermal LOEL for RE-45601 Technical under the conditions of this study is 1000 mg/kg/day, and the systemic NOEL is 100 mg/kg/day. On the basis of a dose-related incidence and severity of skin irritation in males and females of the 10-, 100-, and 1,000-mg/kg/day groups, the LOEL for dermal irritation under the conditions of this study is 10 mg/kg/day; a NOEL for dermal irritation was not established.

Classification: CORE Supplementary. The study may be upgraded pending submission of information on size of application site and percent of body surface covered.

A. MATERIALS:

1. Test Compound: RE-45601 Technical; description: dark amber liquid; batch No.: SX-1688; purity: 83.2%.
2. Test Animals: Species: rat; strain: Sprague-Dawley; age: 60 days (males) and 81 days (females) at study initiation; weight: 266-305 g (males) and 211-252 g (females) at study initiation; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 17 days, identified by ear tag and color-coded cage card, and assigned randomly (assignment parameters not defined) to the following test groups:

Test Group	Dose Concentration ^a (%)	Dose Level ^b (mg/kg/day)	No. of Animals	
			Males	Females
1 Control	0	0	6	6
2 Low (LDT)	1	10	6	6
3 Mid (MDT)	10	100	6	6
4 High (HDT)	100	1000	6	6

^aw/w in 0.7% CMC and 1.0% Tween 80 in distilled water; no adjustment was made for purity.

^bApproximate dose of RE-45601 Technical.

2. Diet Preparation: RE-45601 was suspended in 0.7% carboxymethyl cellulose (CMC) and 1% Tween 80 in distilled water (w/w) at the dose concentrations shown in the above table of animal assignment. Dosing mixtures were prepared weekly on the day prior to their first use and stored in the refrigerator. Homogeneity analyses were conducted during study week 1, and concentration and stability analyses were conducted weekly.

Results: Analyses by HPLC (UV detector) of 1% and 10% dose concentrations during the first week of the study indicated that the dosing mixtures were homogeneous with coefficients of variation of 2.3% at the lower and 1.4% at the higher concentration. Analyses of the in-use 1% and 10% dose concentrations at study initiation and during week 3 indicated that actual concentrations ranged from 93.3 to 103% of target. The test material was stable under in-use conditions in that 93.3-102% and 99.9-102% of the test material was still present after 7-8 days for the 1% and 10% dose concentrations, respectively.

3. Preparation of Animal Skin: The fur on the dorsal area of the trunk was clipped 1 day prior to the start of dosing, weekly thereafter, and as needed the day prior to skin irritation scoring. Twenty-one repeated doses of test material or vehicle were administered to each animal over

a 29-day period. The vehicle or dosing suspension was applied with a micropipet at a dose volume of 1 mL/kg; the size of the test area and percent of body surface area were not indicated. Plastic film and Peg elastic bandage were then wrapped around the torso, and these were secured with porous tape. A plastic collar was placed around the neck of each animal to prevent damage to the wrap. After the first week of the study, the animals became accustomed to the wrapping procedure and collaring was discontinued. Approximately 6 hours after the application, the dressing and collars, if applicable, were removed and the application site was wiped with a gauze sponge moistened with distilled water and then a dry gauze sponge. Twice weekly, doses were adjusted to the most recent body weight.

4. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.
5. Statistics: The following procedures were utilized in analyzing the numerical data: body weights and weight gains, food consumption, food efficiency, hematology and serum chemistry values, and absolute and relative organ weights were analyzed using one-way analysis of variance. Statistically significant differences among groups were further analyzed using Dunnett's test; however, food efficiency data were analyzed by the Least Significant Difference test. The Kruskal-Wallis and Mann-Whitney U-tests were used to analyze differential white blood cell counts. Any statistically significant results for serum chemistry parameters were compared with historical control values in rats of approximately the same age in 4- to 5-week dermal studies to assist in the interpretation of the findings.
6. Quality Assurance: A quality assurance statement and a statement of compliance with FIFRA Good Laboratory Practice Standards were signed and dated December 22, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were observed once daily for signs of toxicity. A viability check was also made once daily, 6 hours before or after the animal was observed. Detailed observations for signs of toxicity were performed once weekly. The pupil response of each animal was examined on the first and last days of dosing after the test material was removed.

Skin irritation was scored 30 minutes following removal of the test material on days 0, 2, 9, 16, 23, and 28. The following modification of the method of Draize was used:

Evaluation of Skin Reactions

Erythema and eschar formation:

- 0 = No erythema
- 1 = Slight erythema (barely perceptible)
- 2 = Well-defined erythema
- 3 = Moderate erythema
- 4 = Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Edema formation:

- 0 = No edema
- 1 = Slight edema (barely perceptible)
- 2 = Well-defined edema (edges of area well-defined by definite raising)
- 3 = Moderate edema (raised approximately 1 millimeter)
- 4 = Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)

Results: No deaths occurred during the study. Incidences of anogenital discharge and staining of the anogenital fur are shown in Table 1. Anogenital discharge occurred in 6/6 high-dose males and 2/6 high-dose females over study days 7-28. Corresponding staining of the anogenital fur occurred in 3/6 high-dose males and 1/6 high-dose females. The reviewers agree with the study author that these clinical signs, which were not observed in other groups, were compound-related. Red ocular and/or red nasal discharge occurred in dosed and control animals; the study author indicated that these observations were probably the result of the collaring and/or wrapping procedures. Occasional observations of diarrhea, hyperactivity, hunched posture, and alopecia were reported to be unrelated to dosing. Pupil response appeared normal in all animals during the study.

The incidence and severity of skin irritation are shown in Tables 2 (males) and 3 (females). The severity of skin irritation on days 2, 9, 16, and 23 appeared to be dose related in both sexes; on day 28, irritation scores were elevated in control and treated groups and no dose-response relationship was evident. In high-dose animals, maximum irritation scores (8.0 is maximum possible score) were 5.7 on day 23 in males and 5.5 on day 16 in females; these animals exhibited well-defined to moderate erythema (with dry and/or flaky skin) and well-defined to moderate edema.

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TABLE 1. Incidence of Anogenital Discharge and Staining of the Anogenital Fur in Rats Treated Dermally With RE-45601 Technical for 4 Weeks^a

Parameter	Incidence							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Anogenital discharge	0/6	0/6	0/6	6/6	0/6	0/6	0/6	2/6
Stained anogenital fur	0/6	0/6	0/6	3/6	0/6	0/6	0/6	1/6

^aData extracted from tables 1 and 2 of the study report.

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TABLE 2. Incidences and Severity of Skin Irritation in Male Rats Treated Dermally With RE-45601 Technical for 4 Weeks^a

Day	Dose/Level (mg/kg/day)	Irritation	Dry and/or Flaky Skin	Scab(s)	Mean Score ^b
0	0	1/6	0/6	0/6	0.2
	10	2/6	0/6	0/6	0.3
	100	1/6	0/6	0/6	0.2
	1000	6/6	0/6	0/6	2.0
2	0	2/6	1/6	0/6	0.5
	10	3/6	0/6	0/6	0.5
	100	4/6	0/6	0/6	0.3
	1000	6/6	2/6	0/6	3.7
9	0	3/6	1/6	0/6	0.5
	10	6/6	0/6	0/6	1.7
	100	6/6	0/6	3/6	1.3
	1000	6/6	0/6	0/6	2.7
16	0	4/6	2/6	0/6	1.2
	10	6/6	1/6	0/6	2.0
	100	6/6	1/6	0/6	2.7
	1000	6/6	3/6	0/6	4.5
23	0	4/6	3/6	0/6	1.2
	10	6/6	5/6	1/6	3.0
	100	6/6	0/6	0/6	3.3
	1000	6/6	2/6	0/6	5.7
28	0	6/6	2/6	1/6	2.7
	10	6/6	4/6	0/6	4.0
	100	6/6	2/6	0/6	2.7
	1000	6/6	4/6	0/6	5.0

^aModified from study author's Table 3.^bMean of the sum of scores for erythema and edema; maximum mean score possible: 8.0.

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TABLE 3. Incidences And Severity of Skin Irritation in Female Rats Treated Dermally with RE-45501 Technical for 4 Weeks^a

Day	Dose/Level (mg/kg/day)	Irritation	Dry and/or Flaky Skin	Scab(s)	Mean Score ^b
0	0	1/6	0/6	0/6	0.2
	10	0/6	0/6	0/6	0.0
	100	2/6	0/6	0/6	0.3
	1000	6/6	0/6	0/6	1.7
2	0	2/6	0/6	0/6	0.3
	10	2/6	0/6	0/6	0.5
	100	3/6	0/6	0/6	0.8
	1000	6/6	0/6	0/6	2.7
9	0	6/6	1/6	0/6	1.3
	10	3/6	0/6	0/6	0.8
	100	6/6	0/6	0/6	1.5
	1000	6/6	1/6	0/6	3.8
16	0	6/6	3/6	0/6	2.2
	10	6/6	4/6	0/6	3.3
	100	6/6	4/6	0/6	4.3
	1000	6/6	3/6	0/6	5.5
23	0	5/6	2/6	0/6	2.0
	10	6/6	4/6	1/6	3.0
	100	6/6	5/6	1/6	4.5
	1000	6/6	2/6	0/6	5.3
28	0	6/6	3/6	0/6	3.0
	10	6/6	3/6	0/6	2.2
	100	6/6	5/6	0/6	3.8
	1000	6/6	4/6	0/6	4.7

^aModified from study author's Table 3.^bMean of the sum of scores for erythema and edema; maximum mean score possible: 8.0.

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The incidence of skin irritation appeared to be dose-related on day 2 in males and females; thereafter, incidences were either maximal (6/6) in control and dosed animals or were not dose-related. Skin irritation in the controls was reported to be the result of the wrapping procedure; on day 28 slight to severe erythema and no to well-defined edema were reported in these animals.

2. Body Weight: Rats were weighed prior to dosing, and twice weekly thereafter. At the end of the study, fasting terminal body weights were measured prior to sacrifice.

Results: Final prefasting mean body weights and weekly and total mean body weight gains of male rats are presented in Table 4. No statistically significant differences in mean body weights between control and treated animals were observed at termination or during any interval of the study (other intervals not shown). However, the final prefasting mean body weight of high-dose males was slightly (7%) but not significantly less than that of controls. Weekly body weight gain of high-dose males was less than control at each study interval, and differences were significant at days 7 ($p < 0.01$) and 14 ($p < 0.05$); total body weight gain of high-dose males (days 0-28) was also significantly less than control ($p < 0.01$). The reviewers consider these significantly lower body weight gains and the slightly lower terminal prefasting weight in males to be related to dosing. No differences in body weight gains were observed in other groups.

3. Food Consumption and Compound Intake: Food consumption was measured weekly. Water consumption was not determined.

Results: No significant differences were observed between treated and control animals in mean food consumption at any interval of the study.

Food efficiency data are presented in Table 5. The reviewers note that the significantly lower food efficiencies in high-dose males in weeks 1 and 2 ($p \leq 0.01$) correlate with the significantly lower body weight gains at these times and agree with the study author that the lower food efficiencies are compound-related. The reviewers also agree with the study author that the significant ($p < 0.01$) increases in food efficiency in all dosed female groups at week 2 were the result of a depressed control value and are not treatment-related.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

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TABLE 4. Final Prefasting Mean Body Weight and Weekly and Total Body Weight Gains in Male Rats Treated Dermalily With RE-45601 Technical for 4 Weeks^{a,b}

Dose Level	Weekly Body Weight Gain (g $\pm$ S.D.) For Week Ending:				Total Body Weight Gain (g $\pm$ S.D.) 0-28	Final (day 28) Prefasting Mean Body Weight (g $\pm$ S.D.)
	Day 7	Day 14	Day 21	Day 28		
0	23.8 $\pm$ 7.1	25.8 $\pm$ 4.4	22.7 $\pm$ 4.2	18.6 $\pm$ 6.9	91 $\pm$ 12	378 $\pm$ 20
10	20.7 $\pm$ 6.0	25.8 $\pm$ 2.3	28.6 $\pm$ 6.0	11.3 $\pm$ 5.3	86 $\pm$ 10	377 $\pm$ 14
100	18.9 $\pm$ 8.8	22.5 $\pm$ 4.9	25.4 $\pm$ 2.2	15.0 $\pm$ 3.6	82 $\pm$ 14	370 $\pm$ 25
1000	8.4 $\pm$ 5.7**	18.5 $\pm$ 4.8*	21.7 $\pm$ 7.8	10.7 $\pm$ 6.0	59 $\pm$ 16**	352 $\pm$ 18

^aModified from study author's Tables 5 and 7.

^bBased on six animals/dose.

*Significantly different from control, $p < 0.05$.

**Significantly different from control, $p < 0.01$.

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TABLE 5. Mean Food Efficiency in Rats Treated Dermally With RE-45601 Technical for 4 Weeks^a

Dose Level mg/kg/day	Mean (± S.D.) Food Efficiency (%) ^{b,c}			
	week 1	week 2	week 3	week 4
<u>Males</u>				
0	13.0±2.9	13.6±2.1	11.5±2.2	9.2±3.4
10	11.6±3.1	13.7±1.1	14.2±2.7	5.7±2.5
100	10.2±4.1	11.7±1.9	12.7±1.0	7.5±1.5
1000	4.8±3.2 ^{**}	9.7±2.5 ^{**}	10.9±3.8	5.4±2.9
<u>Females</u>				
0	2.9±2.9	-2.5±2.4 ^{**}	7.8±4.6	3.2±6.4
10	3.5±4.3	4.3±1.1 ^{**}	6.4±4.9	0.2±6.3
100	5.0±8.2	2.7±3.7 ^{**}	4.9±2.3	-0.2±1.5
1000	6.4±4.4	2.4±3.6 ^{**}	4.7±4.9	-0.3±2.5

^aModified from study author's Tables 11 and 12.^bMean food efficiency (%) = $\frac{\text{Body weight gain (grams/week)}}{\text{Food consumed (grams/week)}} \times 100$.^cBased on six rats/sex/dose.^{**}Significantly different from control, p < 0.01.

5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta of all animals at study termination for hematology and clinical chemistry analyses. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count [†]
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	X Coagulation:thromboplastin time (PT) [†]
X Reticulocyte count (RETIC)	
X Red cell morphology	

Results: No treatment-related changes in hematology parameters were found.

b. Clinical Chemistry:

<u>Electrolytes</u>	<u>Other</u>
X Calcium [†]	X Albumin [†]
X Chloride [†]	X Albumin/globulin ratio
X Magnesium	X Blood creatinine [†]
X Phosphorus [†]	X Blood urea nitrogen [†]
X Potassium [†]	X Cholesterol
X Sodium	X Globulins
	X Glucose [†]
<u>Enzymes</u>	X Total bilirubin [†]
X Alkaline phosphatase (ALP)	X Direct bilirubin
X Cholinesterase	X Total protein [†]
X Creatine phosphokinase	X Triglycerides
X Lactic acid dehydrogenase	X Uric acid
X Serum alanine aminotransferase (SGPT) [†]	
X Serum aspartate aminotransferase (SGOT) [†]	
X Gamma glutamyltransferase (GGT)	

[†]Recommended by Subdivision F (October 1982) Guidelines for 21-day repeated dose dermal toxicity studies.

Results: There were no compound-related changes in clinical chemistry parameters. The reviewers agree with the study author that the following changes were not treatment related on the basis that either they fell within the range of historical control data (except blood urea nitrogen), or that differences occurred in one sex only and there were no histopathological correlates: decreased chloride in high-dose males and females ($p < 0.05$); decreased blood urea nitrogen (BUN) in high-dose males ($p < 0.01$); or increased BUN/creatinine ratios in mid- and high-dose males ($p < 0.01$). Elevated triglyceride levels were seen in females of the mid-dose ($p < 0.05$) and high-dose ($p < 0.01$) groups; however, because the values in both the control and dosed groups were much lower than historical control data, the reviewers do not consider these changes to be treatment related.

6. **Urinalysis:** Urinalyses were not performed.
7. **Sacrifice and Pathology:** All animals were subjected to gross pathological examination at sacrifice and the CHECKED (X) tissues were collected. The brain, adrenals, ovaries or testes, spleen, and skin (treated and untreated) from animals in the control and high-dose groups and the lungs, liver, kidneys, and gross lesions from animals in all groups were examined microscopically. In addition, the (XX) organs were weighed.

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	Aorta	XX Brain
Salivary glands	Heart	Peripheral nerve
Esophagus	Bone marrow	(sciatic nerve)
Stomach	Lymph nodes	Spinal cord
Duodenum	X Spleen	(3 levels)
Jejunum	Thymus	Pituitary
Ileum		Eyes
Cecum		(optic nerve)
Colon		
Rectum	<u>Urogenital</u>	<u>Glandular</u>
XX Liver	XX Kidneys	XX Adrenals
Gallbladder	Urinary bladder	Lacrimal gland
Pancreas	XX Testes	Mammary gland
	Epididymides	Thyroids
	Prostate	Parathyroids
	Seminal vesicle	Harderian glands
<u>Respiratory</u>	XX Ovaries	
Trachea	Uterus	<u>Other</u>
X Lung		Bone (sternum and femur)
		Skeletal muscle
		X Skin (treated and untreated)
		X All gross lesions and masses

Recommended by Subdivision F (October 1982) Guidelines for 21-day repeated dose dermal toxicity studies.

Results:

- a. Organ Weights: Fasting terminal body weights and selected absolute and relative organ weights are presented in Table 6. In high-dose females, significant increases in absolute liver weight ($p < 0.05$), the liver-to-body weight ratio ($p < 0.01$), and the liver-to-brain weight ratio ($p < 0.05$) in the absence of a depression of the terminal mean body weight were considered by the study author to be compound-related.

The reviewers agree with the study author that the following changes in high-dose males were artifacts of depressed terminal body weights: increased kidney-to-body weight ratio ($p < 0.05$); and increased testes-to-body weight ratio ($p < 0.01$).

- b. Gross Pathology: There were no compound-related findings at gross necropsy.
- c. Microscopic Pathology: There were no compound-related microscopic lesions. Dose-related incidences of trace regeneration of the kidney were found (control, 0/6; low-dose, 0/6; mid-dose, 2/6; high-dose, 3/6). However, the reviewers agree with the authors of the pathology report that this component of spontaneous progressive nephropathy is expected in male rats of this strain and age.

D. STUDY AUTHOR'S CONCLUSIONS:

Groups of six young adult Sprague-Dawley rats of each sex were treated with 21 6-hour dermal applications over a 4-week period of 1 mL/kg of RE-45601 Technical at concentrations of 1%, 10%, or 100% (w/w) in 0.7% carboxymethyl cellulose (CMC) and 1% Tween 80 in distilled water, which corresponded approximately to dose levels of 10, 100, or 1,000 mg/kg/day, respectively. Six rats/sex were treated with 1 mL/kg 0.7% CMC and 1% Tween 80 in distilled water and served as controls. No mortality was observed. Compound-related anogenital discharge/stain occurred in high-dose animals. The incidence and severity of skin irritation increased through day 23 in a dose-related fashion in all groups; irritation in the high-dose animals on days 16 and 23 consisted of well-defined to moderate erythema and edema. Treatment-related depressions in food efficiency and body weight gain were observed in high-dose males. Compound-related increases were observed in liver weight, the liver/body weight ratio, and the liver/brain weight ratio in high-dose females. No significant treatment-related effects were seen on body weight, food consumption, hematology, clinical chemistry,

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TABLE 6. Fasting Terminal Body Weights and Selected Absolute and Relative Organ Weights in Rats Treated Dermally With RE-45601 Technical for 4 Weeks^{a,b}

Parameter	Males				Females			
	0	10	100	1,000	0	10	100	1,000
Terminal body weight(g)	342± 22 ^c	342± 13	337± 27	318± 17	226± 13	234± 15	226± 12	223± 16
Liver weight Absolute(g)	10.31± 0.74	9.88± 0.89	10.01± 0.85	10.22± 1.19	6.45± 0.65	6.58± 0.76	6.77± 0.61	7.77± 1.01 ^c
Relative ^c	3.01± 0.15	2.88± 0.19	2.97± 0.12	3.20± 0.23	2.95± 0.18	2.32± 0.35	3.00± 0.27	3.48± 0.28 ^{***}
Relative ^d	4.90± 0.29	4.80± 0.47	4.88± 0.28	5.00± 0.59	3.23± 0.37	3.39± 0.29	3.50± 0.29	3.99± 0.65 ^c
Kidney weight Absolute(g)	2.92± 0.16	2.84± 0.19	3.00± 0.21	2.99± 0.24	1.82± 0.19	1.86± 0.18	1.87± 0.19	1.97± 0.27
Relative ^c	0.86± 0.08	0.83± 0.06	0.89± 0.02	0.94± 0.05 ^c	0.81± 0.06	0.80± 0.06	0.83± 0.04	0.88± 0.07
Relative ^d	1.39± 0.09	1.38± 0.11	1.47± 0.08	1.46± 0.14	0.91± 0.10	0.96± 0.07	0.97± 0.09	1.01± 0.17
Testes weight Absolute(g)	3.13± 0.26	3.16± 0.24	3.20± 0.15	3.28± 0.07				
Relative ^c	0.92± 0.06	0.92± 0.06	0.95± 0.05	1.03± 0.06 ^{***}				
Relative ^d	1.49± 0.11	1.53± 0.10	1.56± 0.04	1.60± 0.05				

^aBased on six animals/dose/sex.^bData extracted from tables T-21 and T-22 of the study report.^cOrgan weight as percent of body weight.^dg organ weight/g brain weight.^cSignificantly different from control. p < 0.05.^{***}Significantly different from control. p < 0.01.

or gross or microscopic lesions. A clear no-effect level of 10% (100 mg/kg/day) was established for the systemic toxicity of RE-45601 Technical.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The following deficiencies were noted when this study was compared with general testing requirements and the specific requirements for conducting the repeated-dose dermal toxicity study (EPA Pesticide Assessment Guidelines, 1982): (1) although the guideline specifies that not less than 10 percent of the body surface area should be clipped clear for application of the test substance and that the test substance should be applied uniformly over an area equivalent to approximately 10 percent of the total body surface area, this study did not indicate the size of the treatment sites; and (2) the homogeneity and concentration of the test compound in the dermal dosing solutions was not determined before the start of the study. The study, however, exceeded the guideline as follows: (1) the preserved and examined tissues included the required tissues (normal and treated skin, liver, kidneys) as well as several not required by the guideline (lungs, spleen, brain, adrenals, gonads); (2) treatment groups consisted of six animals/sex rather than the five/sex required by the guideline; and (3) the study examined one hematology parameter (red cell morphology) and several clinical chemistry parameters not required by the guideline.

The reviewers agree with the study author that (1) compound-related anogenital discharge and staining of the anogenital fur occurred in high-dose animals of both sexes; (2) treatment-related depressions in food efficiency and body weight gain occurred in high-dose males; (3) compound-related increases in absolute and relative (to body and brain) liver weight occurred in high-dose females; and (4) no treatment-related effects were seen on mortality, body weight, food consumption, hematology, clinical chemistry, or gross or microscopic lesions.

Although the reviewers agree with the study author that the severity of skin irritation increased through day 23 in a dose-related fashion in all groups, the reviewers do not agree that the incidence of skin irritation increased in a similar fashion. The reviewers note that the incidence of skin irritation was clearly dose-related only on day 2 in both males and females; thereafter, the incidence in dosed groups was maximal (6/6) in both sexes (except for a 3/6 incidence in low-dose females on day 9).

On the basis of anogenital discharge and staining of the anogenital fur in both sexes, depressions in food efficiency and body weight gain in males, and increases in absolute and

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relative liver weight in females dosed at 1,000 mg/kg/day, the systemic repeated-dose dermal LOEL for RE-45601 Technical under the conditions of this study is 1000 mg/kg/day, and the systemic NOEL is 100 mg/kg/day. On the basis of a dose-related incidence and severity of skin irritation in males and females of the 10-, 100-, and 1000-mg/kg/day groups, the LOEL for dermal irritation under the conditions of this study is 10 mg/kg/day; a NOEL for dermal irritation was not established.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12063)

EPA No.: 68D80056
DYNAMAC No.: 245-P
TASK No.: 2-45P
April 25, 1990

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DATA EVALUATION RECORD

CLETHODIM

Four-Week Repeated Dose Dermal
Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

Roman J. Penta for

Date:

April 25, 1990

EPA No.: 68D80056
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DATA EVALUATION RECORD

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Toxicity Study in Rats

REVIEWED BY:

William L. Richards, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 25, 1990

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Independent Reviewer
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Signature: _____
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Date: 5/24/90

Mike Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: _____
Date: _____

007972

DATA EVALUATION RECORD

GUIDELINE §82-2

STUDY TYPE: Four-week repeated-dose dermal toxicity study in rats.

MRID NUMBER: 410302-01.

TEST MATERIAL: Select 2.0 EC.

SYNONYMS: Clethodim; RE-45601; (E,E)-(+/-)-2-[1-[[(3-chloro-2-propenyl)oxy] imino]propyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclo-hexen-1-one.

STUDY NUMBER: S-2862.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Richmond, CA.

TITLE OF REPORT: Four-Week Repeated Dose Dermal Toxicity Study in Rats with Select 2.0 EC (SX-1722).

AUTHOR: Cushman, J. R.

REPORT ISSUED: November 17, 1987.

CONCLUSIONS:

Groups of six male and six female Sprague-Dawley rats were dosed with twenty-one 6-hour dermal applications of Select 2.0 EC in distilled water over a 4-week period at dose levels of 0, 10, 50, or 100/200 mg/kg/day. The high-dose group received a dose level of 200 mg/kg/day for the first four applications; because of observed skin irritation in males and phonation due to skin irritation in females, the dose level of subsequent applications was reduced to 100 mg/kg/day. Controls received the vehicle alone. There were no treatment-related effects on clinical signs, body weight, body weight gain, food consumption, food efficiency, clinical chemistry, absolute or relative organ weights, or gross or microscopic lesions. Treatment-related increases in the incidence and severity of skin irritation were observed in males and females at all dose levels. Incidences of skin irritation increased with time and were dose related through day 16 in males and day 9 in females; thereafter, irritation was seen in every control and treated animal. Irritation in controls was possibly a result of application technique or animal scratching. The severity of skin irritation increased with time and was dose related through day 16 in both sexes; thereafter, no dose-related differences in the severity of skin irritation could be distinguished. Reticulocytosis in high-dose males and females may be indicative of the initial stages of chronic posthemorrhagic anemia. On the basis of the increased reticulocyte count in high-dose males and females, the 4-week repeated-dose dermal systemic LOEL is 100 mg/kg/day, and the systemic NOEL is 50 mg/kg/day. On the basis of treatment-related increases in the incidence and severity of skin irritation at all dose levels, the 4-week repeated-dose LOEL for dermal irritation is 10 mg/kg/day; a NOEL for dermal irritation was not established.

Classification: CORE Supplementary. The study may be upgraded pending submission of information on surface area exposure.

A. MATERIALS:

1. Test Compound: Select 2.0 EC; description: dark amber liquid; batch No.: SX-1722; purity: 26.3% active ingredient by HPLC analysis. Expected composition: 29.76% PE-45601 (Select; 84.0% pure).
2. Test Animals: Species: rat; strain: Sprague-Dawley; age: 58 days (males) and 65 days (females) at start of study; weight: 239-257 g (males) and 193-208 g (females) at start of study; source: Charles River Breeding Laboratory, Portage, MI.

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B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 8 days, identified by ear tag and color-coded cage card, and assigned randomly (assignment parameters not defined) to the following test groups:

Test Group	Dose Concentration ^a (w/v in distilled water)	Dose Level (mg/kg/day)	No. of Animals	
			Males	Females
1 Control	0.0%	0	6	6
2 Low (LDT)	1.0%	10	6	6
3 Mid (MDT)	5.0%	50	6	6
4 High (HDT)	10.0/20.0% ^b	100/200	6	6

^aNo adjustment was made for purity.

^bThe first 4 applications were administered at the 20.0% level; because of skin irritation during dosing, the dose level was reduced to 10.0% for the 17 remaining applications.

2. Dose Preparation: Select 2.0 EC was dissolved or suspended in distilled water (w/v) at the dose concentrations shown in the above table of animal assignment. Dosing mixtures were prepared weekly and stored at 5°C; homogeneity and stability analyses were conducted at study initiation.

Results: Analyses by HPLC (UV detector) of 5% and 50% dose concentrations prior to study initiation indicated that the dosing mixtures were homogeneous with coefficients of variation of 4.2% at the lower and 3.2% at the higher concentration; the coefficient of variation of the in-use 1% dose concentration was 7.7%. Analyses of the in-use 1%, 5%, 10%, and 20% dose concentrations indicated that actual concentrations ranged from 92 to 105% of target and that the test material was stable under in-use conditions (storage at 5°C) for 7-8 days.

3. Preparation of Animal Skin: The fur on the dorsal area of the trunk was clipped 1 day prior to the start of dosing and twice weekly thereafter. Twenty-one repeated doses of test material or vehicle were administered to each animal over a 29-day period. The vehicle or test material was applied with a micropipet at a dose volume of 1 mL/kg; the

size of the test area and percent of body surface were not indicated. Plastic film and Peg wrap were then wrapped around the torso, and these were secured ~~with tape~~. A plastic collar was placed around the neck of each animal to prevent damage to the wrap. After the first 2 weeks of the study, the animals became accustomed to the wrapping procedure and collaring was discontinued. Approximately 6 hours after the application, the dressing and collars, if applicable, were removed and the application site was wiped with a gauze sponge moistened with distilled water.

4. Food and Water Consumption: Animals received food (Furina Certified Rodent Chow #5002) and water ad libitum.
5. Statistics: The following procedures were utilized in analyzing the numerical data:

Body weights and weight gains, food consumption, food efficiency, hematology and serum chemistry values, and absolute and relative organ weights were analyzed using one-way analysis of variance. Statistically significant differences among groups were further analyzed using Dunnett's test. The Kruskal-Wallis and Mann-Whitney U-tests were used to analyze differential white blood cell counts. Any statistically significant results were compared with historical control values in rats of approximately the same age in 4- to 5-week dermal studies to assist in the interpretation of the findings.

6. Quality Assurance: A quality assurance statement was signed and dated November 13, 1987, and a statement of compliance with FIFRA Good Laboratory Practice Standards was signed and dated November 16, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were observed once daily for signs of toxicity. A viability check was also made once daily, 6 hours before or after the animal was observed. Detailed observations for signs of toxicity were performed once weekly. The pupil response of each animal was examined on the first and last days of dosing after the test material was removed.

Skin irritation was scored 30 minutes following removal of the test material using the following modification of the method of Draize:

Evaluation of Skin ReactionsErythema and eschar formation:

- 0 = No erythema
- 1 = Slight erythema (barely perceptible)
- 2 = Well-defined erythema
- 3 = Moderate erythema
- 4 = Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Edema formation:

- 0 = No edema
- 1 = Slight edema (barely perceptible)
- 2 = Well-defined edema (edges of area well-defined by definite raising)
- 3 = Moderate edema (raised approximately 1 millimeter)
- 4 = Severe edema (raised more than 1 millimeter and extending beyond the area of exposure).

Results: No deaths occurred during the study. Phonation accompanied by skin irritation was observed in the high-dose females on day 5. The phonation did not continue after the dose level was reduced on day 6. Pupil response appeared normal in all animals during the study. Clinical observations that occurred in some animals at all dose levels included red ocular and/or red nasal discharge. The study author indicated that these observations were probably the result of the collaring and/or wrapping procedures. Scabs were noted on the forepaws or head of two high-dose males and did not appear to be test material related.

The incidence and severity of skin irritation are shown in Tables 1 (males) and 2 (females). The severity of skin irritation on days 0, 2, 9, and 16 appeared to be dose related in both sexes; on days 23 and 28, irritation scores were similarly elevated in control and treated groups. The study author reported that irritation in controls was possibly the result of the wrapping procedure. High-dose males at day 9 exhibited no to severe erythema (with scabs and dry and/or flaky skin) and slight to moderate edema; at day 16, males of this dose group exhibited well-defined to severe erythema (with scabs and dry and/or flaky skin) and slight to moderate edema. High-dose females at day 9 exhibited no to slight erythema (with dry and/or flaky skin) and no to slight edema; at day 16, females of this

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TABLE 1. Incidence and Severity of Skin Irritation in Male Rats Treated Dermally With Select 2.0 $\pm$ for 4 Weeks^a

Day	Dose Level (mg/kg/day)	Irritation	Dry and/or Flaky Skin	Scab(s)	Mean Score ^b
0	0	0/6	0/6	0/6	0.0
	10	0/6	0/6	0/6	0.0
	50	2/6	0/6	0/6	0.3
	200	5/6	0/6	0/6	1.0
2	0	1/6	1/6	0/6	0.2
	10	3/6	1/6	0/6	0.7
	50	5/6	0/6	0/6	2.0
	200	~~6/6~~	6/6	0/6	~~3.8~~
9	0	3/6	2/6	0/6	0.7
	10	4/6	3/6	0/6	2.0
	50	6/6	1/6	0/6	4.2
	100 ^c	6/6	1/6	1/6	5.8
16	0	4/6	3/6	0/6	2.0
	10	6/6	3/6	0/6	2.0
	50	6/6	2/6	0/6	4.3
	100	6/6	1/6	2/6	5.0
23	0	6/6	4/6	1/6	3.8
	10	6/6	4/6	0/6	4.5
	50	6/6	1/6	0/6	3.8
	100	6/6	3/6	1/6	3.8
28	0	6/6	3/6	0/6	3.3
	10	6/6	3/6	1/6	5.7
	50	6/6	2/6	0/6	4.8
	100	6/6	2/6	1/6	5.0

^aModified from study author's Table 3.^bMean of the sum of scores for erythema and edema; maximum mean score possible: 8.0.^cAfter fourth application, high-dose level reduced to 100 mg/kg/day.

TABLE 2. Incidence and Severity of Skin Irritation in Female Rats Treated Dermally With Select 2.0 EC for 4 Weeks^a

Day	Dose Level (mg/kg/day)	Irritation	Dry and/or Flaky Skin	Scab(s)	Mean Score ^b
0	0	0/6	0/6	0/6	0.0
	10	0/6	0/6	0/6	0.0
	50	0/6	0/6	0/6	0.0
	200	1/6	0/6	0/6	0.2
2	0	2/6	3/6	0/6	0.3
	10	3/6	1/6	0/6	0.7
	50	3/6	2/6	0/6	1.2
	200	6/6	3/6	0/6	3.0
9	0	2/6	2/6	0/6	0.5
	10	4/6	2/6	0/6	1.0
	50	6/6	0/6	0/6	2.5
	100 ^c	6/6	2/6	0/6	3.0
16	0	6/6	3/6	0/6	1.7
	10	6/6	3/6	0/6	2.5
	50	6/6	1/6	0/6	2.8
	100	6/6	2/6	0/6	4.8
23	0	6/6	5/6	0/6	3.5
	10	6/6	4/6	0/6	3.7
	50	6/6	1/6	0/6	2.2
	100	6/6	3/6	0/6	3.7
28	0	6/6	4/6	0/6	3.0
	10	6/6	3/6	0/6	2.8
	50	6/6	1/6	0/6	3.0
	100	6/6	1/6	0/6	3.7

^aModified from study author's Table 4.^bMean of the sum of scores for erythema and edema; maximum mean score possible: 8.0^cAfter fourth application, high-dose level reduced to 100 mg/kg/day.

dose group exhibited no to well-defined erythema (with dry and/or flaky skin) and slight to well-defined edema. The incidence of skin irritation appeared to be dose-related on days 0, 2, 9, and 16 in males and days 0, 2, and 9 in females; thereafter, incidences were maximal (6/6) in control and dosed animals.

2. Body Weight: Rats were weighed prior to dosing at study initiation and twice weekly thereafter. At the end of the study, fasting terminal body weights were measured prior to sacrifice.

Results: No significant differences in mean body weights between control and treated animals were observed at any interval of the study.

3. Food and Water Consumption and Food Efficiency: Food consumption was measured weekly. Water consumption was not determined.

Results: No significant differences were observed between treated and control animals in mean food consumption or mean food efficiency at any interval of the study.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.
5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta of all animals at study termination for hematology and clinical chemistry analyses. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count [†]
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X [✓] Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	Coagulation: thromboplastin
X Reticulocyte count (RETIC)	time (PT) [†]
X Red cell morphology	

Recommended for 21-day repeated-dose dermal toxicity studies by Subdivision F (October 1982) Guidelines.

Results: Representative hematology data are presented in Table 3. Mean reticulocyte counts were significantly elevated ($p < 0.01$) beyond historical control ranges for the laboratory in both males and females of the high-dose group. The study author indicated that the significance of this finding is unclear, since no changes in other erythrocyte parameters or in the platelet count were observed. Mean eosinophil counts were significantly decreased in females at the low- ($p < 0.05$), mid- ($p < 0.02$), and high-dose ($p < 0.02$) levels. The study author indicated that since these values fell within the range of historical control data and the differences in eosinophil counts were small, they were not considered compound related.

b. Clinical Chemistry

<u>Electrolytes</u>		<u>Other</u>	
X	Calcium [†]	X	Albumin [†]
X	Chloride [†]	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine [†]
X	Phosphorus [†]	X	Blood urea nitrogen [†]
X	Potassium [†]	X	Cholesterol
X	Sodium [†]	X	Globulins
		X	Glucose [†]
		X	Total bilirubin [†]
X	Alkaline phosphatase (ALP)	X	Direct bilirubin
	Cholinesterase	X	Total protein [†]
X	Creatine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase	X	Uric acid
X	Serum alanine aminotransferase (SGPT) [†]		
X	Serum aspartate aminotransferase (SGOT) [†]		
	Gamma glutamyltransferase (GGT)		

Results: There were no compound-related changes in serum chemistry. The reviewers agree with the study author that the following changes were not treatment related on the basis that they fell within the range of historical control data (except blood urea nitrogen), were not consistent between sexes, and had no histologic correlates: decreased total bilirubin in mid-dose males ($p < 0.01$) and decreased indirect bilirubin in mid-dose ($p < 0.01$) and high-dose ($p < 0.05$) males; significant increases in calcium in females of the high-dose ($p < 0.01$) and mid- and low-dose groups ($p < 0.05$); decreased blood urea nitrogen in high-dose females ($p < 0.01$); and a decreased albumin/globulin ratio in high-dose females ($p < 0.01$).

Recommended for 21-day repeated-dose dermal toxicity studies by Subdivision F (October 1982) Guidelines.

TABLE 3. Representative Mean Hematology Values ($\pm$ S.D.) in Rats Treated Dermally With Select 2.0 EC for 4 Weeks^{a,b}

Parameter ^c	Dose (mg/kg/day)			
	0	10	50	100/200 ^d
Males				
Reticulocytes (%)	1.8 $\pm$ 0.3	2.4 $\pm$ 0.4	2.0 $\pm$ 0.8	4.1 $\pm$ 0.9**
RBC (10E6/mm ³)	7.30 $\pm$ 0.23	7.49 $\pm$ 0.15	7.51 $\pm$ 0.14	7.42 $\pm$ 0.45
HGB (g/dL)	14.4 $\pm$ 0.5	14.7 $\pm$ 0.4	15.0 $\pm$ 0.4	14.8 $\pm$ 0.9
HCT (%)	40.2 $\pm$ 1.1	41.0 $\pm$ 1.0	41.6 $\pm$ 1.0	41.1 $\pm$ 2.8
MCH (pg)	19.7 $\pm$ 0.9	19.7 $\pm$ 0.5	20.0 $\pm$ 0.5	20.0 $\pm$ 0.3
MCHC (%)	35.7 $\pm$ 0.3	35.9 $\pm$ 0.3	36.1 $\pm$ 0.3	36.1 $\pm$ 0.2
PLT (10E3/mm ³)	987 $\pm$ 178	927 $\pm$ 80	812 $\pm$ 86	811 $\pm$ 145
EOS (%)	0 $\pm$ 1	0 $\pm$ 0	1 $\pm$ 1	1 $\pm$ 1
Females				
Reticulocytes (%)	2.2 $\pm$ 0.6	2.3 $\pm$ 0.8	3.2 $\pm$ 0.9	4.1 $\pm$ 1.1**
RBC (10E6/mm ³)	7.11 $\pm$ 0.33	7.31 $\pm$ 0.21	7.22 $\pm$ 0.34	7.21 $\pm$ 0.17
HGB (g/dL)	14.2 $\pm$ 0.5	14.7 $\pm$ 0.5	14.2 $\pm$ 0.6	14.3 $\pm$ 0.4
HCT (%)	39.7 $\pm$ 1.4	40.6 $\pm$ 1.5	39.6 $\pm$ 1.6	40.0 $\pm$ 1.6
MCH (pg)	20.0 $\pm$ 0.6	20.0 $\pm$ 0.5	19.7 $\pm$ 0.2	19.8 $\pm$ 0.4
MCHC (%)	35.8 $\pm$ 0.2	36.1 $\pm$ 0.3	35.8 $\pm$ 0.4	35.8 $\pm$ 0.4
PLT (10E3/mm ³)	802 $\pm$ 115	822 $\pm$ 160	927 $\pm$ 173	874 $\pm$ 173
EOS (%)	3 $\pm$ 1	1 $\pm$ 1*	0 $\pm$ 0**	1 $\pm$ 1**

^aBased upon six rats/sex/dose.^bData extracted from tables 11 to 14 of the study report.^cRBC, erythrocytes; HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; EOS, eosinophils.^dFirst four applications administered at 200 mg/kg/day.^eHistorical control values for males:

Reticulocytes: Mean = 2.2
 Range of means = 1.0-2.9
 No. of males = 89
 No. of studies = 9

^fHistorical control values for females:

Reticulocytes: Mean = 2.4
 Range of means = 1.4-4.0
 No. of females = 89
 No. of studies = 9

^gHistorical control values for females:

Eosinophils: Mean = 0.9
 Range of means = 0-2
 No. of females = 120
 No. of studies = 12

*Significantly different from control ($p < 0.05$).**Significantly different from control ($p < 0.01$), except for eosinophils in females ($p < 0.02$).

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals were subjected to gross pathological examination at sacrifice, and the CHECKED (X) tissues were collected. The brain, adrenals, ovaries or testes, and spleen from animals in the control and high-dose groups and the lungs, liver, kidneys, and gross lesions from animals in all groups were examined microscopically. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	Aorta	XX Brain
Salivary glands	Heart	Peripheral nerve
Esophagus	Bone marrow	(sciatic nerve)
Stomach	Lymph nodes	Spinal cord
Duodenum	X Spleen	(3 levels)
Jejunum	Thymus	Pituitary
Ileum		Eyes
Cecum		(optic nerve)
Colon		
Rectum	<u>Urogenital</u>	<u>Glandular</u>
XX Liver†	XX Kidneys†	XX Adrenals
Gallbladder	Urinary bladder	Lacrimal gland
Pancreas	XX Testes	Mammary gland
	Epididymides	Thyroids
	Prostate	Parathyroids
	Seminal vesicle	Harderian glands
<u>Respiratory</u>	XX Ovaries	
Trachea	Uterus	
XX Lung		<u>Other</u>
		Bone (sternum
		and femur)
		Skeletal muscle
		X Skin (treated
		and untreated)†
		X All gross
		lesions
		and masses†

Recommended for 21-day repeated-dose dermal toxicity studies by
Subdivision F (October 1982) Guidelines.

Results:

- a. Organ Weights: No statistically significant differences were seen in the absolute or relative (to body or brain) weights of the liver, kidneys, testes, ovaries, brain, or adrenals of treated rats compared with controls.
- b. Gross Pathology: There were no compound-related findings at gross necropsy.
- c. Microscopic Pathology: There were no compound-related microscopic lesions. Trace lymphocytic infiltration of the lungs was seen in animals of all groups. The pathology report indicated that the alveolar hemorrhage seen in the lungs of control and dosed animals and the tubular degeneration of the kidneys seen in mid-dose males were related to the method of sacrifice (exsanguination under pentobarbital anesthesia). Acanthosis was present in the treated skin of control and dosed groups but was not seen in untreated skin; this finding was considered to be a result of application technique.

D. STUDY AUTHOR'S CONCLUSIONS:

Over a 4-week period, groups of six young adult Sprague-Dawley rats of each sex were treated with twenty-one 6-hour dermal applications of 1 mL Select 2.0 EC/kg at concentrations of 1, 5, or 10% (w/v) in distilled water, which correspond to dose levels of 10, 50, or 100 mg/kg/day, respectively. The high-dose group was initially dosed with a concentration of 20% Select 2.0 EC; after four applications, the dose level was decreased to 10% due to observed skin irritation. Six rats/sex were treated with 1 mL/kg distilled water and served as controls. Irritation due to skin irritation occurred in some high-dose females on day 5, but was not observed after the dose level was reduced. The incidence and severity of skin irritation increased through day 16 in a dose-related fashion in all groups; irritation in the high-dose group on day 16 consisted of slight to severe erythema and slight to moderate edema. No dose-related differences in skin irritation could be distinguished at days 23 and 28. Mean reticulocyte values for both males and females were significantly elevated in the high-dose group and were possibly compound-related; however, the significance of this effect is unclear since no compound-related differences in other hematology parameters and no corresponding pathological findings were observed. No treatment-related effects were seen on mortality, clinical signs, body weight, body weight gain, food consumption, food efficiency, clinical chemistry, absolute or relative organ

weights, or gross or microscopic lesions. A clear no-effect level of 5% (50 mg/kg/day) was established for the systemic toxicity of Select 2.0 EC.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The following deficiency was noted when this study was compared with requirements for conducting the repeated-dose dermal toxicity study (EPA Pesticide Assessment Guidelines, 1982): Although the guideline specifies that not less than 10% of the body surface area should be clipped clear for the application of the test substance and that the test substance should be applied uniformly over an area that is approximately 10% of the total body surface area, this study did not indicate the size of the treatment sites. The study can be upgraded from supplementary to minimum pending submission of this information on surface area exposure. The study, however, exceeded the guideline as follows: (1) the preserved and examined tissues included the ~~required~~ tissues (normal and treated skin, liver, kidneys) as well as several not required by the guideline (lungs, spleen, brain, adrenals, gonads); (2) treatment groups consisted of six animals/sex rather than the five/sex required by the guideline; (3) the study examined one hematology parameter (red cell morphology) and several clinical chemistry parameters not required by the guideline; and (4) dosing was for 28 days rather than the required 21 days.

The reviewers agree with the study author that no treatment-related effects were seen on mortality, clinical signs, body weight, ~~body~~ weight gain, food consumption, food efficiency, clinical chemistry, absolute or relative organ weights, or gross or microscopic lesions. The increased incidence of skin irritation in control animals on study days 23 and 28 was possibly a result of application technique (application and spreading of the test material with a micropipet, wrapping procedures) or animal scratching.

The reviewers also agree with the study author that the severity of skin irritation increased through day 16 in a dose-related fashion in all groups. However, whereas the study author concluded that the incidence of skin irritation also increased through day 16 in a dose-related fashion in all groups, the reviewers note that the incidence was dose-related in females only through day 9.

The study author stated that the significance of the increased reticulocyte count in high-dose rats of both sexes is unclear, since no compound-related differences in other hematology parameters and no corresponding pathological findings were observed. The reviewers, however, suggest that this reticulocytosis in high-dose animals may be indicative of the initial

stages of chronic posthemorrhagic anemia. Wintrobe (1974, as cited in Nelson 1979) reported that if blood is lost in small amounts over an extended period of time, the reticulocyte count may be slightly increased in the absence of the clinical and hematologic features that characterize acute hemorrhagic anemia, and that significant anemia does not usually develop until after storage iron is depleted.

On the basis of the reticulocytosis in males and females of the 100-mg/kg/day group, the reviewers assess that, under the conditions of this study, the systemic LOEL is 100 mg/kg/day, and the systemic NOEL is 50 mg/kg/day. On the basis of dose-related increases in the incidence and severity of skin irritation in males and females of the 10-, 50-, and 100-mg/kg/day groups, the reviewers assess that, under the conditions of this study, the LOEL for dermal irritation is 10 mg/kg/day; a NOEL for dermal irritation was not established.

REFERENCES:

Nelson DA. 1979. Erythrocytic disorders. In: Henry JB (ed.), Todd-Sanford-Davidsohn Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: W.B. Saunders Company, pp. 964-1035.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-Q
TASK No.: 2-45Q
April 19, 1990

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DATA EVALUATION RECORD

CLETHODIM

Metabolism in Rats

STUDY IDENTIFICATION: Rose, A. F., and Griffis, L. C. The in vivo metabolism of [propyl-1-¹⁴C]clethodim in rats. (Unpublished study No. CEHC 2515 performed by Chevron Chemical Company, Richmond, CA; dated December 22, 1988.) MRID No. 410301-32.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: *Robert J. Weir*

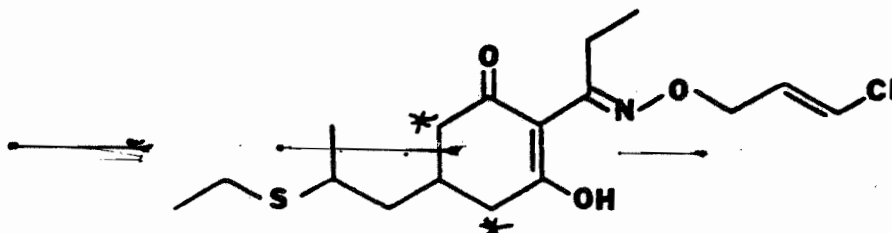
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1. **CHEMICAL:** Clethodim; SELECT 2EC; 2-[1-[[[E-3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one. (Note: nomenclature corrected per CBI p. 52.)

2. **TEST MATERIAL:** Based on the structure provided by the study authors, the correct chemical name for the radiolabeled material is [cyclohexenone-4,6-¹⁴C]clethodim. (The study authors reported that animals were dosed with [propyl-1-¹⁴C]clethodim.) The [¹⁴C]-labeled material (stock No. 145, radiochemical purity >90 percent, specific activity 56 Ci/mM) was diluted with analytically pure (99 percent) clethodim (lot No. RE-45601-31) for the low-, high-, and repeated-dose groups. Analytically pure (99 percent) clethodim (lot No. RE-45601-43) was used to dilute [¹⁴C]clethodim (as above) to formulate the supplemental dose. All dosing solutions were prepared using TWEEN-80 and carboxymethyl cellulose (CMC). The structure and radiolabel positions (*) for [¹⁴C]clethodim are (as provided by the study authors) shown below:



3. **STUDY/ACTION TYPE:** Metabolism in rats.
4. **STUDY IDENTIFICATION:** Rose, A. F., and Griffis, L. C. The in vivo metabolism of [propyl-1-¹⁴C]clethodim in rats. (Unpublished study No. CEHC 2515 performed by Chevron Chemical Company, Richmond, CA; dated December 22, 1988.) MRID No. 410301-32.

5. **REVIEWED BY:**

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Date: 4/13/90

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(H-7509C)

Signature: *James N. Rowe*
Date: *5/24/90*

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7. CONCLUSIONS:

- A. Groups of five male and five female Sprague-Dawley rats were given (by gastric intubation) a single oral dose of 4.5 or 450 mg [^{14}C]clethodim/kg (low- and high-dose groups, respectively). An additional group of rats (10/sex) received single daily oral doses of unlabeled clethodim (4.5 mg/kg/day) for 14 days; five animals/sex chosen randomly from this group were then administered a single oral dose of 4.5 mg [^{14}C]clethodim/kg (repeated-dose group). Another group, consisting of 10 male rats, was given a single oral dose of 450 mg [^{14}C]clethodim/kg (supplemental-dose group); urine from these animals was used for the identification of metabolites.

Clethodim was readily absorbed and eliminated by all animals. Nearly all (87 to 92 percent) of each [^{14}C] dose was recovered from the urine within 7 days after compound administration. Smaller amounts were extracted from the feces (9 to 17 percent) and expired air (≤ 1.0 percent as [^{14}C]CO₂). The low- and repeated-dose rats excreted clethodim at a slightly faster rate (98 percent in 40 hours) than did high-dose rats (98 percent in 50 hours). Gastrointestinal absorption of clethodim was estimated to be between 89 and 96 percent; no sex-, dose-, or treatment-related differences were apparent.

At 7 days postdosing, all organs and tissues combined contained <1.0 percent of the [^{14}C] administered to each group. The adrenals contained the highest concentrations of radioactivity (0.07 to 0.22 ppm for low- and repeated-dose rats; 5.4 to 13 ppm for high-dose rats). Low levels of radioactivity in the tissues of repeated-dose rats indicated that clethodim does not bioaccumulate appreciably following multiple exposures to low levels of the compound.

Clethodim was extensively metabolized by rats, with <1 percent of the original dose eliminated as the unchanged parent compound. Eleven compounds, including clethodim, were identified in the excreta; between 5 and 9 percent of each dose was not identified. Urinary and fecal metabolite profiles were similar for all animals. The predominant metabolite recovered was clethodim sulfoxide, which accounted for 48 to 63 percent of the administered [^{14}C] (46 to 61 percent in the urine) in excreta collected for up to 48 hours after dosing. Recovery of other metabolites indicated that in addition to oxidation to clethodim sulfoxide (and, subsequently, to clethodim sulfone), clethodim can (1) be converted to S-methyl sulfoxide and sulfone, (2) undergo N-O bond cleavage to form imine sulfoxide and sulfone, or (3) be hydroxylated at the C5 position to form the 5-OH sulfoxide and sulfone.

- B. This study is acceptable and was conducted adequately according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation; Human and Domestic Animals, 1984, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, pp. 152-156).

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS:

A. Materials and Methods:

1. [^{14}C]Clethodim (stock No. 145, specific activity 56 Ci/M) was purified just before use by preparative silica gel thin-layer chromatography (TLC). The compound's radiochemical purity, as determined by TLC autoradiography and liquid scintillation counting (LSC), was >96 percent. Unlabeled clethodim (lot Nos. RE-45601-31 and RE-45601-43) was reported to be 99 percent pure; no other details were provided.
2. Male and female Sprague-Dawley Crl:CD(SD)BR rats purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA) were used. Animals were quarantined for 14 days before being dosed with clethodim.
3. Purified [^{14}C]clethodim was diluted with unlabeled clethodim to give a specific activity of 1.974×10^{11} , 2.22×10^8 , 2.056×10^{10} , or 2.51×10^8 dpm/g for the low, high, repeated, and supplemental doses, respectively. Diluted [^{14}C]clethodim was suspended in a mixture of TWEEN-80, CMC, and deionized water to give the following final concentrations: 440; 46,390; 453; and 126,700 μg [^{14}C] equivalents/g suspension, respectively. These values corresponded to doses of 4.4, 468, 4.8, and 450 (nominal concentration) mg clethodim/kg. The unlabeled suspension used in the repeated-dose study was prepared once each week by adding analytically pure clethodim and then CMC to a TWEEN-80/deionized water solution to give a final concentration equivalent to a nominal dose of 4.5 mg/kg/day. Radiolabeled suspensions for the low-, high-, and repeated-dose groups were prepared the day before dosing and refrigerated until used. The supplemental-dose suspension was prepared the day of dosing.

Only the items appropriate to this DER have been included.

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All radioactive suspensions were checked for homogeneity via LSC; standard deviations of 5.4, 2.9, and 7.7 percent for the low, high, and repeated doses, respectively, indicated that dosing suspensions were homogeneous. The radiochemical purity of [^{14}C]clethodim, as determined by TLC, was 98.2, 98.3, 96.6, and 96.9 percent for the low, high, repeated, and supplemental doses, respectively. The unlabeled suspension was found to be homogeneous (coefficients of variation: 6.3, 2.9, and 1.8 percent at 0, 5, and 9 days after preparation, respectively) and somewhat unstable (90 and 85 percent of the original calculated concentration of clethodim remained after 5 and 9 days, respectively).

It was noted that in solution, clethodim is a tautomeric equilibrium mixture of syn- and anti-oxime isomers. (The isomer ratio is influenced by pH, moisture content, and solvent polarity.) The anti-isomer was the predominant form seen on autoradiograms of the [^{14}C]clethodim used to prepare the various dosing solutions. It was also reported that the sulfur in clethodim is susceptible to autoxidation by atmospheric oxygen. No attempt was made to exclude oxygen from the TLC plates or developing tank. A small amount of material, most likely clethodim sulfoxide, remained at the origin site on the autoradiograms.

Two oral dosing studies were conducted (low and high dose; repeated dose), and an additional group of animals was given a supplemental dose approximately equivalent to the high dose. An intravenous study was not conducted owing to clethodim's poor solubility in water and saline. Animals were randomly assigned to one of the treatment groups described below.

In the first study, groups of five male and five female rats were given, by gastric intubation, a single oral dose of either 4.5 or 450 mg [^{14}C]clethodim/kg (actual calculated doses 4.4 and 468 mg [^{14}C]clethodim/kg, respectively). Males were 56 to 57 days old and females were 70 to 71 days at the time of dosing; body weights at dosing were 222 to 241 g and 186 to 222 g for males and females, respectively.

For the repeated-dose study, rats (10/sex) received single daily oral doses of nonlabeled clethodim at a nominal level of 4.5 mg/kg for 14 days. At 24 hours after the final dose of unlabeled clethodim was administered, five animals/sex were randomly selected to receive a single oral dose of 4.5 mg [^{14}C]clethodim/kg (actual calculated dose 4.8 mg/kg). Males and

females were approximately 57 and 71 days old, respectively, when they were given the radiolabeled test material; corresponding body weight ranges were 272 to 287 g and 189 to 199 g.

An additional group of 10 male rats was given a single oral dose of 450 mg [^{14}C]clethodim/kg (nominal dose). These animals were approximately ~~62 days~~ old at the time of dosing; 12- to 24-hour urine samples from these animals were used in the isolation and identification of metabolites.

For all groups, the administered doses were calculated by weighing the dosing syringe before and after dosing. All animals were placed in individual metabolism cages immediately after dosing with [^{14}C]clethodim. Urine and feces were collected separately on ice at 6, 12, 24, 36, 48, 72, and 168 hours postdosing from low-, high-, and repeated-dose animals for [^{14}C] balance studies. Excreta collected between 0 and 48 hours from repeated- and high-dose rats were used for identifying metabolites, as was the 12- to 24-hour urine fraction from the supplemental-dose males. At the end of the collection period, each cage was washed with methanol. Expired CO_2 was collected at 6, 12, 24, and 36 hours after dosing for all groups; an additional collection at 48 hours was included for the low- and repeated-dose groups. Body weights and food consumption were determined, and animals were observed for signs of toxicity. For the low-, high-, and repeated-dose groups, animals were sacrificed 7 days after being dosed with radiolabeled clethodim, and a general gross necropsy was performed. The following were examined, weighed, combusted, and then counted (by LSC): whole blood, brain (with brain stem), heart, liver, kidneys, lungs, gonads/ovaries, uterus, spinal cord (entire length), fat (peritoneal), spleen, bone (sternum), muscle, adrenals, salivary glands, and the remaining carcass. No tissues were analyzed from the supplemental-dose group.

5. Large organ and large tissue samples were homogenized individually, and small organ and small tissue samples were minced prior to combustion and radioassay. Expired CO_2 was trapped in NaOH before LSC; cage washes were counted directly. Appropriate measures were taken to determine counting efficiencies for each sample and to minimize color quenching.

6. Clethodim metabolites in methanol, dichloromethane, and diethyl ether:ethanol extracts of urine were

determined by TLC with reference standards in two solvent systems (chloroform:2-propanol:glacial acetic acid, 250:50:1, v/v/v; and n-hexane:acetone:glacial acetic acid, 50:50:1, v/v/v). Urinary metabolites were further identified by chemical ionization and electron impact mass spectrometry (MS), high-performance liquid chromatography (HPLC) with coinjection of authentic standards, and/or HPLC-MS.

Clethodim metabolites in acetone and methanol extracts of feces were isolated and identified by TLC cochromatography and HPLC and HPLC-MS with coinjection of reference standards. TLC plates were developed and viewed under short wavelength ultraviolet light; radioactive spots were scraped off the plates and radioassayed by LSC. Samples from males and females were analyzed separately; for metabolite identification, urine from the 6- to 48-hour collection periods was pooled, as were feces from the 0- to 48-hour collection periods.

Liver and blood from high-dose rats were also analyzed for clethodim metabolites. Samples pooled by sex were extracted with chloroform and methanol and then methanol; these extracts were combined and partitioned between acetonitrile and hexane before TLC and LSC. Samples were also treated with HCl or methanol and HCl, extracted with ethyl acetate, and quantified for radiocarbon.

- B. Protocol: A protocol for this study is presented in Appendix A.

12. REPORTED RESULTS:

- A. A few high-dose rats (number and sex not reported) exhibited salivation, decreased motor activity, and collapse within 2 hours after dosing. These animals recovered quickly, however, and no other signs of toxicity were observed in these or any other rats throughout the study. Weight gain was normal for all animals.
- B. Mean total recovery of administered radioactivity for all groups was between 103 and 110 percent at 7 days after administration of [^{14}C] clethodim (Tables 1 and 2). Most radioactivity (87 to 93 percent) was excreted in the urine during this time; the feces contained between 9 and 17 percent of the administered [^{14}C]. Approximately 1 percent of each dose was recovered as [^{14}C]CO₂ within 36 to 48 hours. No statistically significant (by the t-test)

TABLE 1. Mean Percent Recoveries of Radioactivity in Rats 7 Days after Administration of a Single Oral Dose of 4.5 or 450 mg [^{14}C]Clethodim/kg

Fraction	Percent of [^{14}C] administered to rats dosed at:			
	4.5 mg/kg		450 mg/kg	
	Males	Females	Males	Females
Urine	93.2 ^a	91.3	87.9	93.0
Feces	15.3	11.3	13.1	9.3
CO ₂ ^b	1.0	0.9	0.7	0.7
Carcass	0.3	0.2	0.7	0.7
Blood	<0.1	<0.1	<0.1	<0.1
Kidney	<0.1	<0.1	<0.1	<0.1
Liver	<0.1	<0.1	<0.1	<0.1
Other tissues	<0.1	<0.1	<0.1	<0.1
Cage wash	0.2	0.2	0.6	0.4
Total	110.1	104.0	103.1	104.1

^aEach value represents the mean of five animals.

^b43-hour (low dose) or 36-hour (high dose) collection period.

Source: CBI Tables VII and IX, CBI pp. 60 and 62.

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TABLE 2. Mean Percent Recoveries of Radioactivity in Rats Following Repeated Oral Dosing with Clethodim^a

Fraction	Percent of administered [¹⁴ C] dose	
	Males	Females
Urine	90.7 ^b	87.2
Feces	15.3	17.0
CO ₂ ^c	0.6	0.5
Carcass	0.2	0.2
Blood	<0.1	<0.1
Kidney	<0.1	<0.1
Liver	<0.1	<0.1
Other tissues	<0.1	<0.1
Cage wash	0.1	0.2
Total	106.9	105.2

^aAnimals were treated orally with 4.5 mg unlabeled clethodim/kg/day for 14 days and then were given a single oral dose of 4.5 mg [¹⁴C]clethodim/kg. Values are for 7 days after administration of radioactive material.

^bEach value represents the mean of five animals.

^c48-Hour collection period.

Source: CBI Table VIII, CBI p. 61.

treatment-, dose-, or sex-related differences were observed in the excretion of radioactivity. Urinary and fecal elimination of [^{14}C] was lower for high-dose rats (56.3 to 64.2 percent) than for low- and repeated-dose rats (92.4 to 97.1 percent) during the first 24 hours after dosing (Table 3). However, excretion of [^{14}C] was similar for all groups within 48 hours after administration of the radiolabeled test material. At 7 days postdosing, animal carcasses contained 0.2 to 0.7 percent of the administered [^{14}C]; blood, liver, kidney, and other tissues combined each contained <0.1 percent; and cage washes contained no more than 0.6 percent (Tables 1 and 2).

On the basis of urinary, tissue, and cage-wash levels of [^{14}C], gastrointestinal absorption of clethodim at 7 days postdosing was estimated to be approximately 95, 90, and 92 percent for low-, high-, and repeated-dose males, respectively, and 93, 96, and 89 percent for the corresponding female groups.

- C. Tissue levels of [^{14}C] were low for all groups (Tables 4 and 5). Concentrations were less than 0.08 ppm for all tissues of low- and repeated-dose animals, except the adrenals of repeated-dose females and males, which had residual [^{14}C] levels of 0.221 and 0.104 ppm, respectively. Tissue [^{14}C] concentrations of high-dose rats were proportionately higher than those of low- and repeated-dose rats and were between 0.4 and 5.4 ppm in males and 0.5 and 13.0 ppm in females; the highest levels in both sexes were found in the adrenals.
- D. A total of 11 compounds (including clethodim) plus several unidentified compounds were recovered from the pooled urine and pooled feces (acetone extracts) of high- and repeated-dose animals within 48 hours after dosing (Table 6; Table 7 provides reference standards). Metabolite profiles were similar for both dosing regimes and for both males and females. Most of the administered [^{14}C] (46 to 61 percent) was recovered from the urine as clethodim sulfoxide; feces contained 2 to 5 percent. Approximately 6 to 11 percent and 0.4 to 3 percent were identified in the urine and feces, respectively, as either S-methyl sulfoxide or imine sulfoxide; smaller amounts (2 to 5 percent in the urine and <1 percent in the feces) were recovered as 5-OH sulfoxide or oxazole sulfoxide. Trace amounts (<1 percent) of the following also were identified: trione sulfoxide, 5-OH sulfone, clethodim sulfone, aromatic sulfone, and S-methyl sulfone. Unchanged parent compound was recovered from the urine of high-dose females and the feces of all other animals and accounted for <1 percent of the administered [^{14}C] in each of these fractions. Approximately 5 to 9 percent of the [^{14}C] dose was not identified but was

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TABLE 3. Cumulative Excretion of Radioactivity in the Urine and Feces of Rats Given Single or Repeated Oral Doses of Clethodim^a

Experimental Group	Mean Percent Recovery of Administered [¹⁴ C] Dose in:					
	Urine			Feces		
	24 hr ^b	48 hr	168 hr	24 hr	48 hr	168 hr
Low-dose females	83.9 ^c	89.9	91.2	8.5	10.7	11.3
Low-dose males	86.2	91.9	93.2	10.9	14.5	15.3
High-dose females	53.1	88.4	93.1	3.2	7.7	9.4
High-dose males	59.3	83.6	87.9	4.9	10.8	13.1
Repeated-dose females	79.8	85.7	87.2	13.9	16.3	17.1
Repeated-dose males	82.9	88.8	90.7	11.9	15.2	16.0

^aAnimals were given a single oral dose of either 4.5 or 450 mg [¹⁴C]clethodim/kg (low- and high-dose groups, respectively) or a single oral dose of 4.5 mg [¹⁴C]clethodim/kg after being dosed orally with unlabeled clethodim (4.5 mg/kg/day) for 14 days (repeated-dose groups).

^bTime after administration of radioactive test material.

^cEach value represents the cumulative mean of five animals, except for urine from low- and high-dose males, which includes the mean of only four animals for the 24- to 36-hour collection period.

Source: CBI Tables XIII, XIV, XV, XVI, XVII, and XVIII, CBI pp. 66-71.

TABLE 4. Distribution of Radioactivity in Tissues of Rats 7 Days after Administration of a Single Oral Dose of 4.5 or 450 mg [^{14}C]Clethodim/kg

Organ/ Tissue	^{14}C Clethodim equivalents (ppm) for rats dosed at:			
	4.5 mg/kg		450 mg/kg	
	Males	Females	Males	Females
Adrenals	0.068 ^a	0.079	5.36	13.0
Bone (sternum)	0.008	<0.009 ^b	1.07	<1.56 ^b
Brain plus stem	<0.005 ^b	<0.005 ^b	0.474	<0.459 ^b
Peritoneal fat	<0.009 ^b	<0.007 ^b	0.712	0.775
Gonads/ovaries	0.003	<0.023 ^b	0.441	<2.43 ^b
Heart	<0.008 ^b	<0.010 ^b	1.27	1.16
Kidney	0.030	0.021	2.56	2.26
Liver	0.063	0.057	5.19	3.82
Lung	<0.008 ^b	0.009	1.56	1.45
Muscle	<0.005 ^b	<0.006 ^b	1.17	0.852
Salivary glands	<0.008 ^b	<0.010 ^b	0.834	<0.888 ^b
Spinal cord	0.012	<0.011 ^b	1.42	1.06
Spleen	<0.008 ^b	<0.010 ^b	1.52	2.84
Uterus	.. ^c	<0.010 ^b	.. ^c	1.13

Each value represents the mean of five animals.

.. = minimum quantifiable limit.

Not applicable.

Source: CBI Tables X and XII, CBI pp. 63 and 65.

TABLE 5. Distribution of Radioactivity in Tissues of Rats
Following Repeated Oral Dosing with Clethodim^a

Organ/ Tissue	<u>[¹⁴C]Clethodim equivalents (ppm)</u>	
	Males	Females
Adrenals	0.104 ^b	0.221
Bone (sternum)	0.008	0.009
Brain plus stem	<0.003 ^c	<0.005 ^d
Peritoneal fat	0.006	<0.010 ^d
Gonads/ovaries	0.004	<0.021 ^d
Heart	0.009	<0.010 ^d
Kidney	0.036	0.020
Liver	0.058	0.048
Lung	0.012	0.010
Muscle	<0.005 ^d	<0.005 ^d
Salivary glands	0.008	<0.007 ^d
Spinal cord	<0.010 ^d	<0.011 ^d
Spleen	0.017	<0.009 ^d
Uterus	-- ^e	<0.009 ^d

^aAnimals were treated orally with 4.5 mg unlabeled clethodim/kg/day for 14 days and then were given a single oral dose of 4.5 mg [¹⁴C]clethodim/kg. Values are for 7 days after administration of radioactive test material.

^bEach value represents the mean of five animals, except for the concentration of [¹⁴C] in the adrenals of females, which is the mean of four animals.

^cThe minimum quantifiable limit listed by study authors is <0.005 (CBI p. 179). However, the value reported (CBI p. 64) is less.

^dThe minimum quantifiable limit.

^eNot applicable.

Source: CBI Table XI, CBI p. 64.

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TABLE 6. Mean Percent Recovery and Distribution of Metabolites in the Urine and Feces of Rats Following Single or Repeated Oral Dosing with Clethodim

Metabolite Identified	Percent of [^{14} C] administered to rats dosed at:							
	450 mg/kg ^a				4.5 mg/kg ^b			
	Males		Females		Males		Females	
	Urine 6-48hr ^c	Feces 0-48hr	Urine 0-48hr ^c	Feces 0-48hr	Urine 0-48hr ^c	Feces 0-48hr	Urine 0-48hr ^c	Feces 0-48hr
Clethodim sulfoxide	46 ^d	2	61	2	52	3	56	5
S-Methyl sulfoxide	6	0.4	8	0.4	9	1	11	1
Unidentified	5	1.6	5	1	9	3	7	3
Imine sulfoxide	7	1	6	1	9	1	6	2
5-OH sulfoxide ^e	4	0.3	2	0.1	5	0.8	3	0.4
Oxazole sulfoxide	3	0	3	0	2	0.4	2	0.2
Trione sulfoxide	1	0.3	1	0.1	1	0.4	1	0.2
Clethodim	0	0.8	0.4	0.8	0	1	0	1.3
5-OH sulfone	0.3	0.1	0.3	0.1	1	0.1	1	1.1
Clethodim sulfone	0.7	0.4	1	0.1	0	0.5	0	0.1
Aromatic sulfone	0	0.4	0.5	0.2	0	0.4	0	1.2
S-Methyl sulfone	0	0.2	0	0.1	0	0.4	0	0

^aAnimals were given a single oral dose of 450 mg [^{14} C]clethodim/kg.^bAnimals were treated orally with 4.5 mg unlabeled clethodim/kg/day for 14 days and then were given a single oral dose of 4.5 mg [^{14} C]clethodim/kg.^cNo 0- to 6-hour samples for high-dose males were available for metabolite analysis; this fraction contained 3.9 percent of the [^{14} C] dose. Urine fraction headings were changed from 12-48 hour to 6-48 hour for high-dose males and from 6-48 hour to 0-48 hour for high-dose females and repeated-dose males and females by the reviewers (see section 14 for discussion).^dEach value represents the mean of five animals; compounds were identified by high-performance liquid chromatography (HPLC) except where noted.^eIdentified by thin-layer chromatography (TLC).

Source: CBI Table XIX, CBI p. 72.

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TABLE 7. Designations, Chemical Names, and Structures for Clethodim and Reference Standards

DESIGNATION	CHEMICAL NAME	STRUCTURE
CLETHODIM (RE-45601)	2-[1-[(E-3-chloro-2-propenyl)oxy]-imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one	
CLETHODIM SULFOXIDE (RE-45924)	2-[1-[(E-3-chloro-2-propenyl)oxy]-imino]propyl]-5-[2-(ethylsulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one	
CLETHODIM SULFONE (RE-47253)	2-[1-[(E-3-chloro-2-propenyl)oxy]-imino]propyl]-5-[2-(ethylsulfonyl)propyl]-3-hydroxy-2-cyclohexen-1-one	
S-METHYL (RE-46474)	2-[1-[(E-3-chloro-2-propenyl)oxy]-imino]propyl]-5-[2-(methylthio)propyl]-3-hydroxy-2-cyclohexen-1-one	
S-METHYL SULFOXIDE (RE-47506)	2-[1-[(E-3-chloro-2-propenyl)oxy]-imino]propyl]-5-[2-(methylsulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one	

TABLE 7 (continued)

DESIGNATION	NAME	STRUCTURE
S-METHYL SULFONE (RE-47507)	2-[1-[(E-3-chloro-2-propenyl)oxy]- imino]propyl]-5-[2-(methylsulfonyl) propyl]-3-hydroxy-2-cyclohexen-1-one	
IMINE SULFOXIDE (RE-47718)	5-[2-(ethylsulfonyl)propyl]-3-hydroxy- 2-(1-iminopropyl)-2-cyclohexen-1-one	
IMINE SULFONE (RE-47719)	5-[2-(ethylsulfonyl)propyl]-3-hydroxy- 2-(1-iminopropyl)-2-cyclohexen-1-one	
S-OH SULFOXIDE (RE-51229)	2-[1-[(E-3-chloro-2-propenyl)oxy]- imino]propyl]-5-[2-(ethylsulfonyl)propyl]- 3,5-dihydroxy-2-cyclohexen-1-one	
S-OH SULFONE (RE-51228)	2-[1-[(E-3-chloro-2-propenyl)oxy]- imino]propyl]-5-[2-(ethylsulfonyl)propyl]- 3,5-dihydroxy-2-cyclohexen-1-one	

TABLE 7 (continued)

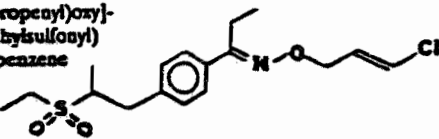
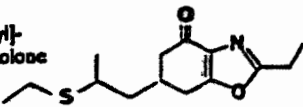
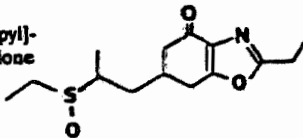
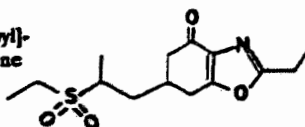
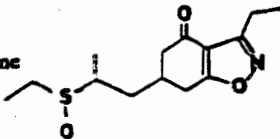
DESIGNATION	NAME	STRUCTURE
AROMATIC SULFONE (RE-50419)	2-{1-[(E-3-chloro-2-propenyl)oxy]-imino}propyl-5-[2-(ethylsulfonyl)propyl]-1,3-dihydroxybenzene	
OXAZOLE (RE-47365)	2-ethyl-6-[2-(ethylthio)propyl]-6,7-dihydro-4(5H)-benzoxazolone	
OXAZOLE SULFOXIDE (RE-47396)	2-ethyl-6-[2-(ethylsulfinyl)propyl]-6,7-dihydro-4(5H)-benzoxazolone	
OXAZOLE SULFONE (RE-47397)	2-ethyl-6-[2-(ethylsulfonyl)propyl]-6,7-dihydro-4(5H)-benzoxazolone	
ISOXAZOLE SULFOXIDE (RE-47614)	3-ethyl-6-[2-(ethylsulfinyl)propyl]-6,7-dihydro-1,2-benzoxazol-4(5H)-one	

TABLE 7 (continued)

DESIGNATION	NAME	STRUCTURE
ISOXAZOLE SULFONE (RE-47615)	3-ethyl-6-[2-(ethylsulfonyl)propyl]- 6,7-dihydro-1,2-benzisoxazol-4(5H)-one	
TRIONE SULFOXIDE (RE-47386)	5-[2-(ethylsulfinyl)propyl]-3-hydroxy- 2-(1-oxopropyl)-2-cyclohexen-1-one	
TRIONE SULFONE (RE-47387)	5-[2-(ethylsulfonyl)propyl]-3-hydroxy- 2-(1-oxopropyl)-2-cyclohexen-1-one	

Source: CBI Table 1, CBI pp. 51-54.

composed of several metabolites; these generally were polar, solvent-front-eluting compounds. Metabolite profiles for liver and blood were not presented. The proposed metabolic pathways for clethodim in rats are shown in Figure 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The authors concluded that clethodim was readily absorbed and eliminated by rats following administration of a single oral low (4.5 mg/kg) or high (450 mg/kg) dose or after repeated oral dosing (4.5 mg/kg/day for 14 days). For every treatment group, nearly all (87.2 to 92.3 percent) of the [^{14}C] dose was recovered from the urine within 7 days after dosing; smaller amounts were excreted in the feces (9.3 to 17.1 percent) and expired air (0.5 to 1.0 percent, as [^{14}C]CO₂). Approximately 93.5 to 98.2 percent of the [^{14}C] administered was eliminated within 48 hours after dosing; the low- and repeated-dose animals excreted clethodim somewhat faster (98 percent in 48 hours) than did high-dose animals (98 percent in 50 hours). No sex-related differences in the rate of elimination of clethodim were reported for any dose group.

Tissue levels of [^{14}C] were very low. At 7 days postdosing, the total amount of radiocarbon recovered from all organs and tissues combined was <1 percent of that administered to all groups. Tissue concentrations of [^{14}C] were similar for rats in the low- and repeated-dose groups, indicating that clethodim probably would not bioaccumulate to an appreciable extent following multiple exposures to low doses. Tissue [^{14}C] levels in high-dose animals were proportionately higher than those in low- and repeated-dose animals. The highest concentrations of radiocarbon were found in the adrenals (0.068 to 0.221 ppm for low- and repeated-dose rats; 5.36 to 13.0 ppm for high-dose rats).

The urinary and fecal metabolite profiles for males and females within a dose group and between dose groups were similar. Eleven compounds (including clethodim) were identified in the excreta. The predominant metabolite, clethodim sulfoxide, accounted for 48 to 63 percent of the administered [^{14}C] in urine (46 to 61 percent) and feces (2 to 5 percent in acetone extracts) samples collected for up to 48 hours after dosing. Other major excretory metabolites included S-methyl sulfoxide (6 to 12 percent in the urine and feces combined), imine sulfoxide (7 to 10 percent), and 5-OH sulfoxide (2 to 6 percent). Minor metabolites were oxazole sulfoxide (2 to 3 percent), trione sulfoxide (about 1 percent), 5-OH

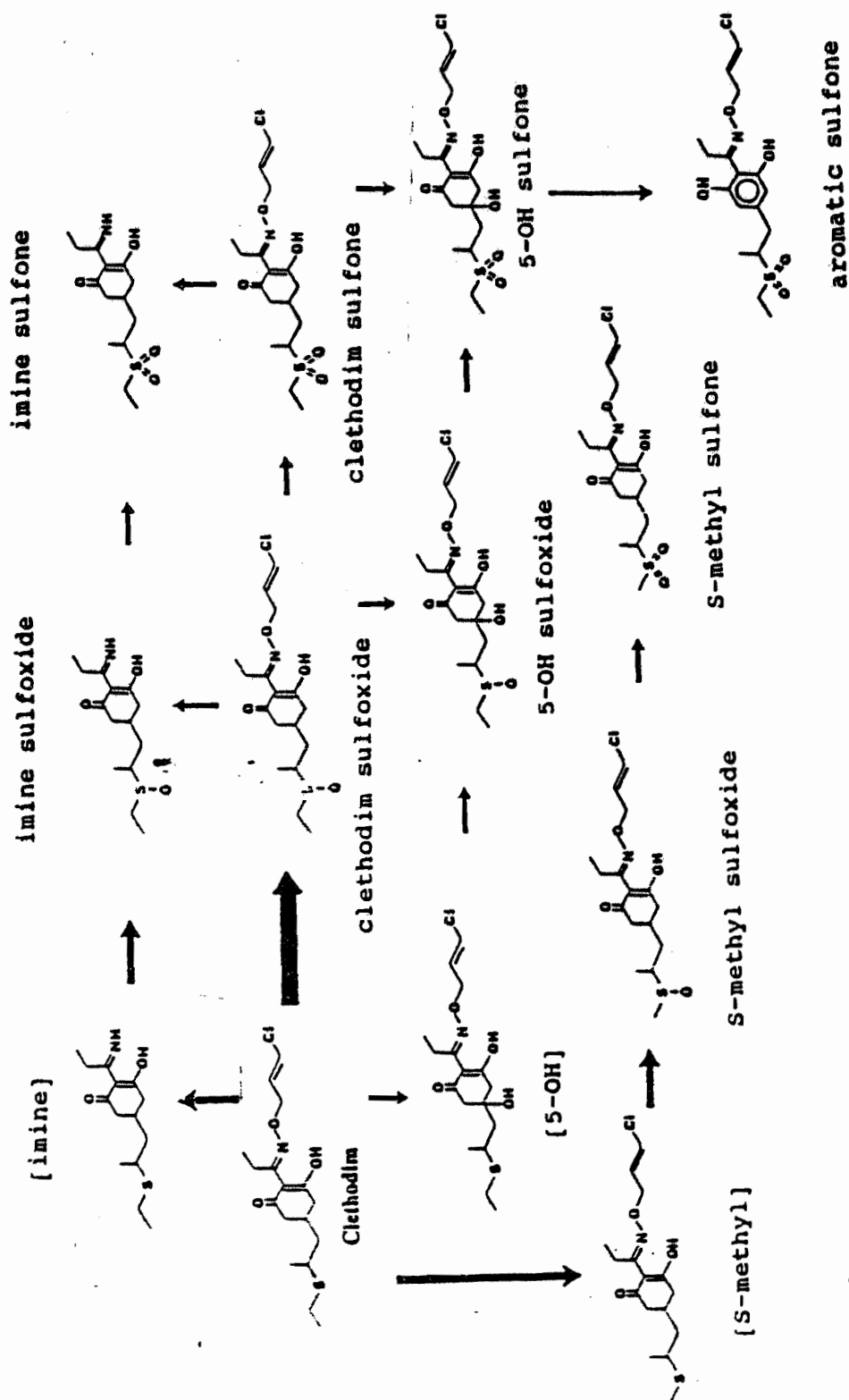


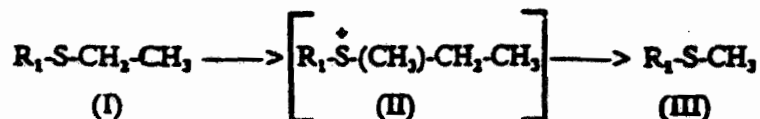
Figure 1. Proposed metabolic pathways for clethodim in rats. Compounds in brackets are proposed metabolites or intermediates of clethodim; they have not been isolated.

Source: CBI Figure 20, CBI p. 91.

sulfone (about 0.5 to 1.0 percent), clethodim sulfone (0.1 to 1.1 percent), aromatic sulfone (≤ 0.7 percent), and S-methyl sulfone (0 to 0.4 percent). Less than 1 percent of the [^{14}C] dose was recovered as unchanged parent compound, and approximately 5 to 9 percent was not identified. The authors concluded that orally administered clethodim was completely metabolized by rats.

The proposed primary pathway for clethodim in rats is via oxidation to clethodim sulfoxide and then to clethodim sulfone. Clethodim sulfone, in turn, could be converted to imine sulfone or to 5-OH sulfone and aromatic sulfone; however, the latter metabolites probably are generated by one of the other pathways described below. Before oxidation to the sulfoxide, clethodim can (1) be converted to S-methyl via a sulfonium cation intermediate, (2) undergo N-O bond cleavage to form imine, or (3) undergo hydroxylation at the C5 position. The proposed S-methyl, imine, and 5-OH compounds -- none of which were detected in the urine or feces -- would then undergo the dominant oxidative processes to form S-methyl sulfoxide and sulfone, imine sulfoxide and sulfone, and 5-OH sulfoxide and sulfone, respectively. The authors suggested that oxazole sulfoxide could have been formed by a Beckmann rearrangement of clethodim sulfoxide or, alternatively, by oxidation of the oxazole, which may have formed via a Beckmann rearrangement of clethodim. The authors noted, however, that oxazole sulfoxide may be a degradative product (rather than a metabolite) because its concentration in some extracts increased with time. Trione sulfoxide was probably formed via hydrolysis of imine sulfoxide.

The presence and source of S-methyl metabolites in the rats was questioned. Since S-methyl (i.e., the sulfide) was not detected in the excreta, but S-methyl sulfoxide and S-methyl sulfone were, S-methyl was thought to have been an impurity in the radiolabeled clethodim. Dosing solutions were not available for analysis, but analysis of the stock solution of [^{14}C]clethodim (No. 145) by HPLC and HPLC-MS failed to detect any S-methyl, S-methyl sulfoxide, or S-methyl sulfone. Thus, S-methyl and S-methyl sulfoxide were not considered contaminants, and the S-methyl metabolites were assumed to be generated via biometabolism. According to the study authors, formation of the S-methyl metabolites via a sulfonium cation intermediate is supported by the literature and could proceed as follows:



where I is clethodim, II is the cation intermediate, and III is the S-methyl sulfide. Any S-methyl formed would then be readily converted by atmospheric or biological oxidation to S-methyl sulfoxide.

- B. A quality assurance statement and a statement of compliance with EPA's Good Laboratory Practices, both signed and dated December 22, 1988, were included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study was conducted adequately according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, 1984, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, pp. 152-156), and the authors' conclusions are supported by the data. Sufficient numbers of animals (five/sex/dose level) were used, and the doses administered and the treatment schedules employed were appropriate. An intravenous study was not conducted because of clethodim's poor solubility in water and saline.

As noted by the study authors, orally administered clethodim is rapidly absorbed and eliminated by rats given a single low or high dose or repeated low doses of the test material. Most (84 to 92 percent) radioactive material was excreted in the urine within 48 hours postdosing, and on the basis of urine, tissue, and cage-wash levels of [^{14}C] measured during the first 7 days after dosing, gastrointestinal absorption of clethodim was estimated to be between 89 and 95 percent for all treatment groups and for both sexes. Approximately 9 to 17 percent of the [^{14}C] dose was recovered in the feces within 7 days. Combined tissue levels of radioactivity accounted for less than 1 percent of each of the three doses, including the repeated dose, indicating that the compound does not accumulate to a marked extent following multiple low exposures. Relatively high tissue levels (particularly in the adrenals, bone, heart, muscle, gonads (males), spleen, lung, and spinal cord) may be of concern, however, following an acute high exposure to clethodim. At 7 days after dosing, [^{14}C] levels in these tissues were more than 100 times greater in high-dose animals than in the low-dose group.

Clethodim was extensively metabolized by rats, with the primary metabolic pathway involving oxidation of the parent compound. The principal metabolite recovered from all animals was

clethodim sulfoxide, that accounted for approximately 46 to 61 and 2 to 5 percent of the administered [^{14}C], as recovered from the urine and feces, respectively, within 48 hours postdosing. Metabolites that accounted for more than 5 percent of the radiolabeled dose included: S-methyl sulfoxide (6 to 12 percent, urine and feces combined), imine sulfoxide (7 to 10 percent), and 5-OH sulfoxide (2 to 6 percent). Minor metabolites (≤ 3 percent, urine and feces combined) were oxazole sulfoxide, trione sulfoxide, 5-OH sulfone, clethodim sulfone, aromatic sulfone, S-methyl sulfone, and the unchanged parent compound. Approximately 5 to 9 percent of the dose was unidentified and consisted of materials that were primarily polar in nature in the TLC systems used by the study authors.

In comparing recovery and metabolite distribution data, it appeared that the study authors provided the wrong headings (i.e., the incorrect sampling times) for metabolite distribution in the urine (see Table 6 of this DER and CBI Table XIX, CBI p. 72). According to reviewer calculations, the metabolite and [^{14}C] recovery data balanced for 0- to 48-hour urine samples (rather than for 6- to 48-hour fractions) for high-dose females and repeated-dose males and females; similarly, urinary metabolite quantification data for high-dose males balanced with percent-recovery data for 6 to 48-hour samples (rather than for the 12- to 48-hour fractions) (Table 8, next page). The study authors noted that inadequate amounts of the 0- to 6-hour urine from high-dose males were available for metabolite analyses and that this fraction contained 8.9 percent of the administered [^{14}C].

Information on metabolite distribution in the blood and liver may have been helpful in possibly isolating and identifying, among other compounds, the imine, 5-OH, and S-methyl intermediates of clethodim that were proposed by the study authors but that were not recovered in the urine or feces. However, recovery of small amounts of radioactivity from these tissues made identification of metabolites difficult. In addition, only one TLC autoradiogram of extractable residue from the acidified male rat liver was provided, and the solvent system used for this analysis was not described.

Another concern of the reviewers is the spontaneous generation of clethodim sulfoxide. The study authors reported that clethodim is readily oxidized by atmospheric oxygen and that no attempt was made to exclude oxygen from the TLC plates or developing tank. The compound appears to be somewhat unstable (stability tests on unlabeled test material showed that approximately 90 and 85 percent of the original calculated concentration of clethodim remained after 5 and 9 days, respectively), and radioactive suspensions were prepared either the day before or on the day of dosing. Air oxidation of the test material and its sulfoxide probably proceeds slowly, since small amounts of the sulfone were identified. However, since clethodim sulfoxide was the primary metabolite excreted

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TABLE 8. Comparison of Percent Recovery Data for Metabolite Distribution in the Urine of Rats Exposed Orally to a Single High Dose or to Repeated Doses of Clethodim

Recovery	Percent of [^{14}C] administered to:			
	High-dose rats ^a		Repeated-dose rats ^b	
	Males	Females	Males	Females
Total recovery: distribution of metabolites in urine	73 (12-48 hr) ^c	88.2 (6-48 hr) ^c	88 (6-48 hr) ^c	87 (6-48 hr) ^c
Total recovery: urine	58.1 ^d (12-48 hr)	77.4 ^d (6-48 hr)	47.4 ^d (6-48 hr)	53.2 ^d (6-48 hr)
Total recovery: urine	74.7 (6-48 hr) ^e	88.4 (0-48 hr) ^e	88.8 (0-48 hr) ^e	85.7 (0-48 hr) ^e

^aAnimals were given a single oral dose of 450 mg [^{14}C]clethodim/kg.

^bAnimals were treated orally with 4.5 mg unlabeled clethodim/kg/day for 14 days and then received a single oral dose of 4.5 mg [^{14}C]clethodim/kg.

^cTime fractions as reported by study authors, CBI Table XIX, CBI p. 72.

^dTotal recovery of [^{14}C] in the urine for time fractions reported by the study authors for metabolite distribution.

^eTime fractions that appear to correspond to data for the distribution of metabolites in the urine, as suggested by the reviewers.

by rats, a more precise measurement of clethodim sulfoxide production and elimination could have been obtained by monitoring the time-dependent atmospheric conversion of clethodim to clethodim sulfoxide, by assaying for clethodim sulfoxide believed to be at the origin site on TLC plates, and by checking the concentration of clethodim sulfoxide at the time of dosing.

Finally, it appears either that the radioactive test material was incorrectly named as [propyl-1-¹⁴-C]clethodim (by the study authors) or that the sites of the radiolabel position were placed incorrectly in the structure provided by the study authors. The radiolabeled structure provided can be named [cyclohexenone-4,6-¹⁴C]clethodim.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 148-153.

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APPENDIX A

Materials and Methods
(CBI pp. 148-153)

CLETHODIM

Page _____ is not included in this copy.

Pages 328 through 333 are not included.

The material not included contains the following type of information:

- ____ Identity of product inert ingredients.
 - ____ Identity of product impurities.
 - ____ Description of the product manufacturing process.
 - ____ Description of quality control procedures.
 - ____ Identity of the source of product ingredients.
 - ____ Sales or other commercial/financial information.
 - ____ A draft product label.
 - ____ The product confidential statement of formula.
 - ____ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - ____ The document is a duplicate of page(s) _____.
 - ____ The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-H
TASK No.: 2-45H
April 25, 1990

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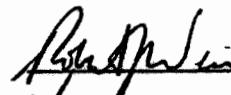
DATA EVALUATION RECORD

RE-45601 TECHNICAL

One-Year Oral Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: 
Date: 4/24/90

EPA No.: 68D80056
DYNAMAC No.: 245-H
TASK No.: 2-45H
April 25, 1990

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DATA EVALUATION RECORD

RE-45601 TECHNICAL

One-Year Oral Toxicity Study in Dogs

REVIEWED BY:

John Liccione, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: John J. Liccione

Date: April 25, 1990

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan

Date: April 25, 1990

APPROVED BY:

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Signature: Roman J. Pienta

Date: April 25, 1990

James Rowe, Ph.D.
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Toxicology Branch II
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Signature: James Rowe

Date: 5/22/90

Mike Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: J. M. Ioannou

Date: 5/31/90

DATA EVALUATION RECORD

GUIDELINE § 83-1

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STUDY TYPE: One-year dietary toxicity study in dogs.

MRID NUMBER: 410301-11.

TEST MATERIAL: RE-45601 Technical.

SYNONYMS: Clethodim; (E,E)-(+/)-2[1[[[(3-chloro-2-propenyl)oxy]imino]-propyl]-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one; select.

STUDY NUMBER: HLA 2107-153.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA.

TITLE OF REPORT: One-Year Oral Toxicity Study in Dogs with Chevron RE-45601 Technical (SX-1688).

AUTHOR: Cox, R. H.

REPORT ISSUED: October 28, 1988.

CONCLUSIONS:

RE-45601 technical was fed to male and female dogs at dose levels of 0, 1, 75, or 300 mg/kg/day for 1 year. Absolute and relative (to body weight or brain weight) liver weights were significantly increased in male and female dogs receiving 300 mg/kg/day. Histological changes in the liver correlated with the increased liver weights at 300 mg/kg/day. Although absolute and relative liver weights were also increased in females receiving 75 mg/kg/day, there were no correlative histopathological changes in the livers at this dose. In addition, mean thyroid/parathyroid weights (absolute and relative to body weight and brain weight) were significantly increased in males administered the high dose. The toxicological importance of the increased thyroid/parathyroid weights is equivocal, since no corresponding histopathological lesions were found and a similar weight increase was not detected in females. Platelet, leukocyte, and corrected leukocyte counts were significantly increased, and apparently treatment-related, in females receiving 75 mg/kg/day. In addition, glucose levels were decreased in females treated with 75 mg/kg/day. In the high-dose animals, statistically significant decreases from control values were noted for mean erythrocyte values (both sexes), hemoglobin levels (females only), hematocrit values (females only), and glucose levels (both sexes). Increases in total cholesterol levels, alanine aminotransferase activity, alkaline phosphatase activity, and triglyceride levels were seen in both sexes following oral treatment with 300 mg/kg/day.

The LOEL is 75 mg/kg/day based on increased absolute and relative liver weights in females, and alterations in hematology and clinical chemistry parameters in males and females. The NOEL is 1 mg/kg/day.

Classification: CORE Supplementary pending submission of data on stability and concentration of test material in the capsules.

A. MATERIALS:

1. Test Compound: RE-45601 technical; description: brown viscous liquid; batch No.: SX-1688; purity: 83.3%.
2. Test Animals: Species: dog; strain: beagle; age: at initiation of treatment, all animals were greater than 4 and less than 6 months of age; weight: at initiation of treatment, the males weighed between 4.7 and 10.7 kg, and the females weighed between 4.4 and 7.1 kg; source: Hazleton Research Animals, Cumberland, VA.

B. STUDY DESIGN:

1. Animal Assignment: Dogs were held in quarantine and acclimated to laboratory conditions for at least 2 weeks. During the quarantine and acclimation period, each dog was examined by a veterinarian. The dogs released from quarantine and considered acceptable for use in the study were considered to be free of clinical signs of contagious, infectious, or communicable diseases. The records of each dog indicated that parvovirus, distemper, hepatitis, parainfluenza, and leptospirosis vaccines had been received. During the final week of acclimation, i.e., the week immediately prior to test material administration, all dogs received an empty gelatin capsule daily in order to acclimate them to the dosing procedure. The animals were randomly placed into the following groups in which the variance and mean body weight data were homogeneous:

Test group	Dose in diet (mg/kg/day)	Number of Animals	
		Males	Females
1 Control	0	6	6
2 Low (LDT)	1	6	6
3 Mid (MDT)	75	6	6
4 High (HDT)	300 ^a	6	6

^aReceived 200 mg/kg/day during weeks 1-7 but the dosage level was increased to 300 mg/kg/day after evaluation of the day 31 clinical pathology data did not reveal any changes in alkaline phosphatase levels.

Dose levels were selected on the basis of the results of a previous 90-day subchronic oral toxicity study in groups of four dogs/sex. As reported in the 90-day study, significant increases in absolute liver weights were observed in males (34% increase) and females (30% increase) receiving 125 mg/kg/day. Liver lesions, consisting of cytoplasmic vesiculation and vacuolation, were found in males and females following treatment with 125 mg/kg/day. Both alkaline phosphatase activity and mean cholesterol levels were significantly increased in females receiving 125 mg/kg/day. There was no effect of dosing on mortality, body weight, or food consumption.

In the present study, the animals were housed individually in stainless steel cages within rooms with temperature and

relative humidity ranges of 64-87°F and 25-98%, respectively, with a 12-hour light/dark cycle. One day prior to initiation of dosing, the dogs were assigned permanent identification numbers embossed on eartags secured to the ear.

2. Diet Preparation: Dogs were administered the test substance orally via gelatin capsules. The test material was measured with an appropriate syringe (μ L or mL) and placed in 1/4-oz gelatin capsules. Capsules were refrigerated until administration. Individual doses were adjusted weekly based on the body weights recorded at the end of the previous week. The test material and control capsules were administered until the animals were killed following 52 weeks of treatment.

Results: Data on analyzed concentrations and stability of test material in the capsules were not provided.

3. Food and Water Consumption: Animals received food (Purina Certified Canine Diet Meal No. 5007) for 4 hours/day immediately following test material administration and water ad libitum.
4. Statistics: Body weight and body weight change, food consumption, clinical pathology parameters, and organ weights were evaluated by one-way analysis of variance (ANOVA) and Levine's test for homogeneity of variance. The t-tests for equal or unequal variances were used to compare dosed and control groups, and Dunnett's test was used for levels of significance. When variances of untransformed data were heterogeneous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data.
5. Quality Assurance: A quality assurance statement was signed and dated October 27, 1988; an updated GLP compliance statement was also present.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and signs of toxicity.

Results: Clinical observations during the study included frequently noted signs for dogs at the laboratory, for example, emesis, soft feces, alopecia, sores, lacrimation, few or no feces, thinness, and evidence of estrous activity. However, none of these observations appeared to be related to treatment with the test material. No mortality occurred during the course of the study.

2. Body Weight: Dogs were weighed before treatment and once each week thereafter.

Results: Mean body weight values and body weight changes were similar among all groups throughout the study, and no statistically significant differences were noted.

3. Food Consumption and Compound Intake: Food consumption values of individual animals were determined weekly.

Results: Mean food consumption values were similar among all groups throughout the study, and no statistically significant differences were noted.

4. Ophthalmological Examinations: An indirect ophthalmoscopic examination was conducted for each dog prior to study initiation and on days 180 and 363. A 1% mydriatic was used.

Results: There were no ocular findings noted for dogs which were attributed to treatment with RE-45601 technical.

5. Hematology and Clinical Chemistry: Blood samples were collected from the jugular vein of each dog prior to study initiation and on days 31, 90, 180, 270, and 360 for hematology and serum chemistry analyses. Dogs were fasted overnight prior to blood collections. The CHECKED (X) parameters were examined:

- a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	X Coagulation:thromboplastin time (PT)
X Reticulocyte count (RETIC)	X Coagulation:partial thromboplastin time (PPT)
X Red cell morphology	

[†]Recommended by Subdivision F (October 1982) Guidelines.

Results: Table 1 summarizes data on selected hematology parameters. Several treatment-related changes in hematology values were noted. Mean erythrocyte values were significantly decreased ($p \leq 0.05$) in males receiving 300 mg/kg/day on days 270 and 360, and in females receiving 300 mg/kg/day on days 180, 270 and 360. Mean hemoglobin level and hematocrit values were also significantly decreased ($p \leq 0.05$) in high-dose females on days 180, 270, and 360. There were no consistent effects on MCV, MCH, or MCHC. Statistically significant increases ($p \leq 0.05$) were noted for mean platelet counts for mid-dose females (days 180 and 360), high-dose females (days 31, 90, 180, 270, and 360); and high-dose males (days 31, 180, 270, and 360). Mean leukocyte values were significantly elevated ($p \leq 0.05$) in mid-dose females (day 90), high-dose females (days 90, 180, 270, and 360), and high-dose males (day 180). The changes in mean leukocyte values for high-dose females was primarily due to significant increases in segmental neutrophil counts on days 31, 90, and 270. Mean values for corrected leukocytes (i.e., when nucleated red blood cells were a controlled factor) reflected a similar pattern of the uncorrected leukocyte value. Other statistically significant differences from control values were of little or no biological importance.

b. Clinical Chemistry:

<u>Electrolytes</u>		<u>Other</u>	
X	Calcium [†]	X	Albumin [†]
X	Chloride [†]	X	Albumin/globulin ratio
	Magnesium [†]	X	Blood creatinine [†]
X	Phosphorus [†]	X	Blood urea nitrogen [†]
X	Potassium [†]	X	Cholesterol [†]
X	Sodium [†]	X	Globulins
		X	Glucose [†]
		X	Total bilirubin [†]
		X	Direct bilirubin
		X	Total protein [†]
		X	Triglycerides
<u>Enzymes</u>			
X	Alkaline phosphatase (ALP)		
	Cholinesterase		
X	Creatinine phosphokinase [†]		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT) [†]		
X	Serum aspartate aminotransferase (SGOT) [†]		
X	Gamma glutamyltransferase (GGT)		

[†]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 1. Selected Hematology Parameters (Mean $\pm$ S.D.) for Dogs Dosed Orally with RE-45601 for 1 Year^{a,b}

Parameter/ Interval	Dose Level (mg/kg/day)				
	0	1	75	300	300
Males					
RBC					
(MI/ μ L)					
Day -10	6.03 $\pm$ 0.594	6.18 $\pm$ 0.422	6.07 $\pm$ 0.220	6.05 $\pm$ 0.172	6.19 $\pm$ 0.442
Day 31	6.01 $\pm$ 0.485	6.28 $\pm$ 0.568	6.06 $\pm$ 0.475	5.98 $\pm$ 0.199	6.40 $\pm$ 0.329
Day 90	6.50 $\pm$ 0.657	6.49 $\pm$ 0.414	6.38 $\pm$ 0.397	6.20 $\pm$ 0.322	6.79 $\pm$ 0.424
Day 180	6.56 $\pm$ 0.524	6.85 $\pm$ 0.489	6.55 $\pm$ 0.280	6.11 $\pm$ 0.489	6.97 $\pm$ 0.411
Day 270	6.82 $\pm$ 0.539	7.03 $\pm$ 0.545	6.57 $\pm$ 0.183	6.17 $\pm$ 0.249*	7.11 $\pm$ 0.319
Day 360	7.25 $\pm$ 0.511	7.76 $\pm$ 0.675	7.06 $\pm$ 0.291	6.50 $\pm$ 0.440*	7.59 $\pm$ 0.464
HGB					
(G/DL)					
Day -10	13.7 $\pm$ 1.23	13.8 $\pm$ 1.11	13.5 $\pm$ 0.51	13.3 $\pm$ 0.47	14.0 $\pm$ 0.99
Day 31	13.5 $\pm$ 1.01	13.8 $\pm$ 0.88	13.5 $\pm$ 1.31	12.9 $\pm$ 0.76	14.1 $\pm$ 0.58
Day 90	15.1 $\pm$ 1.65	14.7 $\pm$ 0.86	14.7 $\pm$ 1.30	13.6 $\pm$ 1.05	15.5 $\pm$ 1.16
Day 180	15.0 $\pm$ 1.38	15.4 $\pm$ 0.81	15.0 $\pm$ 0.99	14.2 $\pm$ 1.04	15.8 $\pm$ 0.89
Day 270	15.6 $\pm$ 1.33	16.0 $\pm$ 1.20	15.1 $\pm$ 0.53	14.5 $\pm$ 0.33	16.4 $\pm$ 0.77
Day 360	16.7 $\pm$ 1.20	17.7 $\pm$ 1.32	16.5 $\pm$ 0.68	15.3 $\pm$ 0.65	17.2 $\pm$ 1.53
HCT					
(%)					
Day -10	40.8 $\pm$ 3.37	41.3 $\pm$ 3.18	40.2 $\pm$ 1.37	39.6 $\pm$ 1.31	41.5 $\pm$ 2.80
Day 31	39.8 $\pm$ 2.96	41.0 $\pm$ 3.22	39.2 $\pm$ 3.91	37.8 $\pm$ 2.49	42.1 $\pm$ 1.74
Day 90	43.3 $\pm$ 4.40	42.5 $\pm$ 2.29	41.9 $\pm$ 3.37	39.7 $\pm$ 3.04	44.4 $\pm$ 3.19
Day 180	43.8 $\pm$ 4.18	45.1 $\pm$ 3.10	43.8 $\pm$ 2.79	41.4 $\pm$ 3.29	46.2 $\pm$ 2.67
Day 270	45.8 $\pm$ 3.74	46.4 $\pm$ 3.08	44.1 $\pm$ 1.64	42.8 $\pm$ 1.05	47.6 $\pm$ 1.63
Day 360	47.5 $\pm$ 3.15	50.4 $\pm$ 3.77	46.7 $\pm$ 2.00	43.7 $\pm$ 1.66	49.0 $\pm$ 4.00
Females					
RBC					
(MI/ μ L)					
Day -10	6.12 $\pm$ 0.257	6.08 $\pm$ 0.234	6.02 $\pm$ 0.564	6.03 $\pm$ 0.595	6.11 $\pm$ 0.735
Day 31	5.75 $\pm$ 0.802	6.83 $\pm$ 0.702	6.82 $\pm$ 0.750	6.83 $\pm$ 0.702	6.81 $\pm$ 0.621
Day 90	5.90 $\pm$ 0.490	5.58 $\pm$ 0.678*	6.95 $\pm$ 0.601	6.91 $\pm$ 0.652	7.28 $\pm$ 0.446
Day 180	5.99 $\pm$ 0.618*	7.30 $\pm$ 0.512	7.16 $\pm$ 0.782	7.16 $\pm$ 0.782	7.30 $\pm$ 0.512
Day 270	6.20 $\pm$ 0.452*				
HGB					
(G/DL)					
Day -10	13.9 $\pm$ 0.86	13.8 $\pm$ 0.56	13.6 $\pm$ 1.17	13.6 $\pm$ 0.99	13.8 $\pm$ 1.13
Day 31	13.1 $\pm$ 1.49	16.1 $\pm$ 1.38	15.8 $\pm$ 1.39	15.8 $\pm$ 1.39	16.1 $\pm$ 1.38
Day 90	14.0 $\pm$ 1.21	16.2 $\pm$ 1.74	16.0 $\pm$ 0.80	16.0 $\pm$ 0.80	16.2 $\pm$ 1.74
Day 180	13.5 $\pm$ 1.76*	17.6 $\pm$ 1.16	16.1 $\pm$ 1.30	16.1 $\pm$ 1.30	17.6 $\pm$ 1.16
Day 270	14.2 $\pm$ 1.72*	17.4 $\pm$ 1.22	16.6 $\pm$ 1.77	16.6 $\pm$ 1.77	17.4 $\pm$ 1.22
Day 360	14.8 $\pm$ 1.43*				
HCT					
(%)					
Day -10	41.0 $\pm$ 2.45	41.0 $\pm$ 1.74	40.4 $\pm$ 3.00	39.8 $\pm$ 3.19	40.2 $\pm$ 3.94
Day 31	37.9 $\pm$ 5.20	46.0 $\pm$ 4.01	45.1 $\pm$ 3.60	45.1 $\pm$ 3.60	46.0 $\pm$ 4.01
Day 90	39.9 $\pm$ 3.34	46.9 $\pm$ 5.27	46.4 $\pm$ 2.53	46.4 $\pm$ 2.53	46.9 $\pm$ 5.27
Day 180	39.2 $\pm$ 5.27*	50.8 $\pm$ 3.06	46.4 $\pm$ 4.11	46.4 $\pm$ 4.11	50.8 $\pm$ 3.06
Day 270	41.5 $\pm$ 4.76*	49.7 $\pm$ 3.51	47.5 $\pm$ 4.88	47.5 $\pm$ 4.88	49.7 $\pm$ 3.51
Day 360	42.3 $\pm$ 4.05*				

(continued)

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TABLE 1. (continued)

Parameter/ Interval	Dose Level (mg/kg/day)				
	0	1	75	300	300
Males					
PLATELET (1H/ μ L)					
Day -10	464 $\pm$ 106.8	461 $\pm$ 82.3	381 $\pm$ 112.0	517 $\pm$ 1	5
Day 31	319 $\pm$ 35.4	319 $\pm$ 81.1	343 $\pm$ 114.1	484 $\pm$ 89.5*	
Day 90	317 $\pm$ 91.7	304 $\pm$ 22.7	381 $\pm$ 38.0	516 $\pm$ 110.4	
Day 180	310 $\pm$ 52.0	319 $\pm$ 44.6	395 $\pm$ 61.4	530 $\pm$ 105.3*	
Day 270	324 $\pm$ 73.8	294 $\pm$ 46.1	393 $\pm$ 57.5	532 $\pm$ 93.7*	
Day 360	332 $\pm$ 67.5	341 $\pm$ 43.6	399 $\pm$ 111.3	562 $\pm$ 89.3*	
Females					
Day -10			428 $\pm$ 57.8	428 $\pm$ 114.6	350 $\pm$ 76.1
Day 31			297 $\pm$ 62.9	297 $\pm$ 59.6	326 $\pm$ 61.2
Day 90			268 $\pm$ 52.8	266 $\pm$ 60.8	334 $\pm$ 48.4
Day 180			264 $\pm$ 33.5	217 $\pm$ 67.9	367 $\pm$ 64.4*
Day 270			300 $\pm$ 82.5	284 $\pm$ 57.3	396 $\pm$ 85.8
Day 360			294 $\pm$ 78.5	304 $\pm$ 62.5	410 $\pm$ 42.0*
WBC					
(1H/ μ L)					
Day -10	12.8 $\pm$ 1.53	12.0 $\pm$ 1.60	11.0 $\pm$ 4.86	14.5 $\pm$ 6.87	10.9 $\pm$ 1.37
Day 31	12.0 $\pm$ 1.80	12.6 $\pm$ 1.24	13.3 $\pm$ 3.59	12.6 $\pm$ 4.62	13.0 $\pm$ 1.05
Day 90	11.4 $\pm$ 1.75	13.3 $\pm$ 1.90	11.8 $\pm$ 2.93	10.5 $\pm$ 1.00	13.3 $\pm$ 1.52*
Day 180	11.8 $\pm$ 1.81	13.0 $\pm$ 1.29	12.1 $\pm$ 1.99	12.1 $\pm$ 2.49	14.0 $\pm$ 3.43
Day 270	11.7 $\pm$ 3.76	13.1 $\pm$ 2.76	14.2 $\pm$ 3.99	11.9 $\pm$ 1.90	12.8 $\pm$ 2.08
Day 360	11.3 $\pm$ 2.45	11.5 $\pm$ 2.20	11.1 $\pm$ 2.26	9.9 $\pm$ 1.73	14.0 $\pm$ 4.02

^aBased on six animals/sex/group. Data were extracted from Table 6-A of the study report.^bAbbreviations are as follows:

RBC = erythrocyte count

HGB = hemoglobin

HCT = hematocrit

WBC = total leukocyte count

*Significantly different from control values (p $\leq$ 0.05).**Significantly different from control values (p $\leq$ 0.01).

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Results: Table 2 summarizes data on selected clinical chemistry parameters. Mean glucose levels for the high-dose males and females were generally less than control values, with statistically significant decreases occurring for the males at day 270 and for the females at days 180 and 360. The mean glucose level for females receiving 75 mg/kg/day was also significantly decreased at day 360. Mean total cholesterol values were significantly elevated for the high-dose males (day 360) and high-dose females (days 31, 90, 180, 270, and 360). Beginning with the day 90 interval, mean alanine aminotransferase values for high-dose males and females were generally higher than control values. Statistically significant increases occurred for males at days 180, 270, and 360, and for females at days 180 and 360. When compared to pretreatment values, mean alkaline phosphatase values for the control group and the 1- and 75-mg/kg/day dose groups (both sexes) generally decreased over time throughout the study. The mean alkaline phosphatase values for the 300-mg/kg/day dose groups (both sexes) did not follow this pattern and for the most part remained significantly ($p < 0.05$) higher than control values throughout the study starting from day 90. Mean triglyceride values (evaluated at the day 360 interval only) for males and females receiving 300 mg/kg/day were significantly increased when compared to control values at this interval. The aforementioned changes in clinical chemistry parameters were considered by the study authors to be treatment-related. Other statistically significant differences from control values of little or no biological importance.

6. **Urinalysis:** Urine samples were collected from each dog on days 180 and 360 for clinical evaluation. The CHECKED (X) parameters were examined:

X Appearance [†]	X Glucose [†]
X Volume [†]	X Ketones
X Specific gravity [†]	X Bilirubin [†]
X pH	X Blood [†]
X Sediment (microscopic) [†]	X Nitrate
X Protein [†]	X Urobilinogen
	X Reducing substances

[†]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. (continued)

Parameter/ Interval	Dose Level (mg/kg/day)				
	0	1	75	300	0
	Males				
ALX P (U/L)					
Day -10	126 ± 27.9	131 ± 23.1	119 ± 23.7	109 ± 11.1	115 ± 21.9
Day 31	101 ± 25.5	105 ± 18.7	110 ± 31.5	108 ± 20.4	86 ± 17.5
Day 90	68 ± 13.6	70 ± 9.0	78 ± 24.1	116 ± 44.3*	58 ± 10.8
Day 180	41 ± 8.1	41 ± 9.5	59 ± 25.1	103 ± 40.9*	41 ± 10.9
Day 270	38 ± 11.8	38 ± 8.6	48 ± 31.0	101 ± 40.4*	41 ± 16.0
Day 360	30 ± 52	32 ± 9.6	44 ± 21.1	112 ± 66.3*	34 ± 11.3
TRIGLY (MG/DL)					
Day 360 ^c	34 ± 9.4	39 ± 6.6	44 ± 7.5	56 ± 14.0*	32 ± 6.0
					34 ± 7.5
					45 ± 10.4
					59 ± 16.9*
					108 ± 17.8
					109 ± 17.3
					121 ± 29.7*
					138 ± 34.3*
					133 ± 44.0*
					150 ± 34.6*

^aBased on six animals/sex/group. Data were extracted from Table 7 of the study report.^bAbbreviations are as follows:

T CHOL = total cholesterol

ALX = alanine aminotransferase

ALX P = alkaline phosphatase

TRIGLY = triglycerides

^cTriglycerides were evaluated at the day 360 interval only.

*Significantly different from control values (p ≤ 0.05).

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Results: No effects of treatment with RE-45601 technical on urinalysis parameters were seen.

7. Sacrifice and Pathology: All dogs were sacrificed following 52 weeks of treatment, and complete gross necropsies were performed. The CHECKED (X) tissues were collected and fixed. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta [†]	XX Brain
X Salivary glands [†]	XX Heart [†]	X Peripheral nerve (sciatic nerve) [†]
X Esophagus [†]	X Sternal bone marrow [†]	X Spinal cord (3 levels)
X Stomach [†]	X Lymph nodes [†]	XX Pituitary [†]
X Duodenum [†]	XX Spleen	X Eyes (optic nerve) [†]
X Jejunum [†]	X Thymus	
X Ileum [†]		
X Cecum [†]		
X Colon [†]		
X Rectum		
XX Liver [†]	<u>Urogenital</u>	<u>Glandular</u>
X Gallbladder [†]	XX Kidneys [†]	XX Adrenals [†]
X Pancreas [†]	X Urinary bladder [†]	Lacrimal gland
	XX Testes [†]	X Mammary gland [†]
	X Epididymides	XX Thyroids [†]
	X Prostate	XX Parathyroids [†]
	X Seminal vesicle	Harderian glands
<u>Respiratory</u>	XX Ovaries	
X Trachea [†]	X Uterus	
XX Lung [†]		
		<u>Other</u>
		X Bone (femur) [†]
		X Skeletal muscle [†]
		X Skin
		X All gross lesions and masses

Results:

- a. Organ Weights: Table 3 presents mean liver and thyroid/parathyroid weight data. Significant ($p \leq 0.05$) increases in mean absolute liver weight (56% increase in males, 70% increase in females), liver-to-body weight ratio (60% increase in males, 75% increase in females), and liver-to-brain weight ratio (64% increase in males, 75% increase in females) were seen in both sexes administered the high dose. Mean absolute liver weight, liver-to-body weight ratio, and liver-to-brain weight ratio were significantly increased by 34%, 25%, and 36%, respectively, in females receiving 75 mg/kg/day.

Recommended by Subdivision F (October 1982) Guidelines.

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TABLE 3. Selected Organ Weights (Mean $\pm$ SD), Organ-to-Body Weight Ratios, and Organ-to-Brain Weight Ratios in Dogs Fed RE-45601 for 1 Year^a

Dose Level (mg/kg/day)	Liver Weight			Thyroid/Parathyroid Weight		
	Absolute (g)	Relative to Body (g)	Relative to Brain (g)	Absolute (g)	Relative to Body (g)	Relative to Brain (g)
<u>Males</u>						
0	250 $\pm$ 26	2.5 $\pm$ 0.2	3.3 $\pm$ 0.5	0.93 $\pm$ 0.42	.009 $\pm$ 0.004	.012 $\pm$ 0.003
1	259 $\pm$ 31	2.7 $\pm$ 0.2	3.5 $\pm$ 0.4	1.13 $\pm$ 0.32	.012 $\pm$ 0.003	.015 $\pm$ 0.003
75	317 $\pm$ 93	2.9 $\pm$ 0.3*	4.1 $\pm$ 0.9	1.35 $\pm$ 0.54	.012 $\pm$ 0.003	.017 $\pm$ 0.006
300	390 $\pm$ 75*	4.0 $\pm$ 0.3*	5.4 $\pm$ 1.1*	1.78 $\pm$ 0.52*	.018 $\pm$ 0.004*	.025 $\pm$ 0.007*
<u>Females</u>						
0	208 $\pm$ 45	2.4 $\pm$ 0.2	2.8 $\pm$ 0.6	1.04 $\pm$ 0.26	.013 $\pm$ 0.004	.014 $\pm$ 0.003
1	209 $\pm$ 27	2.4 $\pm$ 0.1	2.8 $\pm$ 0.2	1.03 $\pm$ 0.24	.012 $\pm$ 0.003	.014 $\pm$ 0.004
75	279 $\pm$ 56*	3.0 $\pm$ 0.4*	3.8 $\pm$ 0.7*	.99 $\pm$ 0.15	.011 $\pm$ 0.001	.013 $\pm$ 0.002
300	354 $\pm$ 47*	4.2 $\pm$ 0.5*	4.9 $\pm$ 0.8*	1.04 $\pm$ 0.27	.012 $\pm$ 0.001	.014 $\pm$ 0.004

^aData were extracted from Tables 9A, B, and C of the study report.*Significantly different from control values (p $\leq$ 0.05).

A 16% increase in mean liver-to-body weight ratio was seen in males receiving 75 mg/kg/day. In addition, significant ($p \leq 0.05$) increases in mean absolute thyroid/parathyroid weight (91% increase), mean thyroid/parathyroid-to-body weight ratio (100% increase), and mean thyroid/parathyroid ratio (108% increase) were seen in males administered the high dose. However, there was no correlative microscopic finding in thyroids from these dogs. The lack of a microscopic correlate plus the lack of a similar increase in weight in females makes this finding of questionable toxicological significance.

- b. Gross Pathology: The only gross pathological finding which was considered by the study authors to be related to treatment with RE-45601 was the presence of dark liver in 4 of 6 high-dose males and 4 of 6 high-dose females.
- c. Microscopic Pathology: Table 4 summarizes the incidence of frequently occurring nonneoplastic lesions in dogs. Treatment-related changes were present in the liver and sternal bone marrow of both sexes receiving 300 mg/kg/day for 1 year. Minimal-to-moderate centrilobular-to-midzonal hepatocellular enlargement was observed in 5 of 6 high-dose males and 4 of 6 high-dose females. Enlarged hepatocytes often had cytoplasmic clearing. There was slight-to-moderately severe increased pigment in the livers of all high-dose animals. The pigment was granular and golden brown, and was present primarily in the cytoplasm of hepatocytes and, to a lesser degree, in sinusoidal lining cells and bile canaliculi. Special stains consisting of Hall's stain for bile, Prussian blue iron reaction, and acid-fast for lipofuscin were performed on two high-dose and control males each and three high-dose and control females each. By these methods, the pigment was negative for bile, iron, and acid-fast lipofuscin. Therefore, the nature of the pigment is not determined.

Slight hypercellularity was observed in the sternal marrow of all high-dose animals, and was considered by the study authors to be an effect of treatment. Minimal-to-moderate amounts of pigment were observed in the spleens of occasional high- and mid-dose females, high-dose males, and control males. These were considered by the study authors to be sporadic findings due to natural variation and not an effect of treatment.

A variety of spontaneously occurring incidental lesions was observed in all groups, and these were of the type

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TABLE 4. Representative Nonneoplastic Findings in Dogs Fed RE-45601 for 1 Year^a

Organ/Finding	Dose Level (mg/kg/day):							
	Males				Females			
	0	1	75	300	0	1	75	300
<u>Liver</u>								
Mononuclear cell infiltrate	2	0	1	1	2	1	0	0
Hepatocellular enlargement	0	0	0	5	0	0	0	4
Increased pigment	0	0	1	6	0	0	0	6
<u>Marrow, sternum</u>								
Hypercellular	0	0	1	6	0	0	1	6
<u>Spleen</u>								
Increased pigment	1	0	2	2	0	0	2	3

^aBased on six animals/sex/group. Data were extracted from Table 11 of the study report.

and frequency anticipated in this age and breed of dog. No neoplastic lesions were observed.

D. STUDY AUTHOR'S CONCLUSIONS:

RE-45601 was administered via gelatin capsules to groups of six dogs/sex at levels of 1, 75, or 300 mg/kg/day. A similar group of six dogs per sex were given empty capsules daily, and served as a comparative control. All dogs received the appropriate capsules for a minimum of 52 weeks. During the first 7 weeks of study, the high-dose dogs received 200 mg/kg/day; this dose level was changed to 300 mg/kg/day at the beginning of week 8. All dogs were observed twice daily for overt signs of toxicity, and detailed examinations were performed once each week. Individual body weight and food consumption values were determined weekly. Individual body weights were also recorded 1 day prior to the initiation of dosing. Ophthalmoscopic examinations were performed prior to initiation of dosing and on days 180 and 363. Blood and serum samples were collected from each dog prior to initiation of dosing and on days 31, 90, 180, 270, and 360; and urine samples were collected on days 180 and 360 for clinical pathological evaluation. All dogs were killed following 52 weeks of treatment, and complete gross necropsies were performed. Organs were weighed and tissues were processed and evaluated microscopically.

There were no treatment-related effects noted for any of the in-life parameters (i.e., clinical observations, body weight, food consumption, and ophthalmology). Evaluation of hematology data revealed treatment-related decreases in red cell parameters (i.e., erythrocyte counts, hemoglobin concentration, and hematocrit levels) for the high-dose males and females; and treatment-related increases in platelet counts, leukocyte counts, and corrected leukocyte counts for the mid-dose females and high-dose males and females. Apparent treatment-related changes in mean serum chemistry parameters included a decrease in glucose values, and increases in total cholesterol, alanine aminotransferase, alkaline phosphatase, and triglycerides. These changes in mean serum chemistry values were noted for the males and females receiving 300 mg/kg/day. In addition, decreased mean glucose levels were also noted in females of the 75 mg/kg/day dose group at day 360.

Mean liver weights, liver-to-body weight ratios, and/or liver-to-brain weight ratios were significantly higher than control values for the mid-dose and high-dose animals. In addition, mean thyroid/parathyroid weights (absolute and relative to body weight and brain weight) were significantly higher than control values for males receiving 300 mg/kg/day. This latter finding is of questionable significance, since there was no microscopic correlative lesion and a similar weight increase was not noted

in females. Treatment-related gross findings included darkened livers in 4 of 6 high-dose males and 4 of 6 high-dose females. Histopathologic findings included hepatocellular enlargement, increased hepatic pigment, and sternal bone marrow hypercellularity in males and females treated orally with 300 mg/kg/day.

In conclusion, based on the absence of treatment-related effects in the clinical pathology parameters, liver weight data, gross findings in the liver, and microscopic findings in the liver and sternal bone marrow observed at the 75- and/or 300-mg/kg/day levels, the no-observed-effect level (NOEL) for the dog is 1 mg RE-45601 technical/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were well reported. Dose levels were appropriately selected on the basis of a previous 90-day dietary toxicity study. Summary data were supported by individual animal data, and mean values that were validated agreed with the author's values.

The findings of this study suggest that the liver may be a target organ for RE-45601 toxicity. The primary treatment-related alterations observed in the liver of male and female dogs following oral treatment with 300 mg/kg/day consisted of minimal-to-moderate centrilobular-to-midzonal hepatocellular enlargement, and slight-to-moderately severe increased pigment in the liver. Histological changes in the liver correlated with increased liver weights at the high dose. Mean liver weights, liver-to-body weight ratios, and liver-to-brain weight ratios were significantly higher than control values for the high-dose males and females. Absolute and relative (to either body weight or brain weight) liver weights were also increased in females receiving 75 mg/kg/day, but there were no correlative histopathological changes in the livers at this dose. The absolute liver weights of males receiving 75 mg/kg/day were also increased. Although mean absolute thyroid/parathyroid weight, mean thyroid/parathyroid-to-body weight and mean thyroid/parathyroid-to-brain weight ratios were significantly increased in males administered the high dose, there were no corresponding histological lesions in the thyroid following treatment with this dose. Slight hypercellularity was observed in the sternal bone marrow of all high-dose animals, and was considered by the study authors to be an effect of treatment.

Treatment-related findings in hematology and clinical chemistry parameters for females receiving 75 mg/kg/day included increases in mean platelet counts, leukocyte counts, and corrected leukocyte counts, as well as a decrease in

glucose levels. In the high-dose animals, statistically significant decreases from control values were noted for mean erythrocyte values (both sexes), hemoglobin levels (females only), hematocrit values (females only), and glucose levels (both sexes). Increases in total cholesterol levels, alanine aminotransferase activity, alkaline phosphatase activity, and triglyceride levels were seen in both sexes following oral treatment with 300 mg/kg/day. There was no effect of dosing on mortality, body weight, or food consumption.

We agree with the study author's assessment of a NOEL of 1 mg/kg/day for systemic toxicity. The LOEL is 75 mg/kg/day based on increased absolute and relative liver weights in females, and alterations in hematology and clinical chemistry parameters in males and females.

**CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12043)**

EPA No.: 68D80056
DYNAMAC No.: 245-A
TASK No.: 2-45A
April 25, 1990

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DATA EVALUATION RECORD

CLETHODIM (RE-45601)

Chronic Toxicity/Oncogenicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

Date:

Roman J. Penta for
April 25, 1990

EPA No.: 68D80056
DYNAMAC No.: 2-45A
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DATA EVALUATION RECORD

CLETHODIM (RE-45601)

Chronic Toxicity/Oncogenicity Study in Rats

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DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity study in rats. GUIDELINE § 83-5

MRID NUMBER: 410301-21.

TEST MATERIAL: RE-45601 Technical.

SYNONYM(S): (E,E)-(+/)-2[1-[[(3-chloro-2-propenyl)oxy]imino]-propyl]-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one; Sokal 2500 (SX-1688).

STUDY NUMBER: S-2766.

SPONSOR: Chevron Chemical Company-Ortho Agriculture Chemical Division, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc., Richmond, CA.

TITLE OF REPORT: Combined Chronic Oral Toxicity/Oncogenicity Study in Rats With RE-45601 Technical (SX-1688).

AUTHOR: K. K. Dougherty.

REPORT ISSUED: November 1, 1988.

CONCLUSIONS: RE-45601 (Clethodim) was not oncogenic under the conditions of the present study when fed to Sprague-Dawley rats for 2 years at levels of 5, 20, 500, or 2500 ppm (approximately 0.18, 0.64, 19, and 100 mg/kg/day, respectively). Mortality was not significantly increased by dosing; survival in male groups was 33 to 49% and in female groups was 40 to 58%. Mean body weight gains (0-91 days) in high-dose males and females were decreased more than 10% when compared to controls ($p < 0.01$), and mean body weights were significantly lower than controls throughout most of the study in high-dose males and females. Food consumption was marginally decreased in high-dose males and females, and food efficiency was decreased in high-dose males. No compound-related effects were seen for clinical signs, ocular findings, hematology, clinical chemistry, or urinary parameters. Liver weights were increased in high-dose males and females at 12 months compared to controls. This was accompanied by centrilobular hypertrophy of the liver in a few rats. Liver weights were not affected at study termination, and there were no compound-related histologic liver changes in the second year of the study. There were no macroscopic or microscopic findings in other organs that were considered related to dosing. The LOEL for systemic toxicity is 2500 ppm (100 mg/kg/day), and the NOEL is 500 ppm (19 mg/kg/day).

Classification: CORE Guideline.

A. MATERIALS:

1. Test Compound: RE-45601 Technical; description: viscous, clear amber liquid; batch No.: SX-1688; purity: approximately 83%.
2. Test Animals: Species: rat; strain: Sprague-Dawley; age: 42 days at initiation; weight: males--171 to 226 g, females--127 to 169 g; source: Charles River Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated in the study room for 17 days, and those found free of physical and behavioral abnormalities were allocated to the following groups using a computerized randomization method:

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Test Group	Dose in Diet (ppm)	Main Study ^a (24 months)		Interim Sacrifice (12 months)	
		Males	Females	Males	Females
A Control	0	55	55	10	10
B Low (LDT)	5	55	55	10	10
C Inter-mediate	20	55	55	10	10
D Mid (MDT)	500	55	55	10	10
E High (HDT)	2500	55	55	10	10

^aAn additional 20/sex were used for pretest clinical pathology studies.

Rats were housed individually in stainless steel cages in a room with a temperature range of 19.4 to 23.9°C, a humidity range of 20.9 to 90.9%, and a 12-hour light/dark cycle.

2. Diet Preparation: A premix containing 2500 ppm was prepared by adding test material dissolved in nanogram-grade acetone (7 mL/kg diet) to half the amount of weighed diet, rinsing the amber vial that contained diluted test compound with 3 mL acetone, and blending for 5 minutes in a Hobart mixer; remaining chow was added, and diet was mixed for an additional 15 minutes. The diets containing 5, 20, and 500 ppm were prepared by adding rodent chow to the 2500- ppm premix at the appropriate amount and mixing for 15 minutes. Control diets were prepared by adding 10 mL acetone to the lab chow and mixing. Diets were stored frozen in plastic containers with airtight lids until use. Fresh diets were prepared weekly. Diets were dispensed three times per week for 18 weeks; then 5- and 20-ppm diets that had been frozen were dispensed daily.

Samples of diets were tested for homogeneity (nine samples/diet). Concentration and stability of test compound in diets were determined three times/week for 4 weeks, during week 8, and every 4 weeks thereafter.

Results: Homogeneity testing of diets at 500 and 2500 ppm gave coefficients of variation of 1.3 and 3.0%, respectively, and diets were 94.9 and 91.3% of target, respectively. The coefficients of variation for 20-ppm diets (two intervals) were 6.8 and 5.1%, and for 5-ppm diets were 3.1 and 14%; these diets ranged from 87 to 101% of nominal levels.

Test material in diets appeared fairly stable in the freezer for 22 days; however, a slow oxidation to the sulfoxide (RE-45924) was observed over a period of 43 or 64 days. The level of sulfoxide was measured in the diets beginning at study week 65. Diets were analyzed after 1 to 7 days in the freezer and after 1 to 3 days in food hoppers. Table 1 summarizes data on concentration of test material and its sulfoxide in diets. Degradation of test compound was not a serious problem under the conditions of food storage and dispensing. Data for degradation at the 5- and 20-ppm levels are not very reliable because of methodology limitations. Recovery of fortified samples had a range of 90 to 110%; and means ranged from 97.8 to 99.5%.

3. Rationale for Dose Selection: A 5-week pilot feeding study (MRID No. 40301-10) was the basis for dose selection. Groups of 10 rats/sex were fed diets containing 0, 5, 200, 1000, 4000, or 8000 ppm. No signs of toxicity or mortality were observed. The 8000-ppm diets were not palatable throughout the study, and the 4000-ppm diets were initially rejected. Food consumption was decreased 14 and 15% in males receiving 4000 and 8000 ppm, respectively, and 14 and 25% in females at the same doses when compared to controls. Mean body weights in males receiving 8000 ppm were significantly ($p < 0.01$) decreased by day 7 and at 5 weeks were 87% of control body weights. For males receiving 4000 ppm, mean body weight at 5 weeks was 94.6% of control; the decrease was not significant, but the mean weight gain was significantly lower than in controls. Mean body weights in females receiving 8000 ppm were significantly decreased (12% compared to controls) at 7 days and were 84% of control at 5 weeks. For females receiving 4000 ppm, mean body weight was 92% of control at 5 weeks. Mean weight gains were 89 and 72% of the control weight gain (178 g) in males receiving 4000 and 8000 ppm, respectively, and 75 and 56% of the control weight gain (85 g) in females at the same doses. A mild treatment-related anemia was observed; hemoglobin concentrations were significantly lower than controls in males receiving 1000, 4000, and 8000 ppm, and in females receiving 8000 ppm; hematocrit was significantly decreased in males receiving 4000 or 8000 ppm, and erythrocyte counts were decreased in females at the highest dose. No effects of biological importance were observed for clinical chemistry or urinary parameters. A mild centrilobular hypertrophy in the liver, which was considered a reversible adaptive effect, was noted for males receiving 1000, 4000, or 8000 ppm and females at the two highest doses. Absolute liver weights were increased ($p < 0.05$) compared to controls in males dosed at 1000, 4000, and 8000 ppm, and in females receiving 8000 ppm ($p < 0.05$), and liver-to-body weight ratios were increased ($p < 0.01$) in

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TABLE 1. Summary of Analysis of Diets for RE-45601 and Its Sulfoxide

Target Level ^a (ppm)	Target Concentration ^b (ppm)	% of Target ^c	% as Sulfoxide ^d	
			Day 0	Day 3
5	4.15	88.7 ± 8.9 (26)	ND ^e	ND
20	16.4	89.2 ± 12.0 (38)	6.7	16
500	415	91.3 ± 6.0 (36)	8.2	10.2
2500	2080	92.8 ± 4.9 (34)	4.2	5.8

^aBased on technical material.^bCorrected for 83.0% active ingredient in Technical.^cMean values ± coefficient of variation for samples stored frozen for 1 day (the 2500-ppm samples were frozen for 2 days); the numbers in parentheses represent the number of weeks analyses were conducted on samples.^dMean of 14 to 17 samples; day 0 samples were frozen for 1 day or 2 days (2500 ppm), and day 3 samples were in food hoppers for 3 days.^eND -- Not assayed, since the levels were at the limit of detection.

males and females receiving 4000 or 8000 ppm. There were no effects of dosing in either sex fed 5 or 200 ppm Technical RE-45601.

4. Food and Water Consumption: Purina Certified Rodent Chow Meal No. 5002 and water were available ad libitum.
5. Statistics: One-way analysis of variance was used for analysis of body weight, weight gain, food consumption and food efficiency, clinical laboratory values, and organ weight data, and statistical difference among groups was evaluated with Dunnett's test. Differential leukocyte counts were evaluated with the Kruskal-Wallis and Mann-Whitney U-tests. Mortality incidence and selected clinical observation data were evaluated with the Chi-square test. Outlying values for body weight, food consumption, or clinical laboratory values were rejected if the values differed from the mean by more than three standard deviations.
5. Quality Assurance: A Quality Assurance Statement was signed and dated November 1, 1988; a GLP compliance statement was also provided.

C. METHODS AND RESULTS:

1. Observations: Viability checks were made twice daily, and each animal was examined once daily for signs of toxicity. Detailed examinations including palpations for masses were performed weekly.

Results: The mortality incidence was not significantly different from controls in any of the dosed groups. Fifteen accidental deaths, most caused by bleeding, were recorded. Table 2 summarizes mortality data. Survival was greater than 80% in all groups at 18 months, and at termination ranged from 33 to 50% in male groups and 40 to 58% in female groups.

Malocclusion and broken teeth were the most commonly observed clinical findings during the study. The incidence of malocclusion was reported to be significantly increased in high-dose males when compared to controls throughout the study. There was no clear dose-response for males or females. Other frequent signs such as red discharge from the nose, muscular weakness, decreased locomotion, alopecia, and scabs were not more frequent than normal for rats of this strain and age. Table 3 summarizes selected data.

2. Body Weights: Table 4 presents data on mean body weights at selected intervals and mean weight gain data for the first 91 days of the study. Mean body weights in high-dose

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TABLE 2. Mortality and Survival Data for Rats Fed RE-45601 for 105 Weeks

	Dietary Level (ppm):				
	0	5	20	500	2500
<u>Males</u>					
Accidental deaths	2	0	3	1	0
No. dead or moribund	28	28	28	27	37
Mean day to death ^a	596	600	599	545	559
Percent survival ^b at					
18 months	87	91	89	90	82
Termination	47	49	46	50	33
<u>Females</u>					
Accidental deaths	2	3	2	0	2
No. dead or moribund	24	22	26	33	27
Mean day to death ^a	580	578	584	581	566
Percent survival ^b at					
18 months	89	87	87	87	85
Termination	55	58	51	40	49

Source: Tables 1 and 2 of Study No. S-2766.

^aIncludes both animals that died and those sacrificed moribund.^bThere were initially 65 rats/sex/group; 10/sex/group were sacrificed at 12 months. Percent survival is calculated excluding the accidental deaths and rats at the interim sacrifice.

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TABLE 3. Incidence of Selected Clinical Signs in Rats Fed RE-45601 for 2 Years

Observation/Interval	Dietary Level (ppm):									
	Males					Females				
	0	5	20	500	2500	0	5	20	500	2500
<u>Malocclusion</u>										
Year 1	10	19	14	14	23	8	19	10	11	12
Year 2	4	13	12	9	15	10	17	12	15	11
<u>Broken teeth</u>										
Year 1	7	8	10	9	18	4	12	8	4	7
Year 2	5	13	14	8	17	12	20	12	14	12
<u>Nose--red discharge</u>										
Year 1	9	11	10	9	12	8	5	7	3	4
Year 2	13	12	14	8	9	16	9	19	16	13

Source: Tables 3 to 6 of Study No. S-2760.

TABLE 4. Mean Body Weight Data at Selected Intervals and Mean Weight Gains in Rats Fed RE-45601 for 2 Years

Dietary Level (ppm)	Mean Body Weight (g $\pm$ S.D.) at Day:					Weight Gain (g $\pm$ S.D.) 0-91 Days
	0	91	192	360	556	
<u>Males</u>						
0	200 $\pm$ 12	508 $\pm$ 46	591 $\pm$ 64	678 $\pm$ 93	724 $\pm$ 117	698 $\pm$ 117 308 $\pm$ 42
5	200 $\pm$ 12	505 $\pm$ 43	582 $\pm$ 58	651 $\pm$ 81	671 $\pm$ 99*	641 $\pm$ 133 305 $\pm$ 38
20	200 $\pm$ 12	501 $\pm$ 44	576 $\pm$ 62	659 $\pm$ 86	698 $\pm$ 104	634 $\pm$ 156 302 $\pm$ 39
500	200 $\pm$ 12	509 $\pm$ 40	582 $\pm$ 45	645 $\pm$ 54	667 $\pm$ 80*	663 $\pm$ 126 308 $\pm$ 37
2500	199 $\pm$ 12	472 $\pm$ 46**	548 $\pm$ 62**	633 $\pm$ 87**	675 $\pm$ 90	642 $\pm$ 145 273 $\pm$ 41**
<u>Females</u>						
0	151 $\pm$ 9	280 $\pm$ 24	327 $\pm$ 43	401 $\pm$ 79	476 $\pm$ 88	496 $\pm$ 122 129 $\pm$ 20
5	152 $\pm$ 9	274 $\pm$ 24	314 $\pm$ 35	379 $\pm$ 60	448 $\pm$ 73	458 $\pm$ 84 123 $\pm$ 20
20	150 $\pm$ 9	280 $\pm$ 25	325 $\pm$ 39	404 $\pm$ 58	474 $\pm$ 72	488 $\pm$ 112 130 $\pm$ 20
500	150 $\pm$ 9	280 $\pm$ 26	325 $\pm$ 41	393 $\pm$ 63	441 $\pm$ 79	444 $\pm$ 105 129 $\pm$ 21
2500	149 $\pm$ 8	263 $\pm$ 22**	303 $\pm$ 33**	368 $\pm$ 52*	417 $\pm$ 70**	433 $\pm$ 110 113 $\pm$ 18**

Source: Tables 7 to 14 of Study No. S-2760.

*Significantly different from control value, $p < 0.05$.

**Significantly different from control value, $p < 0.01$.

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males and females were significantly less than in controls for most of the study (to day 528 and 696 in males and females, respectively). Body weights were in general similar to controls in the groups receiving up to 500 ppm, although in the second year of the study significant decreases were seen for males receiving 500 ppm (weeks 72 to 88) and for females at the same dose between weeks 92 and 96. There were sporadic decreases in rats receiving 5 ppm.

The mean weight gains during the first 3 months were decreased 11 and 12.5% in males and females receiving 2500 ppm, respectively ($p < 0.01$). Weight gains were not affected in rats receiving 5, 20, or 500 ppm.

3. Food Consumption and Compound Intake: Food consumption for males and females receiving 2500 ppm was marginally lower than for controls. For high-dose males it averaged about 0.6 g/day less than for controls over the first 3 months, and the average consumption was about 26 g/rat/day. When data were expressed as food consumed/100 g body weight/day, the values tended to be higher than controls in males and females receiving 2500 ppm. The decreased weight gains did not correlate with food consumption. Food efficiency (weekly weight gain $\times$ 100 $\div$ food consumed) was decreased in high-dose animals, it was 11% in males receiving 2500 ppm for weeks 1-13 compared to 12.4% for controls, and 6.4% in high-dose females compared to 7.1% for controls; the differences were significant ($p < 0.01$) during 5 of 13 weeks in high-dose males.

The mean compound consumption was 0.15, 0.57, 16, and 86 mg/kg/day for male groups receiving 5, 20, 500, or 2500 ppm, respectively, and 0.2, 0.72, 21, or 113 mg/kg/day for females in the same dose groups.

4. Ophthalmological Examinations: Examinations were performed on all rats prior to initiation and on survivors at 1 and 2 years. Pupillary responses to light were evaluated prior to mydriasis. Then the eyelids, conjunctiva, cornea, and anterior segment were examined with a slit lamp, and the retinal vessels and the optic papillae were examined with a direct ophthalmoscope.

Results: No abnormalities associated with dosing were observed, and no trends for incidence of findings were seen.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus of animals to be continued on the study or from the abdominal aorta of those sacrificed. Samples were collected from 20 rats/sex/group at pretest and at 3, 6, 12, and 24 months for hematology. Serum chemistry was carried out on 10/sex/group at pretest and at 6, 12, and 24 months.

Hematology: The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	Coagulation: thromboplastin time (PT)
X Reticulocyte count (RETIC)	
X Red cell morphology	

Results: No effects of dosing on any of the hematology parameters were observed. Differential leukocyte counts were normal, and there was no effect of dosing on erythrocyte morphology.

b. Clinical Chemistry

<u>Electrolytes</u>	<u>Other</u>
X Calcium [†]	X Albumin [†]
X Chloride [†]	X Albumin/globulin ratio
X Magnesium [†]	X Blood creatinine [†]
X Phosphorus [†]	X Blood urea nitrogen [†]
X Potassium [†]	X Cholesterol [†]
X Sodium [†]	X Globulins
	X Glucose [†]
<u>Enzymes</u>	X Total bilirubin [†]
X Alkaline phosphatase (ALP)	X Direct bilirubin
Cholinesterase	X Total protein [†]
X Creatine phosphokinase [†]	X Triglycerides
X Lactic acid dehydrogenase	X Uric acid
X Serum alanine aminotransferase (SGPT) [†]	
X Serum aspartate aminotransferase (SGOT) [†]	
Gamma glutamyltransferase (GGT)	

[†]Recommended by Subdivision F (October 1982) Guidelines.

Results: No effects that were considered of biological importance were observed for any clinical chemistry parameters. No significant differences were seen at 6 or 12 months. Uric acid was slightly increased at 18 months in males receiving 20 and 2500 ppm, but there was no dose-related effect or change at any other interval. Creatine phosphokinase was significantly decreased ($p < 0.05$) in all dosed groups of females at 2 years when compared to controls, but there was no dose-related trend in females or any effect in dosed males. Other changes that reached a level of significance were sporadic.

6. Urinalysis: Urinalysis was performed on 20 rats/sex at pretest, on 10/sex/group at 6 and 12 months, and on 12/sex/group at 24 months. The CHECKED (X) parameters were examined:

X Appearance [†]	X Glucose [†]
X Volume [†]	X Ketones
X Specific gravity [†]	X Bilirubin [†]
X pH	X Blood [†]
X Sediment (microscopic) [†]	
X Protein [†]	

Results: There were no effects of dosing on any of the urinary parameters.

7. Sacrifice and Pathology: All animals that died or were sacrificed in extremis received a complete necropsy. The CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

[†]Recommended by Subdivision F (October 1982) Guidelines.

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta [†]	XX Brain
X Salivary glands [†]	XX Heart [†]	X Peripheral nerve
X Esophagus [†]	X Bone marrow [†]	(sciatic nerve) [†]
X Stomach [†]	X Lymph nodes [†]	X Spinal cord
X Duodenum [†]	(mesenteric)	(3 levels)
X Jejunum [†]	XX Spleen	X Pituitary [†]
X Ileum [†]	X Thymus	X Eyes
X Cecum [†]		(optic nerve) [†]
X Colon [†]		
X Rectum	<u>Urogenital</u>	<u>Glandular</u>
XX Liver [†]	XX Kidneys [†]	XX Adrenals [†]
X Gallbladder [†]	X Urinary bladder [†]	Lacrimal gland
X Pancreas [†]	XX Testes [†]	X Mammary gland [†]
	X Epididymides	X Thyroids [†]
	X Prostate	X Parathyroids [†]
	X Seminal vesicle	X Harderian glands
<u>Respiratory</u>	XX Ovaries	
X Trachea [†]	X Uterus	<u>Other</u>
XX Lung [†]	(cervix, body, horns)	X Bone (sternum and femur) [†]
		X Skeletal muscle [†]
		X Skin
		X All gross lesions and masses

The above tissues were evaluated microscopically for all animals in the control and 2500-ppm group and for animals in all groups with unscheduled sacrifices or those that died. Lung, liver, kidneys, and all macroscopic lesions were evaluated for the interim and terminal sacrifice animals in the 5-, 20-, and 500-ppm groups. A peer review of histopathology was conducted. Livers of 50% of the control and high-dose animals, as well as benign and malignant liver tumors from all dosage levels, were reviewed. In addition, all protocol tissues were reviewed from four males and four females at the high-dosage level.

Results:

- a. Organ Weights: Table 5 presents data on liver weights. Absolute and relative liver weights tended to be increased in high-dose males and females at both 12 and 24 months. At the 12-month sacrifice, mean liver weight was increased ($p < 0.05$) as compared to control

[†]Recommended by Subdivision F (October 1982) Guidelines.

in high-dose females and liver-to-body weight ratio was increased ($p < 0.01$) in high-dose males. In addition, the liver-to-brain weight ratio was significantly increased ($p < 0.05$) in females receiving 500 or 2500 ppm (data not shown). At termination, the relative liver weight was significantly ($p < 0.01$) greater than controls in high-dose females, but there were no significant effects on absolute or relative liver weights in males. No significant weight changes were observed for brains, kidneys, adrenals, or gonads at either 12 or 24 months in either males or females.

- b. Gross Pathology: No changes in gross findings that were considered compound related were observed for animals sacrificed at 12 or 24 months or for animals sacrificed in extremis or found dead. The findings were typical of those seen in aging rats, or of agonal effects at moribund sacrifice. The number of mammary masses increased in the dosed females (12 months to termination) and was 29, 40, 48, 38, or 51 at dose levels of 0, 5, 20, 500, or 2500 ppm, respectively; however, the incidence of females with masses was not markedly increased (21/51 control females and 24/53 females receiving 2500 ppm.)

c. Microscopic Pathology:

- 1) Nonneoplastic: At the 12-month sacrifice, a trace to mild centrilobular hypertrophy was seen in the liver of 1/10 males and 3/10 females at the highest dose. It was reported that this minimal liver change was possibly related to dosing since it correlated with increased liver weights. The hypertrophy was not found in any other dosed groups or in controls at the interim sacrifice; from 12 months to 2 years, it was present only in 1/51 control females and 2/52 females and in 1/50 males receiving 2500 ppm. No other findings at the interim sacrifice were considered to be related to dosing. Minor increases and decreases in the incidence of other findings were considered to be incidental.

Table 6 summarizes the incidence of nonneoplastic findings from 12 months to study termination. No dose- or compound-related changes were observed. The lesions were those of spontaneous disease or agonal changes. None were unusual for the strain and age of the rats. Centrilobular hypertrophy of the liver was not increased.

TABLE 5. Mean Liver Weights and Liver-to-Body Weight Ratios ($\pm$ S.D.) in Rats Fed RE-45601 for 2 Years

Dietary Level (ppm)	12 Months ^a		24 Months ^b	
	g	% Body Weight	g	% Body Weight
<u>Males</u>				
0	16.58 $\pm$ 3.94	2.64 $\pm$ 0.27	19.06 $\pm$ 5.54	2.93 $\pm$ 0.89
5	18.01 $\pm$ 4.07	2.86 $\pm$ 0.41	16.59 $\pm$ 6.42	2.82 $\pm$ 1.09
20	16.71 $\pm$ 3.19	2.60 $\pm$ 0.21	17.75 $\pm$ 5.24	2.89 $\pm$ 1.24
500	17.52 $\pm$ 2.44	2.85 $\pm$ 0.29	17.63 $\pm$ 3.61	2.78 $\pm$ 0.53
2500	19.08 $\pm$ 3.19	3.21 $\pm$ 0.21**	18.70 $\pm$ 4.12	3.06 $\pm$ 0.84
<u>Females</u>				
0	8.76 $\pm$ 1.42	2.58 $\pm$ 0.21	11.86 $\pm$ 2.20	2.54 $\pm$ 0.53
5	9.22 $\pm$ 1.86	2.65 $\pm$ 0.14	11.10 $\pm$ 2.10	2.62 $\pm$ 0.46
20	9.18 $\pm$ 0.96	2.67 $\pm$ 0.26	11.60 $\pm$ 2.22	2.58 $\pm$ 0.51
500	10.57 $\pm$ 2.32	3.04 $\pm$ 1.26	11.68 $\pm$ 2.66	2.84 $\pm$ 0.47
2500	10.85 $\pm$ 1.11*	3.05 $\pm$ 0.58	12.51 $\pm$ 3.37	3.07 $\pm$ 0.84**

Source: Tables 107 to 110 of Study No. S-2760.

^aBased on 10 rats/group.

^bBased on 13 to 25 males/group and 26 to 30 females/group at study termination.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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TABLE 6. Representative Nonneoplastic Findings in Rats Fed RE-45601 for 2 Years^a

Organ/Finding	Dietary Level (ppm):									
	Males					Females				
	0	5	20	500	2500	0	5	20	500	2500
<u>Adrenal (cortex)</u>	(54) ^b	(31)	(37)	(29)	(50)	(51)	(36)	(38)	(40)	(52)
Atrophy	0	4	5	2	1	0	0	1	0	2
Hematocyst	5	5	5	4	3	43	30	27	29	39
Telangiectasis	3	1	1	0	1	9	8	11	13	10
<u>Bone marrow (sternum)</u>	(53)	(26)	(29)	(25)	(48)	(48)	(19)	(25)	(31)	(52)
Hypocellularity	7	3	8	9	8	4	5	11	12	14
<u>Brain</u>	(54)	(31)	(34)	(27)	(50)	(51)	(34)	(34)	(42)	(52)
Atrophy	14	11	13	12	17	21	26	29	31	22
<u>Heart</u>	(54)	(26)	(29)	(25)	(50)	(51)	(19)	(26)	(32)	(52)
Calcification	4	7	4	6	5	0	2	2	3	0
Chronic myocarditis	49	21	26	19	43	30	8	11	16	31
<u>Kidney</u>	(54)	(53)	(53)	(52)	(50)	(51)	(49)	(52)	(53)	(52)
Chronic progressive nephropathy	50	49	49	49	47	36	29	31	30	37
<u>Liver</u>	(54)	(53)	(53)	(52)	(50)	(51)	(49)	(52)	(53)	(52)
Congestion	12	15	14	7	13	4	7	5	10	7
Bile duct hyperplasia	38	34	35	38	39	31	25	17	33	36
Necrosis:										
Trace/mild	4	5	1	3	1	1	0	1	2	3
Moderate/severe	0	2	3	0	3	0	0	1	1	2
Vacuolar change, fatty	25	15	19	17	12	22	16	15	6	8
Vacuolar change, focus	9	12	10	8	8	11	13	18	11	15
<u>Pancreas</u>	(54)	(29)	(30)	(24)	(50)	(51)	(22)	(25)	(31)	(52)
Pancreatitis	22	7	8	7	13	4	3	3	3	15
<u>Spleen</u>	(54)	(27)	(31)	(26)	(50)	(51)	(19)	(26)	(31)	(52)
Atrophy, lymphoid	1	4	4	6	3	1	4	3	1	0
Increased hemosiderin	21	9	11	8	15	34	12	19	17	28
<u>Testis</u>	(54)	(31)	(34)	(31)	(50)					
Atrophy	11	15	10	11	12					

(continued)

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TABLE 6. (continued)

Organ/Finding	Dietary Level (ppm):									
	Males					Females				
	0	5	20	500	2500	0	5	20	500	2500
<u>Thyroid gland</u>	(54)	(29)	(32)	(27)	(50)	(51)	(21)	(25)	(33)	(52)
Parafollicular hyperplasia	5	1	0	2	2	18	4	5	1	6
Ultimobranchial cyst	9	6	3	5	11	10	1	3	4	8
<u>Uterus</u>						(51)	(25)	(32)	(33)	(51)
Cyst						2	5	2	0	1

Source: Data from Appendix M of Study No. S-2760.

^aIncludes animals that died or were sacrificed after 12 months and those sacrificed at study termination.

^bThe numbers in parentheses represent the number of animals with a specific tissue/organ examined microscopically.

- 2) Neoplastic: Table 7 summarizes the incidence of neoplasms in the main study (12 months to termination). There were no compound-related increases in neoplasms at any site, and the frequency of occurrence was normal for the strain of rat. There were no compound-related effects at 12 months.

D. STUDY AUTHOR'S CONCLUSIONS:

Mortality rates did not differ between groups, and most deaths occurred in the last quarter of the study. No compound-related clinical observations were found; the increased incidence of malocclusion and broken teeth in males receiving 2500 ppm was not compound-related, since there were no associated bone changes and no apparent dose-response trend. The body weight gain decrement was greater than 10% at 3 months in both sexes receiving 2500 ppm, indicating that a maximum tolerated dose was achieved. Food efficiency values were decreased in high-dose males but not females, suggesting that the decreased weights in these males was not due solely to reduced food consumption. No treatment-related changes in ocular findings, clinical pathology, or absolute or relative weights of brain, kidneys, adrenals, or gonads were seen in any dosed group. Significantly increased absolute and/or relative liver weight was observed at the 12-month sacrifice in both sexes at 2500 ppm and in females at 500 ppm. Trace to mild centrilobular hypertrophy was observed in the liver of 3 of 20 high-dose animals at 12 months. There were no increases in histologic liver findings or liver weights at 24 months or in dosed animals that died in the second year of the study. An increase in liver-to-body weight ratio at termination in females receiving 2500 ppm was related to decreased body weight.

No compound-related macroscopic or microscopic lesions were present in this study, with the possible exception of liver hypertrophy (minimal or mild) in high-dose animals at the 12-month sacrifice. No compound-related neoplastic lesions were found. All other lesions were due to spontaneous disease, agonal changes, or traumatic injury. The NOEL was considered to be 500 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. All summary data were supported by individual animal data. A tissue inventory was present, and a tabular correlation of gross and histopathologic findings were available. Very few tissues to be examined were lost or found autolyzed. The correlation between gross and histologic findings was very good; all mammary masses in controls and high-dose females were checked using the correlation table, and all tissues for a small sample of animals were checked.

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TABLE 7. Representative Neoplastic Lesions in Rats Fed RE-45601 for 2 Years^a

Organ/Neoplasm	Dietary Level (ppm):									
	Males					Females				
	0	5	20	500	2500	0	5	20	500	2500
<u>Adrenal</u>	(54) ^b	(31)	(37)	(29)	(50)	(51)	(36)	(38)	(40)	(52)
Cortical adenoma	9	3	1	0	4	6	4	4	2	4
Pheochromocytoma	15	11	10	9	15	8	3	8	2	10
<u>Liver</u>	(54)	(53)	(53)	(52)	(50)	(51)	(49)	(52)	(53)	(52)
Hepatocellular adenoma	10	4	1	3	6	3	3	4	5	5
Hepatocellular carcinoma	5	3	3	3	4	0	0	2	0	2
<u>Pancreas</u>	(54)	(29)	(30)	(24)	(50)	(51)	(22)	(25)	(31)	(52)
Islet cell adenoma	8	2	1	1	1	2	1	1	0	3
<u>Pituitary</u>	(51)	(39)	(41)	(37)	(50)	(49)	(44)	(50)	(51)	(51)
Chromophobe adenoma	24	21	21	22	26	30	32	38	43	27
Chromophobe carcinoma	15	11	8	5	11	16	6	8	6	15
<u>Skin</u>	(53)	(31)	(31)	(29)	(50)	(50)	(20)	(25)	(32)	(51)
Keratoacanthoma	3	2	2	2	3	0	0	0	0	1
Papilloma	1	2	2	2	4	0	0	1	0	1
<u>Testis</u>	(54)	(31)	(34)	(31)	(50)					
Interstitial cell tumor	2	3	4	1	4					
<u>Thyroid</u>	(54)	(29)	(32)	(27)	(50)	(51)	(21)	(25)	(33)	(52)
Follicular cell adenoma	5	2	1	4	4	3	0	0	1	1
C-cell adenoma	9	1	1	3	9	7	2	5	6	9
<u>Mammary gland</u>	(37)	(19)	(17)	(19)	(39)	(49)	(42)	(42)	(40)	(51)
Adenocarcinoma	0	0	0	0	0	7	7	4	3	3
Fibroadenoma	2	0	0	2	1	15	24	17	19	24
<u>Ovary</u>						(50)	(24)	(29)	(37)	(51)
Granulosa cell tumor						0	0	0	0	2
<u>Uterus</u>						(51)	(25)	(32)	(33)	(51)
Leiomyoma						3	0	1	0	0
Polyp						2	3	4	0	2

Source: Data extracted from Appendix M of Study No. S2760.

^aIncludes animals that died or were sacrificed after 12 months and those sacrificed at study termination.^bThe numbers in parentheses represent the number of animals with a specific tissue/organ examined microscopically.

The dietary level selected as the highest dose in the chronic study was based on the pilot study and is justified by decreased food consumption and weight gains at 4000 and 8000 ppm as well as effects on liver weight and the presence of centrilobular hypertrophy in the liver in males receiving 1000 ppm. Although the rats may have been able to tolerate a slightly higher dose in the chronic study, a maximum tolerated dose (MTD) was achieved based on the greater than 10% decrement in body weight gain in both sexes receiving 2500 ppm during the first 90 days of the study.

The authors stated that the liver effects in the chronic study were transitory and were considered to be an adaptive change and not an adverse effect. The results of the pilot study (5 weeks) and a subchronic study support this. In the subchronic study, a recovery period (untreated diet) caused a reversal of the effects on liver weights and the observed centrilobular hypertrophy (DER 245-I). The incidence of centrilobular hypertrophy of the liver at the three highest doses in the pilot study is shown below:

Grade of Centrilobular Hypertrophy	Dietary Level (ppm):			
	0	1000	4000	8000
<u>Males</u>				
	(10) ^a	(10)	(10)	(10)
Trace	0	6	3	1
Mild	0	3	6	3
<u>Females</u>				
	(10)	(10)	(10)	(10)
Trace	0	0	0	0
Mild	0	0	0	3

^aThe numbers in parentheses represent the number of rats with liver examined histologically.

In the pilot study, liver weights were significantly increased ($p < 0.05$) in males receiving 1000, 4000, and 8000 ppm and in females receiving 8000 ppm, and liver-to-body weight ratios were increased ($p < 0.01$) in both sexes receiving 8000 ppm after 5 weeks. At 12 months of the chronic study, one high-dose male had mild hypertrophy at 12 months, 2 females had mild hypertrophy, and 1 had trace hypertrophy. No effects on the liver were seen at 24 months.

Based on the effects on weight gain and the increases in liver weights and the presence of centrilobular hepatic hypertrophy at 12 months, we assess that the LOEL is 2500 ppm and the NOEL is 500 ppm.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12063)

EPA No.: 68D80056
DYNAMAC No.: 245-B
TASK No.: 2-45B
May 1, 1990

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DATA EVALUATION RECORD

RE-45601 TECHNICAL

Chronic Oral Oncogenicity Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McAllen for
Date: May 1, 1990

EPA No.: 68D80056
DYNAMAC No.: 245-B
TASK No.: 2-45B
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DATA EVALUATION RECORD

RE-45601 TECHNICAL

Chronic Oral Oncogenicity Study in Mice

REVIEWED BY:

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Principal Reviewer
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Date: May 1, 1990

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Signature: M. Ioannou
Date: 5/31/90

DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Chronic oral oncogenicity study in mice.

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MRID NUMBER: 410301-12.

TEST MATERIAL: RE-45601 Technical.

SYNONYM(S): Clethodim, (E,E)-(+/-) -2-[1-[[(3-chloro-2-propenyl) oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.

STUDY NUMBERS: Chevron Laboratory Project Identification: S-2867; Hazleton HLA Study No. 2107-145.

SPONSOR: Chevron Chemical Company, Ortho Agricultural Chemicals Division, Richmond, CA.

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA.

TITLE OF REPORT: Chronic Oral Oncogenicity Study in Mice with Chevron RE-45601 (SX-1688).

AUTHOR: Cox, R. H.

REPORT ISSUED: October 25, 1988.

CONCLUSIONS:

RE-45601 technical was administered in the diet to groups of 60 male and female CRL:CD-1(ICR)BR, VAF/Plus mice at levels of 0, 20, 200, 1000, or 3000 ppm for 78 weeks. The high-dose group received 2000 ppm of the test material during the first 15 weeks of the study. No consistent gross clinical signs of toxicity were noted throughout the study. Survival incidence for males and females of the 3000 ppm group was significantly lower than controls at week 78. The only treatment-related effect on hematology parameters was a decrease in erythrocyte counts, hemoglobin, and hematocrit values in mice dosed with 3000 ppm of test material. At week 27, significant ($p < 0.05$) decreases occurred in mean erythrocyte counts in males and females, and in hemoglobin and hematocrit values in males only. No differences relative to respective control values were noted at week 53. The only treatment-related change in red cell mass at week 79 was a significant ($p < 0.05$) decrease in mean erythrocyte counts in 3000 ppm male mice. Absolute and relative liver weight values were increased for 1000-ppm and 3000-ppm male and female mice after 52 weeks of treatment and for 3000 ppm female mice after 78 weeks of treatment. The liver appears to be a target organ for RE-45601 toxicity. Microscopic findings included centrilobular hypertrophy, increased pigment, and bile duct hyperplasia in both male and female mice dosed at 1000 and 3000 ppm. Foci of amphophilic alveolar macrophages were also noted in the lung of these same groups. A treatment-related increase in systemic amyloidosis was noted for unscheduled deaths in the 3000 ppm mice.

RE-45601 was not cncogenic to mice when fed in the diet for 78 weeks. There was an increased incidence of lung tumors in treated male mice when compared to the control group. However, the incidence of lung tumors in treated female mice was similar to that of the control group. The increase in lung tumors was not considered to be related to treatment because a dose response was absent, incidence was similar to historical data, and because there was a lack of statistical significance.

Based on treatment-related effects on survival, red cell mass, liver weights, and microscopic findings in the lung and liver the no-observed-effect level (NOEL) for RE-45601 is 200 in mice when administered via the diet.

Classification: CORE Guideline. This study satisfies requirements for Guideline §83-2.

A. MATERIALS:

1. Test Compound: Chevron RE-45601 technical; description: brown liquid; batch No.: SX-1688; purity: 83.3%.
2. Test Animals: Species: mouse; strain: Crl:CD-1 (ICR)BR, VAF/Plus; age: approximately 6 weeks at study initiation; weight: males--23.0-34.4 g, females--18.4-25.8 g; source: Charles River Laboratories, Inc., Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: After acclimation to laboratory conditions for an unstated time, animals were assigned to the following test groups with a computerized randomization procedure using Bartlett's test and one-way analysis of variance and means between body weights:

Test Group	Dose in Diet (ppm)	Main Study (78 weeks)		Interim Sacrifice (53 weeks)	
		Males	Females	Males	Females
1 Control	0	60	60	10 ^b	10 ^b
2 Low (LDT)	20	60	60	10	10
3 Mid (MDT)	200	60	60	10	10
4 Mid (MDT)	1000	60	60	10	10
5 High (HDT) ^a	2000/3000	60	60	10	10

^aDietary level initially was 2000 ppm. After 15 weeks on study, following evaluation of hematology data from week 15, the dietary level was increased to 3000 ppm at the request of the study sponsor.

^bThese animals were randomly selected from the original 60 animals per group.

Mice were housed individually in a room in which temperature and relative humidity ranged from 67 to 73°F and 26 to 72%, respectively, with a 12-hour light/dark cycle. The animals were examined to ensure they were in good health prior to study initiation. During the first week of the study, two group 4 females (Nos. 22269 and 22282) and one group 5 female (No. 22398) were found dead and replaced; permanent identification numbers remained the same for the replacement animals.

2. Diet Preparation: Test material was removed from the freezer, allowed to reach ambient temperature for approximately 1.5 hours, and weighed into glass amber

vials. Pesticide-grade acetone was used to dissolve the test material (1.5 mL/kg). A 2-kg premix was prepared by adding the test material/acetone mixture to 1 kg of feed, mixing for 5 minutes in a Hobart mixture, then adding the remaining 1 kg of feed and mixing for an additional 15 minutes. The premix was added to the required amount of feed to reach desired concentrations and mixed in a Patterson-Kelly twin-shell mixer fitted with an intensifier bar. The control diet was prepared by mixing 1.5 mL/kg of pesticide-grade acetone to the required amount of feed and mixed using the same procedure as for the test diets.

Diets were prepared weekly, frozen immediately, and stored protected from light. Due to the relative instability of the test material, the diet was replaced three times weekly for groups 3, 4, and 5, and daily for group 2.

Results: Samples of the diet were sent to the sponsor for analysis of homogeneity stability and concentration. Homogeneity of the RE-45601 content of the diets ranged from 83.7 to 97.0% of target concentrations with coefficients of variation ranging from 1.7 to 4.8%. Overall means for freshly prepared diets were 75.3% at 20 ppm, 81.9% at 200 ppm, 90.3% at 1000 ppm, 89.8% at 2000 ppm, and 91.1% at 3000 ppm of the respective target concentration. Stability analyses were performed at various storage times: 0 = freshly prepared; 4H = 3 days in freezer + 1 day in hopper for 20 ppm or 1 day in freezer + 3 days in hopper for higher values; 8H = 7 days in freezer + 1 day in hopper for 20 ppm or 6 days in freezer + 2 days in hopper for higher levels. Table 1 summarizes results for sample types 0 and 8H. Since the sulfoxide (RE-45924) has been shown to be a major degradation product in diets and is a major animal metabolite, the total equivalents of RE-45601 were also determined.

Analysis of RE-45601 technical (SX-1688) at 13 intervals from June 1986 to January 1988 showed that the test material was stable over that time period and contained $82.8 \pm 0.5\%$ active ingredient, RE-45601.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and tap water ad libitum.
4. Statistics: Body weight and body weight change, food consumption, clinical pathology parameters, and organ weights were evaluated by one-way analysis of variance (ANOVA) and Levene's test for homogeneity of variance. The t-tests for equal or unequal variances were used to compare dosed and control groups, and Dunnett's test was used for levels of significance. When variances of untransformed data were heterogeneous, analyses were performed on

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TABLE 1. Stability of RE-45601 and Effect of Sulfoxide (RE-45924) Content^a

Target Level of RE-45601 Technical ^b (ppm)	Sample Type ^c	Overall Mean ppm RE-45601 Found	Percent of Target Concentration	Overall Mean ppm RE-45924 Found	Overall Equivalent Total ppm RE-45601 ^d	Percent of Target Concentration
20	O	12.2	73.5	4.68	16.7	101
	8H	11.6	69.9	4.88	16.6	98.8
200	O	133	80.1	22.2	154	92.8
	8H	130	78.3	19.7	148	89.2
1000	O	752	90.8	73.4	822	99.3
	8H	729	83.0	88.0	807	97.5
3000	O	2270	91.5	146	2410	97.2
	8H	2210	89.1	157	2360	95.2

^aData were extracted from CBI document page 2533.^bTechnical contained 82.8% RE-45601.^cO=Freshly prepared diet; 8H=7 days in freezer and 1 day in hopper for 20 ppm, or 6 days in freezer and 2 days in hopper for higher levels.^dThe ratio of molecular weights (RE-45601/RE45924) is 360/376 = 0.957.

transformed data. When the series of transformations (log, square, square root, reciprocal, arcsine) were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. The criterion for significance of group comparisons was at the 5.0% two-tailed probability level.

5. Quality Assurance: A quality assurance statement was signed and dated October 26, 1988. A GLP Statement of Compliance was signed and dated October 25, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity and once daily for overt signs of toxicity. Detailed examinations including palpation for tissue masses were performed weekly.

Results: It was reported that there were no clinical findings that were compound-related. Most common findings included urine stains, alopecia, rough haircoat, sores, squinted or opaque eyes, lacrimation, enlarged abdomen, and pale or swollen eyes or entire body.

The mortality of 3000-ppm group males and females was significantly ($p < 0.05$) increased at week 78. There was a significant negative trend in survival for both male and female mice. The percent survival at the end of 78 weeks of treatment was 58, 66, 60, 52, and 32 for 0-, 20-, 200-, 1000-, and 3000-ppm males, respectively, and 67, 84, 80, 59, and 48 for 0-, 20-, 200-, 1000-, and 3000-ppm females, respectively.

2. Body Weight: Mice were weighed prior to study initiation, weekly for weeks 1 through 14, at week 16, and every 4 weeks thereafter.

Results: There were no significant differences in body weights or body weight gains between treated and control groups of mice. The mean body weight gains were slightly decreased after the dose was increased from 2000 to 3000 ppm. The gain in males receiving 3000 ppm between weeks 16 and 52 was 1.9 ± 1.8 g compared to 2.7 ± 2.2 g for controls, and the corresponding gain in females was 2.5 ± 1.9 g compared to 3.2 ± 1.9 g for controls. Mean body weights and weight gains at selected intervals are summarized in Table 2.

3. Food Consumption and Compound Intake: Food consumption was determined weekly from weeks 1 through 14 and every 4 weeks, thereafter, beginning on week 16.

Results: It was stated that the mean total food consumption value for weeks 16 through 52 was significantly

TABLE 2. Mean Body Weights at Selected Intervals for Mice Fed RE-45601 Technical for 78 Weeks^a

Dose Level (µgm)	Mean Body Weights (g ± S.D.) at Selected Study Weeks:				Mean Body Weight Gain (g)	
	1	13	32	52	0-13	0-78 Weeks
<u>Males</u>						
0	31.3 ± 1.8	37.6 ± 2.6	40.3 ± 3.4	41.2 ± 3.79	40.6 ± 3.8	7.9 ± 2.21
20	30.9 ± 1.6	37.5 ± 2.3	40.5 ± 3.5	41.5 ± 3.8	40.8 ± 4.5	8.0 ± 2.18
200	30.9 ± 1.7	37.9 ± 2.3	40.1 ± 2.5	41.2 ± 3.1	40.1 ± 3.5	8.5 ± 1.83
1000	31.1 ± 1.7	37.6 ± 2.1	40.2 ± 2.7	41.2 ± 3.1	41.0 ± 3.53	7.9 ± 1.66
3000 ^b	30.9 ± 1.3	36.5 ± 1.8	39.0 ± 2.8	39.2 ± 3.1	39.9 ± 4.4	7.4 ± 1.79
<u>Females</u>						
0	23.7 ± 1.7	30.0 ± 2.1	39.0 ± 2.8	34.5 ± 3.0	35.2 ± 3.1	7.7 ± 1.76
20	23.7 ± 1.3	30.1 ± 2.2	32.4 ± 2.7	34.7 ± 2.6	35.3 ± 3.8	7.8 ± 1.95
200	23.7 ± 1.1	30.0 ± 2.2	32.3 ± 2.5	34.8 ± 2.7	35.8 ± 3.6	7.5 ± 1.78
1000	23.5 ± 1.5	29.8 ± 2.2	32.4 ± 2.7	33.9 ± 2.8	34.9 ± 3.7	7.3 ± 2.09
3000 ^b	24.1 ± 1.4	30.2 ± 1.7	32.3 ± 3.4	33.7 ± 2.8	33.7 ± 2.5	8.1 ± 1.67

^aData were extracted from CBI document Table 3A, pages 52-54 and Table 3B, page 55.^bDose level was 2000 ppm through week 15, then increased to 3000 ppm at request of the sponsor.

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higher for 20-ppm males relative to the controls. All other values for treated groups were similar to control values.

Mean values for efficiency of food utilization were variable, but no consistent or treatment-related differences between groups were observed. Mean food consumption values at selected weekly intervals are shown on Table 3.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.
5. Hematology and Clinical Chemistry: Blood was collected by orbital sinus puncture during weeks 15 and 27 for control, 1000- and 3000-ppm groups, and from all groups during weeks 53 and 79, from 10 animals/sex/group selected randomly using a computer randomization procedure. Ten alternative animals/sex/group were selected and used when necessary. Five animals per sex were selected for the prestudy evaluation. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count ^a
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	Coagulation:thromboplastin
X Reticulocyte count (RETIC) ^a	time (PT)
X Red cell morphology ^a	X Absolute reticulocyte count
X Corrected leukocyte count	(A RETIC)
(COR WBC)	X Blood smears ^b

Results: A slight decrease in erythrocyte counts, and hemoglobin and hematocrit values, was noted in mice of the 3000-ppm group. At week 27, statistically significant ($p < 0.05$) differences relative to respective control values occurred for mean erythrocyte count values in males and females, and for hemoglobin and hematocrit values, in males only. No statistically significant differences occurred at week 53. The only treatment-related change in red cell mass at week 79 was a significant ($p \leq 0.05$) decrease in

[†]Recommended by Subdivision F (November 1984) Guidelines.

^aPreinitiation and weeks 53 and 79 only.

^bOn all animals at scheduled necropsy.

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TABLE 3. Mean Food Consumption (g/wk $\pm$ S.D.) at Selected Weekly Intervals For Mice Fed RE-45601 Technical for 78 Weeks^a

Dose Level (ppm)	Selected Weekly Intervals During Study Period:					
	1	13	28	52	64	78
0	40.3 $\pm$ 3.6	36.7 $\pm$ 2.99	37.3 $\pm$ 8.1	36.0 $\pm$ 3.1	33.2 $\pm$ 3.9	35.9 $\pm$ 3.4
20	37.1 $\pm$ 3.7	38.3 $\pm$ 3.9	38.6 $\pm$ 4.6	37.1 $\pm$ 3.2	35.1 $\pm$ 3.4	36.4 $\pm$ 3.5
200	41.1 $\pm$ 3.5	37.2 $\pm$ 3.0	37.3 $\pm$ 3.9	37.6 $\pm$ 4.1	34.9 $\pm$ 4.4	30.9 $\pm$ 2.9
1000	41.2 $\pm$ 5.2	37.0 $\pm$ 2.9	36.3 $\pm$ 2.1	36.3 $\pm$ 3.9	33.1 $\pm$ 3.5	36.4 $\pm$ 4.3
3000 ^b	41.7 $\pm$ 4.9	38.3 $\pm$ 3.6	38.1 $\pm$ 2.8	35.7 $\pm$ 4.0	33.0 $\pm$ 3.5	35.5 $\pm$ 3.6
0	37.9 $\pm$ 5.8	44.5 $\pm$ 4.4	38.2 $\pm$ 4.0	38.6 $\pm$ 4.2	35.3 $\pm$ 3.8	37.4 $\pm$ 3.8
20	39.4 $\pm$ 5.9	40.6 $\pm$ 12.7	41.2 $\pm$ 4.8	37.7 $\pm$ 5.1	38.8 $\pm$ 5.5	40.3 $\pm$ 7.9
200	40.8 $\pm$ 6.2	42.6 $\pm$ 5.8	39.3 $\pm$ 5.0	40.9 $\pm$ 4.5	35.8 $\pm$ 4.2	31.1 $\pm$ 5.3
1000	38.6 $\pm$ 5.98	43.2 $\pm$ 3.9	41.2 $\pm$ 4.8	39.1 $\pm$ 4.4	35.2 $\pm$ 4.1	38.7 $\pm$ 5.4
3000 ^b	43.1 $\pm$ 6.2	42.0 $\pm$ 6.0	40.8 $\pm$ 5.2	38.3 $\pm$ 4.1	33.6 $\pm$ 4.9	35.3 $\pm$ 4.5

^aData were extracted from CBI document Table 4A, pages 56-58.^bDose level was 2000 ppm through week 15, then increased to 3000 ppm at request of the sponsor.

mean erythrocyte counts in 3000-ppm male mice. All other hematological parameters were similar in treated and control animals at all intervals evaluated. Selected hematology parameter values are summarized in Table 4.

b. Clinical Chemistry: Clinical chemistry parameters were not evaluated.

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: At 53 weeks, 10 animals/sex/group were randomly selected for interim sacrifice using a computerized randomization program. All animals that died or were sacrificed on schedule following 78 weeks of treatment were subject to gross pathological examination and CHECKED (X) tissues were collected for histological examination for the control and high-dose animals. The lung, liver, kidneys, and any masses or gross lesions were examined from all animals. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta [†]	XX Brain [†]
X Salivary glands [†]	X Heart [†]	X Peripheral nerve
X Esophagus [†]	X Bone marrow [†]	(sciatic nerve) [†]
X Stomach [†]	X Lymph nodes [†]	X Spinal cord
X Duodenum [†]	(mesenteric)	(3 levels) [†]
X Jejunum [†]	X Spleen [†]	X Pituitary [†]
X Ileum [†]	X Thymus [†]	X Eyes
X Cecum [†]		(optic nerve) [†]
X Colon [†]		
X Rectum		
XX Liver [†]	<u>Urogenital</u>	<u>Glandular</u>
X Gallbladder [†]	XX Kidneys [†]	XX Adrenals [†]
X Pancreas [†]	X Urinary bladder [†]	Lacrimal gland
	XX Testes [†]	X Mammary gland [†]
	XX Epididymides [†]	X Thyroids [†]
	X Prostate [†]	Parathyroids [†]
	X Seminal vesicle [†]	Harderian glands
<u>Respiratory</u>	XX Ovaries	
X Trachea [†]	XX Uterus [†]	
XX Lung [†]		
		<u>Other</u>
		X Bone (sternum and femur) [†]
		X Skeletal muscle [†]
		X Skin [†]
		X All gross lesions and masses [†]

Recommended by Subdivision F (November 1984) Guidelines.

TABLE 4. Selected Hematology Data ($\pm$ S.D.) at Selected Intervals (Week) for Mice Fed RE-45601 Technical for 78 Weeks^a

Parameter/ Interval (Weeks)	Dose Level (ppm):					
	0	20 ^b	Males 200 ^b	1000	3000 ^c	Females 200 ^b 1000 3000 ^c
Erythrocyte count (MI/μL)						
15	9.34 $\pm$ 0.55			9.23 $\pm$ 0.38	9.15 $\pm$ 0.38	9.35 $\pm$ 0.34 9.13 $\pm$ 0.27
27	9.40 $\pm$ 0.38			9.19 $\pm$ 0.47	8.66 $\pm$ 0.44 ^a	9.31 $\pm$ 0.36 8.92 $\pm$ 0.44 ^a
53	8.19 $\pm$ 0.94	8.22 $\pm$ 0.92	8.78 $\pm$ 0.70	8.44 $\pm$ 0.69	7.46 $\pm$ 0.94	8.75 $\pm$ 0.63 8.96 $\pm$ 0.58 7.76 $\pm$ 0.99
79	9.61 $\pm$ 1.09	8.69 $\pm$ 0.94	8.44 $\pm$ 0.97 ^a	9.23 $\pm$ 0.73	8.23 $\pm$ 1.26 ^a	8.34 $\pm$ 1.71 8.07 $\pm$ 1.55 8.56 $\pm$ 1.24
Hemoglobin (g/dL)						
15	16.2 $\pm$ 0.84			16.0 $\pm$ 0.80	15.8 $\pm$ 0.56	16.6 $\pm$ 0.60 16.3 $\pm$ 0.63
27	16.0 $\pm$ 0.56			15.7 $\pm$ 0.68	14.9 $\pm$ 0.52 ^a	16.1 $\pm$ 0.71 16.1 $\pm$ 0.68
53	14.5 $\pm$ 1.72	14.8 $\pm$ 1.32	15.6 $\pm$ 1.30	14.7 $\pm$ 0.85	13.6 $\pm$ 1.34	15.3 $\pm$ 0.88 14.2 $\pm$ 1.99
79	15.2 $\pm$ 1.64	14.2 $\pm$ 1.21	13.7 $\pm$ 1.38	14.8 $\pm$ 0.97	13.4 $\pm$ 2.62	15.1 $\pm$ 1.34 13.2 $\pm$ 2.5 14.3 $\pm$ 1.2
Hematocrit (%)						
15	48.4 $\pm$ 3.9			47.0 $\pm$ 3.1	47.1 $\pm$ 2.99	48.9 $\pm$ 2.2 47.2 $\pm$ 2.6
27	48.7 $\pm$ 3.1			46.6 $\pm$ 3.5	44.7 $\pm$ 3.2 ^a	48.9 $\pm$ 2.6 46.3 $\pm$ 3.5
53	42.7 $\pm$ 5.0	42.7 $\pm$ 5.3	46.0 $\pm$ 4.5	43.6 $\pm$ 4.0	38.9 $\pm$ 4.9	45.9 $\pm$ 3.97 46.9 $\pm$ 2.96 40.4 $\pm$ 4.3
79	45.9 $\pm$ 5.3	42.9 $\pm$ 3.4	41.6 $\pm$ 4.2	44.1 $\pm$ 5.1	40.6 $\pm$ 7.5	44.7 $\pm$ 4.6 40.1 $\pm$ 6.7 42.5 $\pm$ 3.6

^aData were extracted from CBI document Table 6, pages 63-68.

^bOn weeks 15 and 27, only dose levels 0, 1000, and 3000 ppm were evaluated.

^cDose level changed from 2000 ppm to 3000 ppm after week 15.

^aSignificantly different from control, $p \leq 0.05$.

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Results:

- a. Organ Weights: Table 5 presents summary data for liver weights. At 53 and 79 weeks, mean liver weights were increased in males dosed at 1000 and 3000 ppm and females dosed at 3000 ppm. At 53 weeks statistically significant ($p < 0.05$) differences were noted for the mean absolute liver weight value in the 3000-ppm males and in liver-to-brain weight ratios for the 1000 ppm and 3000-ppm males and 3000-ppm females. At 79 weeks, mean liver-to-body weight and liver-to-brain weight ratios were significantly increased ($p < 0.05$) for the 3000-ppm female group.

At the 53-week sacrifice mean adrenal weights (absolute and/or relative to body or brain) were significantly ($p < 0.05$) decreased for all treated male groups and significantly ($p < 0.05$) increased for 20-, 200-, and 1000-ppm females relative to the controls. There were no histopathologic correlations with these findings. The author did not consider this finding to be treatment related owing to a lack of a dose response, the inconsistency of the change between sexes, and the lack of differences relative to the controls at week 79.

- b. Gross Pathology: The study author reported no apparent treatment-related gross lesions in mice sacrificed at weeks 53 and 79. In mice dying or sacrificed when moribund, an increased incidence of pale kidneys was seen in the 3000-ppm group (males--27% in controls versus 44% in dosed animals, females--22% in controls versus 29% in dosed animals).

- c. Microscopic Pathology:

- 1) Nonneoplastic: The liver appears to be a target organ. The most remarkable finding at the 53-week interim sacrifice was centrilobular hypertrophy in nearly all 1000-ppm and 3000-ppm males and 3000-ppm females, which was judged to be moderately severe in the 3000-ppm males and minimal in 1000 ppm males and 3000-ppm females. At 79 weeks, centrilobular hypertrophy was still present in the two highest dose groups, in both males and females, and was graded moderate to moderately severe in most 3000-ppm males and minimal to slight in 1000-ppm males and 3000 ppm males and females. Table 6 summarizes the incidence and severity of centrilobular hypertrophy in mice treated with the test material. Other treatment-related histopathologic changes were observed in 1000-ppm and 3000-ppm mice of both sexes that died during the study or were sacrificed when moribund or at week 79. These changes included increased pigment

TABLE 5. Summary of Absolute and Relative Liver Weights for Mice Fed RE-45601 Technical for 78 Weeks^a

Dose Level (ppm)	53 weeks			79 weeks		
	g	% Body Weight	% Brain Weight	g	% Body Weight	% Brain Weight
Males						
0	1.53 ± 0.15	4.22 ± 0.54	2.90 ± 0.19	1.77 ± 0.48	5.05 ± 1.31	3.50 ± 0.92
20	1.50 ± 0.19	4.15 ± 0.57	2.84 ± 0.39	1.77 ± 0.55	5.0 ± 1.33	3.43 ± 1.1
200	1.58 ± 0.28	4.32 ± 0.59	2.94 ± 0.60	1.81 ± 0.49	5.20 ± 1.46	3.55 ± 1.0
1000	1.71 ± 0.21	4.93 ± 0.56*	3.33 ± 0.40*	1.89 ± 0.59	5.28 ± 1.60	3.76 ± 1.2
3000 ^b	1.78 ± 0.14*	5.35 ± 0.35*	3.51 ± 0.27*	1.98 ± 0.26	5.70 ± 0.61	3.88 ± 0.41
Females						
0	1.47 ± 0.34	4.78 ± 0.64	2.81 ± 0.57	1.64 ± 0.31	5.46 ± 0.82	3.19 ± 0.53
20	1.27 ± 0.10	4.49 ± 0.37	2.44 ± 0.26	1.55 ± 0.29	5.14 ± 0.88	3.05 ± 0.61
200	1.35 ± 0.14	4.61 ± 0.26	2.53 ± 0.30	1.71 ± 0.35	5.45 ± 0.86	3.37 ± 0.66
1000	1.47 ± 0.13	5.05 ± 0.31	2.83 ± 0.28	1.64 ± 0.29	5.48 ± 0.94	3.24 ± 0.57
3000 ^b	1.76 ± 0.20	6.12 ± 0.53*	3.32 ± 0.36*	1.84 ± 0.31	6.26 ± 0.97*	3.77 ± 0.73*

^aData were extracted from CBI document Table 8A, page 87; Table 88, page 90; and Table 8C; page 93.

^bDose level changed from 2000 ppm to 3000 ppm after week 15.

*Significantly different from control, $p < 0.05$.

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TABLE 6. Incidence and Mean Grade of Centrilobular Hypertrophy in the Liver of Mice Fed RE-45601 Technical

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	20	200	1000	3000 ^b	0	20	200	1000	3000 ^b
Interim Sacrifice (53) Weeks										
Number examined	10	10	10	10	10	10	10	10	10	10
Incidence	0	1	1	8	10	1	2	2	1	9
Mean grade	0	0.2	0.1	1.6	3.0	0.1	0.2	0.2	0.1	1.3
Unscheduled Deaths and Terminal Sacrifice (79 weeks)										
Number examined	49	50	50	50	47	49	50	50	49	50
Incidence	3	2	3	32	46	3	1	1	10	28
Mean grade	0.1	0.0	0.1	1.3	2.7	0.1	0.0	0.1	0.3	1.1

^aData were extracted from CBI document Table 2, page 39.^bDose level changed from 2000 ppm to 3000 ppm after week 15.

in the liver in 1000-ppm and 3000-ppm males and 3000 ppm females and increased incidence of bile duct hyperplasia in 1000-ppm and 3000-ppm males. Foci of amphophilic alveolar macrophages in the lung also were observed. For the 0-, 20-, 200-, 1000-, and 3000-ppm groups, the incidences were 0, 0, 1, 10, and 14 for males, and 0, 0, 0, 5, and 22 for females, respectively. (n=60). Kidney findings included mononuclear cell infiltrates, and nephropathy, which are commonly found in CD-1 mice and were considered unrelated to treatment. Amyloidosis, although a common finding in CD-1 mice, appeared to be a treatment-related finding and was noted in 42% of the males and 36% of the females of the 3000-ppm groups compared to 28% of the males and 22% of the females of the respective control groups. Most of the other changes were age related, within the normal background range for this strain of mice, and were considered to be incidental. Table 7 summarizes the incidence of frequently occurring nonneoplastic findings in the liver and kidney of mice fed RE-45601 technical for 78 weeks.

- 2) Neoplastic: Table 8 summarizes neoplastic findings in the lungs and liver of mice fed RE-45601 technical for 78 weeks. In males an increase in lung adenomas was seen in all treated groups when compared to the control group. In females the incidence of lung adenomas was similar to that of the control group. The incidence of lung carcinomas was low and similar in both males and females. The combined incidence of adenomas/carcinomas (5, 10, 14, 13, and 12 in males and 10, 15, 11, 11, and 10 in females dosed at 0, 20, 200, 1000, and 3000 ppm, respectively) showed an increase in treated males when compared to controls but in females the incidence was similar in the controls and treated groups. These tumors were considered by the author to be unrelated to treatment because of an absence of a dose response, similar incidence in historical data, apparent late onset, and lack of statistical significance. The testing laboratory's historical incidence of combined alveolar/bronchiolar tumors in 7 studies with 373 CD-1 mice (see CBI document Text Table 1) was 14.8% for males (range of 5.5 to 26.5%) and 7.8% for females (range of 4.0 to 18.4%). Although the incidence of hepatocellular adenomas was increased in high-dose females, hepatocellular adenomas and carcinomas were low in incidence and considered to be unrelated to treatment. The incidence of tumors at other sites was low and considered to be incidental.

TABLE 7. Nonneoplastic Findings in the Liver and Kidneys of Mice Fed RE-45601 Technical for 78 Weeks^a

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	20	200	1000	3000 ^b	0	20	200	1000	3000 ^b
Interim Sacrifice (53 weeks)										
<u>Liver</u>	(10) ^c	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Centrilobular hypertrophy	0	1	1	8	10	1	2	2	1	9
Amyloidosis	4	3	6	2	4	2	2	2	3	4
Fatty changes	4	3	0	1	2	1	1	0	1	3
Bile duct hyperplasia	0	0	0	0	1	0	0	0	0	0
Increased pigment	0	0	0	0	5	0	0	0	0	0
Inflammation, subacute	8	10	10	10	10	10	10	9	10	10
Individual cell necrosis	0	1	1	2	1	0	0	0	0	0
<u>Kidney</u>	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Mononuclear cell infiltrate	10	10	10	10	10	10	10	10	10	10
Amyloidosis	5	5	5	6	8	6	4	2	3	5
Nephropathy	5	8	8	7	7	7	8	9	9	8
Unscheduled Deaths and Terminal Sacrifice (79 weeks)										
<u>Liver</u>	(49)	(50)	(50)	(50)	(46)	(49)	(50)	(50)	(49)	(50)
Centrilobular hypertrophy	3	2	3	32	46	3	1	2	10	28
Amyloidosis	30	31	29	26	26	26	18	21	21	26
Fatty changes	7	6	5	4	2	7	5	7	8	6
Bile duct hyperplasia	0	0	2	7	12	1	0	0	0	2
Increased pigment	0	0	2	12	25	3	2	4	6	16
Inflammation, subacute	47	45	47	48	43	46	48	45	45	48
Individual cell necrosis	21	15	23	28	23	18	18	26	18	17
<u>Kidney</u>	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Mononuclear cell infiltrate	45	48	45	46	45	46	46	46	44	45
Amyloidosis	32	34	33	29	31	30	27	29	26	28
Nephropathy	45	43	43	43	46	39	38	38	44	44

^aData were extracted from CBI document, Table 10A, pages 153, 154; Table 10B, pages 173, 174; and Table 10C, pages 188-190.

^b2000 ppm was changed to 3000 ppm after week 15.

^cNumbers in parentheses represent the number of tissues examined.

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TABLE 8. Neoplastic Findings in the Lungs and Liver of Mice Fed RE-45601 Technical for 78 Weeks^a

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	20	200	1000	3000 ^b	0	20	200	1000	3000 ^b
<u>Lung</u>	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
Alveolar/bronchiolar adenoma	5	10	12	11	10	9	10	11	10	8
Alveolar/bronchiolar adenoma, multiple	0	0	0	0	2	0	2	0	1	2
Alveolar/Bronchiolar carcinoma	0	0	2	2	0	1	3	1	0	0
Combined adenomas and carcinomas	5	10	14	13	12	10	15	11	11	10
<u>Liver</u>	(59)	(60)	(60)	(60)	(57)	(59)	(60)	(60)	(59)	(60)
Hepatocellular adenoma	2	2	6	3	5	0	0	0	0	4
Hepatocellular adenoma, multiple	0	2	1	2	0	0	0	1	0	0
Hepatocellular carcinomas	2	0	3	2	0	1	1	0	0	0
Combined adenomas and carcinomas	4	4	10	7	5	1	1	1	0	4
Hemangiosarcoma	0	0	0	0	0	0	1	0	2	0
Hemangioma	0	0	0	2	0	0	1	1	0	0

^aData were extracted from CBI document, Table 10A, pages 153-155; Table 10B, pages 173, 174; and Table 10C, pages 188-190.

^b2000 ppm dose level was changed to 3000 ppm after week 15.

D. STUDY AUTHORS' CONCLUSIONS:

Dietary administration of RE-45601 technical to male and female CRL:CD-1(ICR)BR, VAF/Plus mice for 78 weeks resulted in several treatment-related findings. There was a statistically significant decrease in survival of male and female mice fed 3000 ppm of test material. Treatment-related slight decreases in red cell mass were noted in 3000-ppm mice. The mean erythrocyte, hemoglobin values, and hematocrit values for 3000-ppm male and erythrocyte count for 3000-ppm females were lower than respective control values at weeks 27 and 53. The findings were statistically significant ($p < 0.05$) at 27 but not at 53 weeks. Treatment-related changes in red cell mass at week 79 were limited to a significantly lower mean value for erythrocyte count in 3000-ppm male mice.

In 3000-ppm mice that died or were sacrificed when moribund there was an increased incidence of pale kidneys thought to be correlated with the microscopic finding of amyloidosis. Mean absolute or relative (to body weight or brain weight) liver weights were increased for 1000-ppm and 3000-ppm males and females after 52 weeks of treatment and for 3000-ppm females after 78 weeks of treatment.

Treatment-related histopathologic changes included centrilobular hypertrophy in the liver of 1000-ppm and 3000-ppm males and 3000-ppm females. Treatment-related changes observed in the unscheduled deaths and in mice sacrificed at week 79 included foci of amphophilic alveolar macrophages in the lung, and centrilobular hypertrophy, increased pigment, and bile duct hyperplasia in the liver of 1000-ppm and 3000-ppm male and female mice. An increased incidence in systemic amyloidosis was noted for 3000-ppm animals.

The study author stated there was an increased incidence of lung adenomas and carcinomas in the control females and treated males and female relative to control males. The study author did not believe these tumors were related to treatment with RE-45601 technical because of the absence of a dose response, a similar incidence in historical data, the apparent late onset of the tumor, and the lack of statistical support.

Based on the absence of the treatment-related effects on survival, red cell mass, liver weights, and microscopic findings in the lung and liver that were observed at 1000 ppm and 3000 ppm, the no-observed-effect level (NOEL) for dietary administration of RE-45601 technical is 200 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the conduct of the study and reporting of the data were acceptable. Summary data were supported by individual animal data; historical data for lung tumor incidences and mean values that were validated agreed with those of the study author. The MTD was probably achieved, since the incidence of survivors was significantly reduced at the high dose. In a previous 4-week subchronic oral toxicity study in mice (DER 2-45N), erythrocyte counts and hemoglobin values were significantly decreased in animals of both sexes administered 1500 ppm of the test material in the diet; increased liver weights were also noted in male mice at the same dose level. After evaluation of the week 15 hematology results, in which a decrease in red cell mass was anticipated from previous studies but not observed in this study, the high-dose level was increased from 2000 ppm to 3000 ppm at the request of the study sponsor. We agree that there were no significant differences in body weights, body weight gains, or food consumption except for 20 ppm males during weeks 16 through 52. A decrease in red cell mass (e.g., decreased erythrocyte counts, hemoglobin and hematocrit values) occurred in 3000-ppm mice. Other hematology parameters were unremarkable. Amyloidosis was the main cause of death as summarized below:

Dose Levels (ppm)	Males		Females	
	% Survival	Amyloidosis as Cause of Death	% Survival	Amyloidosis as Cause of Death
0	58	14/50 (28%)	67	11/50 (22%)
20	66	15/50 (30%)	84	3/50 (6%)
200	60	14/50 (28%)	80	5/50 (10%)
1000	52	16/50 (32%)	59	11/50 (22%)
3000	32	21/50 (42%)	48	18/50 (36%)

Amyloidosis is frequently noted in aging mice of the CD-1 strain, however, the increase in incidence in the 3000-ppm group was considered a treatment-related exacerbation of the phenomenon.

It is not clear whether the amyloidosis compromised the study or confounded the interpretation of histologic findings.

Mean absolute liver weights and/or relative liver weights (to body or brain weight) were significantly ($p < 0.05$) increased for the 1000-ppm and 3000-ppm males and females after 52 weeks of treatment and for the 3000 ppm female at termination of the study following 78 weeks of treatment. At the 53-week sacrifice mean absolute and relative adrenal weights were significantly decreased for all treated male groups and significantly increased for 20-, 200-, and 1000-ppm females relative to the controls. We agree with the author that the adrenal findings are not treatment related because of lack of dose response, inconsistency of the change between sexes, and no differences relative to the controls at week 79.

The liver appears to be a target organ. Centrilobular hypertrophy was observed in 1000-ppm males and 3000-ppm males and females following 52 weeks of treatment. Treatment-related changes, including centrilobular hypertrophy, increased pigment in the liver, and increased incidence of bile duct hyperplasia were observed in 1000-ppm and 3000-ppm mice that died or were sacrificed when moribund, or at week 79. Foci of amphophilic alveolar macrophages in the lung were also noted in these same groups of mice.

There was a low incidence of hepatocellular adenomas and carcinomas. However, the incidence of adenomas in high-dose females was increased (6.7%) relative to the control group (0%). Historical data from the study author's laboratory (See: Representative Historical Control Data, Part 16, Neoplasia in untreated CD-1 mice. Hazleton Laboratories America, Inc., 2/22/84) in which untreated controls (284 males and 294 females) were examined showed a 6.3% (range of 0-16%) incidence for hepatocellular adenomas in males and a 2.0% (range of 0-6%) incidence in females. The incidence for hepatocellular carcinomas was 14.4% (range of 6-28%) in males and 2.0% (range of 0-12%) for females. We agree with the study author that liver adenomas and carcinomas were unrelated to treatment.

There was an increased incidence of lung adenomas and carcinomas in treated males relative to control males. We agree with the study author that these tumors were not related to treatment with RE-45601 technical owing to the absence of a dose response, a similar incidence in historical data, the apparent late onset of the tumors, and the lack of statistical significance.

Based on treatment-related effects on survival, red cell mass, absolute and relative liver weights, and microscopic findings in the liver and lung the no-observed-effect level (NOEL) for RE-45601 technical is 200 ppm, and the lowest-observed-effect level (LOEL) is 1000 ppm when administered to mice via the diet.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-D
TASK No.: 2-45D
May 1, 1990

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DATA EVALUATION RECORD

CLETHODIM TECHNICAL

Developmental Toxicity Study in Rabbits

STUDY IDENTIFICATION: Dearlove, G.E. Argus Research Laboratories, Inc. Protocol 303-007 teratology study in rabbits with Chevron RE-45601 (Chevron Protocol No. S-2869). (Unpublished study No. S-2869 conducted by Argus Research Laboratories, Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated January 30, 1987.) MRID No. 410301-15.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William J. McEllen for
Date: May 1, 1990

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1. CHEMICAL: (E,E)-(+/)-2-[1-[[3-chloro-2-propenyl)oxy]imino] propyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohex-1-one.
2. TEST MATERIAL: RE-45601 technical, batch No. SX-1688, 82.6% pure, amber liquid.
3. STUDY/ACTION TYPE: Developmental toxicity study in rabbits.
4. STUDY IDENTIFICATION: Dearlove, G.E. Argus Research Laboratories, Inc. Protocol 303-007 teratology study in rabbits with Chevron RE-45601 (Chevron Protocol No. S-2869). (Unpublished study No. S-2869 conducted by Argus Research Laboratories, Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated January 30, 1987.) MRID No. 410301-15.

5. REVIEWED BY:

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Date: 5/31/90

DATA EVALUATION RECORD

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STUDY TYPE: Developmental toxicity. Guideline §83-3.

MRID NUMBER: 410301-15.

TEST MATERIAL: RE-45601 technical.

SYNONYM(S): Clethodim technical, Select.

STUDY NUMBER: S-2869.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham, PA.

TITLE OF REPORT: Argus Research Laboratories, Inc. Protocol 303-007 Teratology Study in Rabbits with Chevron RE-45601 (Chevron Protocol No. S-2869).

AUTHOR: Dearlove, G.E.

REPORT ISSUED: January 30, 1987.

CONCLUSIONS: A developmental toxicity study was conducted in which New Zealand White rabbits were administered RE-45601 via gavage at 0, 25, 100, or 300 mg/kg/day during gestational days (GD) 7 through 19. Maternal toxicity, observed at the mid- and high-dose levels, was manifested as clinical signs of toxicity and reduced weight gain and food consumption during treatment. Based on these results, the maternal NOEL and LOEL were 25 and 100 mg/kg/day, respectively.

Developmental toxicity was not observed in the present study, and therefore, the developmental LOEL cannot be determined. The developmental NOEL was 300 mg/kg/day.

Classification: CORE Minimum Data.

A. MATERIALS:

Test Compound: Purity: 82.6%.
Description: Clear amber liquid.
Lot No.: SX-1688.
Contaminants: Not reported.

Vehicle: 0.7% Carboxymethyl cellulose in water
containing 0.5% Tween 80; Sigma Chemical Co.,
St. Louis, MO.

Test Animals: Species: Rabbit.
Strain: New Zealand White [Hra:(NZW)SPF].
Source: Hazleton Research Products, Inc.,
Denver, PA.
Age: Approximately 6 months on GD 0.
Weight: 2930-4270 g on GD 0.

B. STUDY DESIGN:

This study was designed to assess the potential of RE 45601 to cause developmental toxicity in rabbits when administered daily via gavage from GD 7 through 19, inclusive.

Mating: Does were fertilized by means of artificial insemination. Each female was administered 20 USP Units/kg body weight of Human Chorionic Gonadotrophin (Pregnyl, Organon, Inc., Lot No. 25445, Abbott Lot 86-249-DM-02) approximately 3 hours prior to insemination. An estimated 0.25 mL of semen (from untreated proven breeders of the same strain and source), which had been diluted with normal saline to a concentration of 6.0×10^5 spermatozoa/0.25 mL saline, was used to artificially inseminate each rabbit. The day of insemination was designated as GD 0.

Group Arrangement: Inseminated females were randomly assigned to dose groups based on body weight during the acclimation period as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	20
Low dose	25	20
Mid dose	100	20
High dose	300	20

Dosing: Doses were administered daily on GD 7 through 19 in a volume of 5 mL/kg, based on body weight. Dosage volumes were adjusted daily for observed body weight changes. No correction was made for purity. Dosing suspensions were prepared daily. Analyses for identity and purity were conducted prior to initiation of the study. Stability of the test material was unknown to the testing facility, and the material was considered unstable at ambient temperature and was stored frozen. However, in the addendum to the preliminary study, it was stated that the purity of the test compound stored frozen was 83.3% on November 8, 1985, and 82% on June 2, 1986. Therefore, the compound was considered stable. Homogeneity and concentration of dosing suspensions were analyzed twice during the study.

The selection of doses was based on a preliminary study in rabbits (eight does per group) in which doses of 0, 50, 150, 300, or 500 mg/kg/day of RE-45601 technical were administered via gavage from GD 7 through 19. Dosage-dependent inhibition of body weight gain, reduced feed consumption, increased incidence of dried feces (50 mg/kg/day and above), increased absolute and relative (to body weight) liver weight, increased incidence of gastrointestinal lesions (hairball and/or gastric ulceration), death (300 mg/kg/day and above), and abortion and premature delivery (500 mg/kg/day) were observed. Adverse developmental effects included decreased fetal body weights and increased resorption rates at 300 and 500 mg/kg/day.

Observations: Animals were observed twice daily for mortality and twice daily during the treatment period and once daily during the pre- and posttreatment periods for overt signs of toxicity. Body weights and food consumption were recorded daily during the study. Females dying or aborting during the study were subjected to a gross necropsy, and uterine contents were recorded. Surviving females were sacrificed on GD 29, and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathology assessment;
- Number of corpora lutea;
- Gravid uterine weight;
- Condition of uteri and placentae;
- Number of implantation sites; and
- Number of resorptions and live and dead fetuses.

The viable fetuses were examined in the following manner:

- Individual fetuses were weighed and sexed and their uterine position was recorded;
- External alterations were recorded;
- Visceral alterations were evaluated by dissection and recorded;
- Fetal brains were cross-sectioned freehand; and
- Skeletal alterations were recorded using a modified method of Staples and Schnell (1963).

Statistical Analysis: The following analyses were conducted:

- Maternal physical sign data, incidence of pregnancies, abortion, death, and resorption--variance test for homogeneity of the binomial distribution;
- Body weights (fetal and maternal), body weight change, food consumption, feed utilization, fetal ossification sites, percent dead or resorbed conceptuses, percent male fetuses, percent of fetuses with alterations--Bartlett's test of homogeneity of variances, and (for parametric data) analysis of variance, followed by Dunnett's test, or (for nonparametric data) Kruskal-Wallis test, followed by Dunn's method of multiple comparisons;
- Maternal body weight change, food consumption, and feed utilization for GD 7-10, 7-20, 7-29, 0-29, and corrected weight gain--analysis of covariance;
- Cesarean section data, <75% ties occurring--Kruskal-Wallis and Dunn's tests;

- Cesarean section data, >75% ties occurring--Fisher's Exact test; and
- Proportion data--variance test for homogeneity of binomial distribution.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated November 14, 1988, was provided.
- A signed Statement of Compliance with EPA GLP's, dated February 12 and February 17, 1987, was provided.
- A signed Quality Assurance Statement, dated January 30, 1987, was provided.

C. RESULTS:

The following results were reported by the study author.

1. Dose Analysis: Analyses of homogeneity and concentration of dosing suspensions ranged from 95.6 to 103% and 83.6 to 107%, respectively, of target concentrations. Analysis of stability was not conducted.

2. Maternal Toxicity:

Mortality: One pregnant mid-dose animal was found dead on GD 17. Necropsy revealed gastric ulceration and a red fluid-like substance in the uterus.

Abortion: One low-dose animal aborted five conceptuses on GD 22. Necropsy revealed a parovarian cyst.

Clinical Observations: Dried feces were observed for two control, one low-dose, three mid-dose, and five high-dose animals (high-dose animals significantly different from controls, $p \leq 0.01$). Red substance in the cage pan occurred for two high-dose animals on a total of 3 days (significantly different from controls, $p \leq 0.01$).

Body Weight: A summary of maternal body weight gain and corrected weight gain data is presented in Table 1. In the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups, a significant inhibition of maternal body weight gain was observed during the dosing period. A significant ($p \leq 0.01$) rebound effect on maternal body weight gain occurred for the high-dose group during the postdosing period.

TABLE 1. Mean Body Weight Gains (g $\pm$ S.D.)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-7)	Dosing Period (GD 7-19)	Post-dosing Period (GD 20-29)	Entire Gestation Period (GD 0-29)	Corrected Body Weight Gain ^b
0	160 $\pm$ 63	180 $\pm$ 110	90 $\pm$ 100	440 $\pm$ 180	0 $\pm$ 200
25	150 $\pm$ 100	130 $\pm$ 100	110 $\pm$ 130	390 $\pm$ 190	- 10 $\pm$ 200
100	140 $\pm$ 70	50 $\pm$ 210*	60 $\pm$ 130	240 $\pm$ 230	-200 $\pm$ 220
300	200 $\pm$ 100	-100 $\pm$ 270**	240 $\pm$ 130**	340 $\pm$ 230	- 60 $\pm$ 280

^aData were extracted from study No. S-2869, Table 5.^bCalculated as total body weight gain minus gravid uterine weight.*Significantly different from controls (p $\leq$ 0.05).**Significantly different from controls (p $\leq$ 0.01).

Food Consumption: A summary of food consumption data is presented in Table 2. In the high-dose group, significant decreases in food consumption (g/day, p $\leq$ 0.01) and feed utilization (g/kg/day, p $\leq$ 0.05, data not shown) were observed during the dosing period. Nonsignificant decreases for these parameters were observed in the mid-dose group during the same period.

Gross Pathological Observations: Parovarian cysts were observed among animals from all dose groups. One low-dose animal had ulcerated areas of the gallbladder, and another low-dose animal had discoloration of the liver. One mid-dose animal had an abscess on the neck. One high-dose animal had agenesis of the uterine horn.

TABLE 2. Mean Food Consumption (g/day $\pm$ S.D.)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-7)	Dosing Period (GD 7-19)	Post-dosing Period (GD 20-29)	Entire Gestation Period (GD 0-29)
0	173 $\pm$ 11	152 $\pm$ 18	123 $\pm$ 33	153 $\pm$ 16
25	163 $\pm$ 22	152 $\pm$ 31	126 $\pm$ 25	146 $\pm$ 22
100	165 $\pm$ 15	138 $\pm$ 44	110 $\pm$ 38	134 $\pm$ 32
300	168 $\pm$ 18	117 $\pm$ 49**	136 $\pm$ 33	135 $\pm$ 31

^aData were extracted from study No. S-2869, Table 6.**Significantly different from controls (p $\leq$ 0.01).

Cesarean Section Observations: A summary of cesarean section data is presented in Table 3. By pairwise comparison, none of these parameters was significantly affected by treatment with RE-45601.

3. Developmental Toxicity:

A summary of external, visceral, and skeletal alterations is presented in Table 4.

External Examinations: Downward rotated forelimb(s) were observed for one low-dose, one mid-dose, and one high-dose fetus. The low-dose fetus also exhibited other limb defects. An additional high-dose fetus had an umbilical hernia.

Visceral Examinations: Agenesis of the intermediate lobe of the lungs was observed in one low-dose and three (three litters) mid-dose fetuses. One fetus in the low-dose group had an ectopic kidney.

Skeletal Examinations: Skeletal alterations were observed among animals in all dose groups and included the skull bones, vertebrae, sternebrae, ribs, front limbs, xiphoid, hyoid, pelvis, and manubrium (see Table 4). The number of fetuses with angulated hyoid alae was significantly increased in the low-dose ($p \leq 0.05$) and high-dose ($p \leq 0.01$) animals. The number of fetal ossification sites per litter was similar in all dose groups (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS:

1. Dose Analysis: When analyzed for concentration, the results ranged from 83.6 to 107% of the target concentration. A commonly accepted range is $\pm 10\%$. Because only 4 of the 21 samples analyzed were $<91.5\%$, the reviewers concluded that the range was acceptable.
2. Maternal Toxicity: Maternal toxicity (evidenced by clinical signs of toxicity, decreased weight gain, and decreased food consumption) was observed in does receiving 100 and 300 mg/kg/day of RE-45601. Based on these results, the maternal NOEL and LOEL were 25 and 100 mg/kg/day, respectively.

TABLE 3. Cesarean Section Observations^a

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Parameter	Dose Level (mg/kg/day)			
	0	25	100	300
No. animals assigned	20	20	20	20
No. animals pregnant	19	18	17	17
Pregnancy rate (%)	95	90	85	85
Maternal wastage				
No. died/pregnant	0	0	1	0
No. nonpregnant	1	2	3	3
No. aborted	0	1	1	0
Total corpora lutea ^b	182	149	170	194
Corpora lutea/dam ^c	9.6 ± 2.8	8.8 ± 1.2	10.6 ± 2.9	11.4 ± 2.6
Total implantations ^b	157	118	124	121
Implantations/dam ^c	8.3 ± 2.7	6.9 ± 2.1	7.8 ± 2.4	7.1 ± 3.4
Total live fetuses	138	114	117	111
Live fetuses/dam ^c	7.3 ± 2.8	6.7 ± 2.2	7.3 ± 2.3	6.5 ± 3.0
Total resorptions ^b	18	3	7	10
Early	16	3	5	6
Late	2	0	2	4
Resorptions/dam ^{b,c}	0.9 ± 1.4	0.2 ± 0.4	0.4 ± 0.6	0.6 ± 0.7
Total dead fetuses	1	1	0	0
Dead fetuses/dam ^c	0 ± 0.2	0 ± 0.2	0 ± 0.0	0 ± 0.0
Fetal weight/litter (g) ^c	44.9 ± 7.2	44.2 ± 6.3	43.8 ± 6.4	44.5 ± 9.0
Preimplantation loss (%) ^d	15**	21	25	36
Postimplantation loss (%) ^e	12	5	6	6
Sex ratio (% male)	42	55	52	44

^aData were extracted from study No. S-2869, Tables 8 and 9.^bCalculated by the reviewers.^cMean ± S.D.^dCalculated by the reviewers as $\frac{(\text{No. corpora lutea} - \text{No. implantations})/\text{litter}}{\text{No. corpora lutea/litter}} \times 100$.^eCalculated by the reviewers as $\frac{(\text{No. implantations} - \text{No. viable fetuses})/\text{litter}}{\text{No. implantations/litter}} \times 100$.**Significant trend ($p \leq 0.01$), calculated by the reviewers using linear regression analysis.

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TABLE 4. Summary of Fetal Alterations^a

Findings ^b	Dose Level (mg/kg/day)			
	0	25	100	300
No. fetuses (litters) examined	140 ^c (19)	115 ^c (17)	117 (16)	111 (17)
<u>External examination</u>				
Umbilical hernia	0	0	0	1
Front limb: rotated downward	0	1	1	1
left radius protruding through skin with agenesi of digits 1 and 2	0	1	0	0
agenesi of right digit 1	0	1	0	0
<u>Visceral examination</u>				
Lungs, intermediate lobe, agenesi	0	1	3 (3)	0
Kidney, right, ectopic	0	1	0	0
<u>Skeletal examination</u>				
Skull, ossification				
irregular in various bones	12 (7)	14 (9)	23 (11)	17 (9)
Hyoid, angulated ala(e)	2 (2)	6*(4)	0	7** (6)**
Vertebrae, hemivertebrae, attached rib, fused, asymmetric, bifid, small, or unilateral ossification	6 (3)	2 (2)	1	0
Sternebrae, duplicated, fused, or asymmetric	7 (4)	3 (3)	2 (2)	1
Ribs, fused, split, thickened areas of ossification, or turned into each other at base	3 (3)	2 (2)	0	1
Pelvis, pubes, not ossified	0	0	0	1
Xiphoid, duplicated	0	0	0	2 (1)
Manubrium, duplicated or irregularly ossified	0	0	2 (2)	0

^aData were extracted from study No. S-2869, Tables 11, 12, and 22.^bMore than one type of alteration may be found in one fetus.^cIncludes one dead fetus that was excluded from the statistical evaluation.*Significantly different from controls ($p \leq 0.05$).**Significantly different from controls ($p \leq 0.01$).

3. Developmental Toxicity:

- a. Deaths/Resorptions: In a previous dose range-finding study with RE-45601, developmental toxicity (a nonsignificant increase in number of resorptions, five does per group) had been observed at 300 mg/kg/day. In the present study, this increase was not observed. However, a positive trend was noted in preimplantation loss with an increasing dose of RE-45601. Upon group comparisons, no significant differences were noted. Preimplantation loss is calculated on the basis of the numbers of corpora lutea and implantation sites. These parameters were all within the range of the historical controls. In addition, dosing of the animals did not start until GD 7, at which point the embryos are probably implanted. Therefore, the observed trend is not considered biologically important.
- b. Altered Growth: No compound-related effects were observed.
- c. Developmental Alterations: A variety of alterations, especially skeletal, were observed in all dose groups. A nonsignificant increase in the mid-dose group was noted in the number of fetuses with irregular ossification of various skull bones. However, these alterations were not dose-dependent, and were within the range of the historical control data. In addition, there were no differences between groups when the numbers of fetuses and litters with any alteration(s) were considered. Therefore, the observed alterations were not considered to be compound-related.

Based on these results, no developmental toxicity was observed in this study. The developmental NOEL was 300 mg/kg day; the LOEL was not determined.

4. Study Deficiencies:

No statistical analyses were performed on preimplantation and postimplantation losses. These have been provided by the reviewers.

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E. CLASSIFICATION: CORE Minimum data.

Maternal NOEL = 25 mg/kg/day.
Maternal LOEL = 100 mg/kg/day.
Developmental Toxicity NOEL = 300 mg/kg/day.
Developmental Toxicity LOEL = Not determined.

F. RISK ASSESSMENT: Not applicable.

~~CONFIDENTIAL~~ BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-F
TASK No.: 2-45F
May 1, 1990

007972

DATA EVALUATION RECORD

CLETHODIM 5-HYDROXY SULFONE

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Hoberman, A.M. Oral teratogenicity and developmental toxicity screen in rats with RE-51228. (Unpublished study No. S-3161 conducted by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated November 8, 1988.) MRID No. 410301-18.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William J. McEllan for

Date: May 1, 1990

1. CHEMICAL: 2-[1-[[[E-3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylsulfonyl)propyl]-3,5-dihydroxy-2-cyclohexen-1-one.
2. TEST MATERIAL: RE-51228, lot/batch No. SX-1796, 99.1% pure, off-white powder.
3. STUDY/ACTION TYPE: Developmental toxicity study in rats.
4. STUDY IDENTIFICATION: Hoberman, A.M. Oral teratogenicity and developmental toxicity screen in rats with RE-51228. (Unpublished study No. S-3161 conducted by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated November 8, 1988.) MRID No. 410301-18.

5. REVIEWED BY:

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Date: May 1, 1990

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Date: May 1, 1990

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Date: May 1, 1990

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Date: 5/22/90

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Toxicology Branch II
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Signature: M. Ioannou

Date: 5/31/90

007972

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity. Guideline §83-3.

MRID NUMBER: 410301-18.

TEST MATERIAL: RE-51228.

SYNONYMS: Clethodim 5-OH sulfone; 5-hydroxy Select sulfone.

STUDY NUMBER: S-3161.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham, PA.

TITLE OF REPORT: Oral Teratogenicity and Developmental Toxicity Screen in Rats with RE-51228.

AUTHOR: Hoberman, A.M.

REPORT ISSUED: November 8, 1988.

CONCLUSIONS: A developmental toxicity study was conducted in which Sprague-Dawley rats were administered clethodim 5-hydroxy sulfone via gavage at 0, 10, 100, or 700 mg/kg/day during gestational days (GD) 6 through 15. Neither maternal nor developmental toxicity was observed in this study. Therefore, the NOELs were 700 mg/kg/day. The LOELs could not be determined.

Classification: CORE Supplementary Data.

This study was classified as CORE Supplementary Data because a low number (10) of animals was used, and the dose levels chosen were inappropriate.

A. MATERIALS:

Test Compound: Purity: 99.1% (according to the chemical analysis report).
Description: Off-white powder.
Lot No.: Batch No. SX-1796.
Contaminants: Not reported.

Vehicle: 0.7% carboxymethyl cellulose in water, containing 1.0% Tween 80 (lot Nos. and sources not reported).

Test Animals: Species: Rat.
Strain: Sprague-Dawley [Crl:CD(SD)BR].
Source: Charles River Breeding Laboratories, Inc, Portage, MI.
Age: Approximately 78 days on GD 0.
Weight: 234-268 g on GD 0.

B. STUDY DESIGN:

This study was designed to assess the potential of clethodim 5-hydroxy sulfone to cause developmental toxicity in rats when administered daily via gavage from GD 6 through 15, inclusive.

Mating: Females were mated 1:1 with male rats of the same strain (approximately 114 days old, weighing 428-585 g) for a maximum of 4 days. They were examined daily for signs of mating. The day on which a copulatory plug or sperm in the vaginal smear was found was designated as GD 0.

Group Arrangement: Inseminated females were randomly assigned to dose groups based on body weight during the acclimation period as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	10
Low dose	10	10
Mid dose	100	10
High dose	700	10

Dosing: Doses were administered daily by gavage on GD 6 through 15 in a volume of 10 mL/kg. Dosage volumes were adjusted daily for observed body weight changes. Dosing suspensions were prepared weekly and stored refrigerated. Homogeneity and stability of dosing suspensions were analyzed using the first set of the dosing suspensions collected on the day they were prepared. Concentration was analyzed on the first and last day that each dosing suspension was used. A rationale for the selection of doses was not provided.

Observations: Animals were observed twice daily for mortality during the study. In addition, they were observed twice daily during the treatment period and once daily during the posttreatment period for overt signs of toxicity. Body weights were recorded on GD 0, 3, and 6 through 20. Food consumption was recorded between GD 0 and 3, 3 and 6, and daily on GD 6 through 20. Females were sacrificed on GD 20, and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross assessment of thoracic and abdominal cavities;
- Number of corpora lutea;
- Gravid uterine weight;
- Number and placement of implantation sites; and
- Number of resorptions and live and dead fetuses.

Any gross lesions found during necropsy were preserved for possible histopathologic evaluation. The uteri of apparently nonpregnant animals were stained with ammonium sulfide solution to detect early resorptions.

The viable fetuses were examined in the following manner:

- Individual fetuses were weighed and sexed;
- External alterations were recorded;
- Visceral alterations were determined for all fetuses using an adaptation of the microdissection technique of Staples;
- Approximately half of the fetuses in each litter were decapitated, and the heads were placed in Bouin's solution and later examined for visceral alterations using Wilson's sectioning technique; and
- The remaining fetuses were evaluated for skeletal alterations after staining with Alizarin Red S.

Statistical Analysis: The following analyses were conducted:

- Maternal and fetal incidence data--variance test for homogeneity of the binomial distribution;
- Body weight (fetal and maternal), body weight changes on days 9-12 and 12-16, gravid uterine weight, and food consumption, fetal ossification sites, percent male fetuses, and percent fetuses with alterations--Bartlett's test of homogeneity of variances, and (for parametric data) Analysis of Variance, followed by Dunnett's test, or (for nonparametric data) Kruskal-Wallis test, followed by Dunn's method of multiple comparisons.
- Maternal body weight change for GD 0-6 and 0-20, GD 6 to 7, 9, 12, 16, 18, and 20, GD 16 to 17, 18, and 20, and GD 18 to 19 and 20--analysis of covariance;
- Cesarean section data, <75% ties occurring--Kruskal-Wallis and Dunn's tests;
- Cesarean section data, >75% ties occurring--Fisher's Exact test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated November 28, 1988, was provided.
- A signed Statement of Compliance with EPA GLP's, dated November 8, 1988, was provided.
- A signed Quality Assurance Statement, dated November 3, 1988, was provided.

C. RESULTS:

The following results were reported by the study author.

1. Dose Analysis: Analysis of homogeneity and concentration of dosing suspensions ranged from 91 to 100% and 94.2 to 99.5%, respectively, of target concentrations. The test material was stable for at least 7 days in the refrigerator.

2. Maternal Toxicity:

Mortality: No deaths occurred during this study.

Abortion: No abortions occurred during this study.

Clinical Observations: Two high-dose animals had rales for a total of 13 days during gestation. One high-dose animal experienced excess salivation on GD 14. Localized alopecia was observed in two animals receiving the low dose, one receiving the mid dose, and one receiving the high dose.

Body Weight: A summary of maternal body weight gain and corrected weight gain data is presented in Table 1. During the dosing period, a nonsignificant decrease (9%) was observed among the high-dose animals. During the post-dosing period, the animals in all treated groups gained slightly more (3-10%) than those in the control group. A small, nonsignificant decrease (4%) was observed in the gravid uterine weight among the high-dose animals (data not shown).

Food Consumption: A summary of food consumption data is presented in Table 2. No differences were observed at any time among any dose groups.

Gross Pathological Observations: One pregnant control and one pregnant low-dose animal had mottled kidneys with irregularly shaped pale areas. One nonpregnant low-dose animal had apparent pyometra.

Cesarean Section Observations: A summary of cesarean section data is presented in Table 3. None of these parameters was significantly affected by treatment. A small decrease (2.4%) in fetal body weight was observed among high-dose litters. Male fetuses weighed, on the average, 6% more than female fetuses (data not shown).

3. Developmental Toxicity:

External Examinations: No external alterations were observed in viable fetuses. One late resorption from a

TABLE 1. Mean Body Weight Gain and Corrected Weight Gain (g $\pm$ S.D.)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-16)	Post-dosing Period (GD 16-20)	Entire Gestation Period (GD 0-20)	Corrected Body Weight Gain ^b
0	37 $\pm$ 6	77 $\pm$ 14	71 $\pm$ 11	185 $\pm$ 13	95 $\pm$ 16
10	33 $\pm$ 11	74 $\pm$ 10	74 $\pm$ 10	186 $\pm$ 20	91 $\pm$ 13
100	34 $\pm$ 9	75 $\pm$ 13	78 $\pm$ 7	188 $\pm$ 21	90 $\pm$ 16
700	39 $\pm$ 8	70 $\pm$ 9	73 $\pm$ 5	182 $\pm$ 10	96 $\pm$ 15

^aData were extracted from study No. S-3161, Table 4.^bCalculated as total body weight gain minus gravid uterine weight.TABLE 2. Mean Food Consumption (g/animal/day $\pm$ S.D.)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-16)	Post-dosing Period (GD 16-20)	Entire Gestation Period (GD 0-20)
0	24 $\pm$ 1	27 $\pm$ 3	30 $\pm$ 2	26 $\pm$ 2
10	23 $\pm$ 3	26 $\pm$ 1	30 $\pm$ 1	26 $\pm$ 1
100	22 $\pm$ 2	26 $\pm$ 3	30 $\pm$ 2	26 $\pm$ 2
700	24 $\pm$ 2	26 $\pm$ 2	30 $\pm$ 2	26 $\pm$ 2

^aData were extracted from study No. S-3161, Table 5.

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TABLE 3. Cesarean Section Observations^a

Parameter	Dose Level (mg/kg/day)			
	0	10	100	700
No. animals assigned	10	10	10	10
No. animals pregnant	10	9	9	9
Pregnancy rate (%)	100	90	90	90
Maternal wastage				
No. nonpregnant	0	1	1	1
Total corpora lutea ^b	190	175	170	172
Corpora lutea/dam	19.0	19.4	18.9	19.1
Total implantations ^b	169	149	156	148
Implantations/dam	16.9	16.6	17.3	16.4
Total live fetuses	152	144	148	131
Live fetuses/dam	15.2	16.0	16.4	14.6
Total resorptions ^b	17	5	8	17
Early	17	4	8	16
Late	0	1	0	1
Resorptions/dam	1.7	0.6	0.9	1.9
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Fetal weight/litter (g)	3.77	3.80	3.72	3.68
Preimplantation loss (%) ^c	11	14	8	12
Postimplantation loss (%) ^d	10	4	5	11
Sex ratio (% male)	49	49	49	51

^aData were extracted from study No. S-3761, Tables 7, 8, and 18.^bCalculated by reviewers using individual animal data.^cCalculated by reviewers as $\frac{(\text{No. corpora lutea} - \text{No. implants})/\text{litter} \times 100}{\text{No. corpora lutea/litter}}$ using ANOVA and linear regression analyses.^dPercent dead or resorbed conceptuses/litter.

low-dose litter had multiple external malformations (exencephaly, spina bifida, and gastroschisis). Observations for this dead specimen were excluded from the summary tables and statistical analyses.

Visceral Examinations: Agenesis of the right kidney was observed in one high-dose fetus.

Skeletal Examinations: A summary of fetal skeletal alterations is presented in Table 4. Two low-dose fetuses (one litter) had a bifid centrum in a thoracic vertebra. One fetus from the control group showed delayed ossification of the sternum. Delayed pelvic ossification was seen in one low-dose fetus, two mid-dose fetuses, and one high-dose fetus. The number of fetal ossification sites per fetus per litter was similar in all dose groups (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS:

1. Maternal Toxicity: No maternal toxicity was observed in this study. The changes noted in body weight gain during the dosing (a decrease of 3-10%) and postdosing (an increase of 3-10%) periods in the mid- and high-dose groups were neither statistically significant nor dose-dependent. Therefore, they were attributed to normal variation in the sample population. Based on these results, the maternal NOEL was 700 mg/kg/day; the LOEL was not determined.

2. Developmental Toxicity:

- a. Deaths/Resorptions: No compound-related effects were noted.
- b. Altered Growth: No compound-related effects were noted. The small decrease (2.4%) observed among high-dose fetuses is not considered to be biologically relevant. All fetal body weights are in fact within the upper range of the historical control data (3.19-3.74 g).
- c. Developmental Anomalies: The observed fetal alterations were not attributed to the test material. They were single events occurring in a non-dose-dependent manner and were within the range of historical controls.

No developmental toxicity was observed in this study. The developmental NOEL was 700 mg/kg day; the LOEL was not determined.

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TABLE 4. Summary of Fetal Skeletal Alterations^a

Findings ^b	Dose Level (mg/kg/day)			
	0	10	100	700
No. fetuses (litters)	80 (10)	75 (9)	76 (9)	67 (9)
Thoracic vertebrae:				
Bifid centrum	0	2 (1)	0	0
Sternebrae:				
1st centra, not ossified	1	0	0	0
Pelvis:				
Pubes and/or ischia, incompletely ossified	0	1	2 (2)	1

^aData were extracted from study No. S-3161, Table 12.^bMore than one type of alteration may be found in one fetus.

3. Study Deficiencies:

- a. Only 10 animals per group were used in this study.
- b. The selection of doses appeared to be inappropriate, since no toxicity was observed. No rationale for these doses was reported.
- c. No statistical analysis was performed on preimplantation and postimplantation losses. These have been provided by the reviewers.

E. CLASSIFICATION: CORE Supplementary Data.

Maternal NOEL = 700 mg/kg/day.
Maternal LOEL = Not determined.
Developmental Toxicity NOEL = 700 mg/kg/day.
Developmental Toxicity LOEL = Not determined.

F. RISK ASSESSMENT: Not applicable.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EQ 12045)

EPA No.: 68D80056
DYNAMAC No.: 245-C
TASK No.: 2-45C
May 1, 1990

007972

DATA EVALUATION RECORD

CLETHODIM

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Schroeder, R. Teratology study in rats with Chevron RE-45601 technical--final report. (Unpublished study No. S-280^S conducted by Bio/dynamics, Inc., East Millstone, NJ, and submitted by Chevron Chemical Company, Richmond, CA; dated July 20, 1987.) MRID No. 410301-16.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

William L. McLeenan for

Date:

May 1, 1990

007972

1. CHEMICAL: (E,E)-(±)-2-[1-[[(3-chloro-2-propenyl)-oxy]-imino]propyl-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.
2. TEST MATERIAL: RE-45601 (Clethodim) technical, lot No. SX-1688, amber liquid, 82.6% pure.
3. STUDY/ACTION TYPE: Developmental toxicity study in rats.
4. STUDY IDENTIFICATION: Schroeder, R. Teratology study in rats, with Chevron RE-45601 technical-final report. (Unpublished study No. S-2808 conducted by Bio/dynamics, Inc., East Millstone, NJ and submitted by Chevron Chemical Company, Richmond, CA; dated July 20, 1987.) MRID No. 410301-16.

5. REVIEWED BY:

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Date: May 1, 1990

Pia Lindström, DPH
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Date: 5/31/90

DATA EVALUATION RECORD

007972

STUDY TYPE: Developmental toxicity. Guideline §83-3.

MRID NUMBER: 410301-16.

TEST MATERIAL: RE-45601.

SYNONYMS: Clethodim technical, Select.

STUDY NUMBER: S-2808.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Bio/dynamics, Inc., East Millstone, NJ.

TITLE OF REPORT: Teratology Study in Rats with Chevron RE-45601
Technical--Final Report.

AUTHOR: Schroeder, R.

REPORT ISSUED: July 20, 1987.

007972

CONCLUSIONS: In a developmental toxicity study, groups of mated female rats were administered RE-45601 (clethodim technical) at doses of 0, 10, 100, 350, or 700 mg/kg/day, by gavage, on days 6-15 of gestation. The NOEL and LOEL for maternal toxicity were 100 and 350 mg/kg/day, respectively, based on reductions in body weight gain and clinical signs of toxicity (excess salivation) at 350 and 700 mg/kg/day.

The NOEL and LOEL for developmental toxicity were 100 and 350 mg/kg/day, based on reductions in fetal body weight and increases in the incidence of skeletal anomalies at 350 and 700 mg/kg/day.

Classification: CORE Minimum Data. Deficiencies are listed in Section E, Study Deficiencies.

A. MATERIALS:

Test Compound: Purity: 82.6%.
Description: Clear amber liquid.
Lot No.: SX-1688.
Contaminants: None reported.

Vehicle(s): 0.7% Carboxymethyl cellulose (lot No. 114F-0414) and 0.5 or 1.0% Tween 80 (lot No. 15F-0493) in distilled water; Sigma Chemical Co., St. Louis, MO.

Test Animals: Species: Rat.
Strain: Crl:CD(COBS).
Source: Charles River Breeding Laboratories, Inc., Portage, MI.
Age: Approximately 9 weeks at study initiation.
Weight: 174-253 g at study initiation.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of clethodim technical, when administered by gavage from gestational days (GD) 6 through 15, inclusive.

Mating: Females were mated 1:1 with males (from the laboratory's in-house breeding colony, strain not specified). The day on which evidence of mating, i.e., presence of sperm in the vaginal smear, was observed was designated as day 0 of gestation.

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Group Arrangement: Females were assigned to groups in order to achieve comparable mean body weight among groups as follows:

Test Group	Dose Level* (mg/kg/day)	Number Assigned per Group
I	0	25
II	10	25
III	100	25
IV	350	25
V	700	25

*Dose levels were not adjusted for purity of the chemical.

Dosing: All doses were administered in a volume of 10 mL/kg, and dose suspensions were prepared weekly during the dosing period. The dosing suspensions were analyzed weekly for concentration. Homogeneity of "mock" dosing suspensions (not actual dosing suspensions) was determined prior to study initiation. Stability analysis was conducted by the sponsor. Dosing was based on the most recently recorded gestational body weight.

Dose levels were selected, based on a pilot developmental toxicity study in which mated CD rats (10/group) were administered 0, 50, 150, 300, and 500 mg/kg/day on GD 6-15. Maternal toxicity, as evidenced by a reduction in body weight gain during the dosing (GD 6-15) and postdosing (GD 15-20) periods and an increased incidence of salivation, was observed at 500 mg/kg/day. Developmental toxicity, as evidenced by reduced fetal weight, was also observed at 500 mg/kg/day.

Observations: The animals were checked for mortality and overt signs of toxicity twice daily. Females were given a detailed physical examination on GD 0, 6, 10, 15, and 20. Body weight and food consumption were measured on GD 0, 3, 6-15 (daily), and 20. Animals dying during the study were subjected to a gross necropsy. Surviving dams were sacrificed on GD 20, and litters were delivered by cesarean section. Examination of dams at sacrifice consisted of:

- Observation of external surface, all orifices, cranial cavity, carcass, external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities;

- Measurement of uterine weight;
- Number and location of live and dead fetuses, early and late resorptions, and implantation sites;
- Number of corpora lutea; and
- Staining of uteri of apparently nonpregnant females with ammonium sulfide solution to detect implantation sites.

The fetuses were examined in the following manner:

- Individual body weight and sex (external examination) were determined;
- External body anomalies were recorded;
- Evaluation of soft tissue anomalies was conducted on approximately half of the fetuses using a method similar to that described by Staples.¹
- Heads of fetuses used for the soft-tissue evaluation were examined using a method similar to Wilson's technique; and
- Skeletal anomalies of the remaining fetuses were evaluated using a modification of the method of Crary.¹

Statistical Analysis: The following analyses were conducted:

- Maternal body weight and body weight gain; uterine weight; food consumption; numbers of corpora lutea, resorptions and viable fetuses; fetal body weight; and pre- and post-implantation loss--Bartlett's test, ANOVA, Dunnett's test, and Regression Analysis (parametric) or Kruskal-Wallis, Dunn's Rank Sum test, and Jonckheere's test (nonparametric).
- Mortality rate; pregnancy rate; and fetal and litter incidences of anomalies--Chi-square test, Fisher's Exact test with Bonferroni correction factor, and Armitage trend test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated November 16, 1988, was provided.

¹Crary, D.D. 1962. Modified benzyl alcohol clearing of Alizarin-stained specimens without loss of flexibility.; Stain Technology 37:124-125.

- A signed Statement of Compliance with EPA GLP's, dated August 25, 1987, was provided.
- A signed Quality Assurance Statement, dated February 11, 1987, was provided.

C. RESULTS:

1. Test Material Analysis: For the dosing suspensions, actual concentrations ranged from 92.4 to 103.0% of target concentrations. The test material was found to be stable in the vehicle for the period of use, and the "mock" dosing suspensions were homogeneous.

2. Maternal Toxicity:

Mortality: Of 25 females, 5 (20%) from the 700-mg/kg/day dose group died between GD 11 and 16 of the study. All five were pregnant. These animals had yellow stains on the anogenital area, brown or red stains around the nose and mouth, red areas on the thymus, dark or bright red lungs, and discoloration (brown or black areas) of the stomach mucosa.

Abortion: No abortions occurred during the study.

Clinical Observations: Selected clinical observations are summarized in Table 1. Increased incidences of excess salivation, anogenital staining, excess lacrimation, and red nasal discharge were observed at 700 mg/kg/day. Excess salivation was also observed at 350 mg/kg/day.

Body Weight: Data on body weight gain are summarized in Table 2. The study author supplied the following data. During the dosing period, body weights were significantly reduced ($p < 0.05-0.01$) at 700 mg/kg/day on GD 10, 11, 14, and 15 when compared with controls (data not shown). During the postdosing period, body weight gain was significantly reduced at 350 and 700 mg/kg/day. GD 20 body weight was significantly lower for dams administered 350 ($p < 0.05$) or 700 ($p < 0.01$) mg/kg/day when compared with controls (data not shown). Corrected GD 20 body weight was significantly lower ($p < 0.05$) than controls at 350 mg/kg/day (data not shown). A further discussion of the results is found in Section D, Discussion/Conclusion.

Uterine weight was significantly reduced ($p < 0.01$) in dams administered 700 mg/kg/day when compared with controls (data not shown).

TABLE 1. Summary of Selected Clinical Observations^a

Observation	Dose Level (mg/kg/day):				
	0	10	100	350	700
No. animals observed	25	25	25	25	25
Excess salivation	0 ^b	0	0	11	19
Anogenital staining	0	0	0	2	7
Excess lacrimation	2	1	6	1	12
Red nasal discharge	0	0	0	1	5

^aData were extracted from study No. S-2808, Appendix H.

^bRepresents the number of animals exhibiting the clinical sign at least once during the study.

TABLE 2. Mean Body Weight and Corrected Body Weight Gains (g)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-15)	Post-Dosing Period (GD 15-20)	Entire Gestation Period ^b (GD 0-20)	Corrected Body Weight Gain ^c (GD 6-20)
0	31 ± 9	47 ± 8	70 ± 10	147 ± 17	35 ± 11
10	33 ± 8	47 ± 9	69 ± 11	150 ± 16	37 ± 11
100	33 ± 8	45 ± 10	69 ± 8	147 ± 16	37 ± 13
350	30 ± 7	40 ± 14	58 ± 20*	128 ± 31**	25 ± 21
700	32 ± 6	28 ± 15**	58 ± 13**	120 ± 18**	28 ± 9

^aData were extracted from study No. S-2808, Appendices E and F.

^bCalculated by the reviewers using individual animal data. Data were analyzed using ANOVA.

^cCalculated as body weight gain during GD 6-20 minus gravid uterine weight.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

Food Consumption: Food consumption data are summarized in Table 3. The author supplied the following data (not shown in Table 3). A significant increase ($p < 0.05$) in food consumption was observed on GD 6 in dams administered 700 mg/kg/day. This was possibly due to undocumented spillage. On GD 7-10, significant reductions ($p < 0.01$) were observed at 700 mg/kg/day, when compared with controls (data not shown). A further discussion of the results is found in Section D.

Gross Pathological Observations: No findings were observed in dams surviving to study termination.

Cesarean Section Observations: Cesarean section observations are summarized in Table 4. The number of viable fetuses per litter was slightly, but not significantly, lower at 700 mg/kg/day when compared with concurrent controls. Increases (not statistically significant) in the number of resorptions per dam and postimplantation loss were observed at 700 mg/kg/day when compared with controls. Significant reductions in fetal body weight were observed at 350 and 700 mg/kg/day when compared with controls.

3. Developmental Toxicity:

A summary of selected fetuses and litters with multiple external, visceral, and skeletal anomalies is presented in Tables 5 and 6.

External Examinations: Significant increases in the fetal and litter incidences of total external malformations were observed at 700 mg/kg/day when compared with controls. The majority of malformations were tail defects, and one high-dose fetus had exencephaly. One fetus from the 100-mg/kg/day group had agnathia, which was later changed to micrognathia upon skeletal examination.

Visceral Examinations: Visceral malformations were observed in fetuses from the 700-mg/kg/day group that had external malformations. These included missing kidneys, ureters and bladder, and aortic arch and pulmonary arch defects (Table 5). In addition, one fetus each from the 10- and 100-mg/kg/day groups had distended lateral ventricles.

TABLE 3. Food Consumption Data (g/kg/day)^a

Dose Level (mg/kg/day)	Prior to Dosing Period ^b (GD 0-6)	Dosing Period ^b (GD 6-15)	Postdosing Period (GD 15-20)	Entire Gestation Period ^b (GD 0-20)
0	110 ± 7	99 ± 5	104 ± 7	101 ± 5
10	112 ± 10	99 ± 6	103 ± 7	102 ± 6
100	114 ± 10	97 ± 5	102 ± 8	102 ± 5
350	118 ± 11	99 ± 10	107 ± 26	102 ± 12
700	107 ± 6	83 ± 9**	106 ± 7	90 ± 6**

^aData were extracted from study No. S-2808, Appendix G.

^bCalculated by the reviewers using individual animal data. Data were analyzed using ANOVA.

**Significantly different from controls (p < 0.01).

TABLE 4. Cesarean Section Observations^a

Parameter	Dose Level (mg/kg/day)				
	0	10	100	350	700
No. animals assigned	25	25	25	25	25
No. animals pregnant	25	25	24	25	24
Pregnancy rate (%)	100	100	96	100	96
Maternal wastage					
No. died	0	0	0	0	5
No. died/pregnant	0	0	0	0	5
No. nonpregnant	0	0	1	0	1
No. aborted	0	0	0	0	0
No. premature delivery	0	0	0	0	0
Total corpora lutea	408	408	372	413	279
Corpora lutea/dam	16.3 ± 3.3 ^b	16.3 ± 2.3	15.5 ± 2.3	16.5 ± 2.3	15.5 ± 1.3
Total implantations	373	367	342	364	255
Implantations/dam	14.9 ± 2.2	14.7 ± 1.5	14.3 ± 1.6	14.6 ± 2.0	14.2 ± 1.6
Total live fetuses	353	351	329	350	221
Live fetuses/dam	14.1 ± 1.9	14.0 ± 1.6	13.7 ± 1.5	14.0 ± 2.0	12.3 ± 3.5
Total resorptions	20	16	13	14	34
Early	20	16	12	14	28
Late	0	0	1	0	6
Resorptions/dam	0.8 ± 0.8	0.6 ± 0.9	0.5 ± 0.7	0.6 ± 0.6	1.9 ± 3.5
Total dead fetuses	0	0	0	0	0
Dead fetuses/dam	0	0	0	0	0
Mean fetal weight (g)	3.7 ± 0.2	3.6 ± 0.2	3.5 ± 0.3	3.3 ± 0.5**	2.8 ± 0.4**
Preimplantation loss (%) ^c	7.4 ± 8.9	9.1 ± 9.3	7.2 ± 9.5	11.1 ± 12.4	8.5 ± 8.3
Postimplantation loss (%) ^d	5.2 ± 4.7	4.3 ± 5.7	3.6 ± 4.6	3.8 ± 4.0	12.8 ± 22.2
Sex ratio (% male) ^e	52	49	49	51	46

^aData were extracted from study No. S-2808, Appendix I.^bMean ± S.D.^cRepresents $\frac{\text{No. corpora lutea} - \text{No. implants}}{\text{No. corpora lutea}} \times 100$.^dRepresents $\frac{\text{No. resorptions}}{\text{No. implants}} \times 100$.^eCalculated by the reviewers using individual animal data as: $\frac{\text{No. male pups per litter}}{\text{Total no. pups per litter}} \times 100$.

Data were analyzed using ANOVA.

**Significantly different from controls ($p < 0.01$).

TABLE 5. Summary of Fetuses and Litters with Malformations^a

Dose Level (mg/kg/day)	Dam	Fetus	Malformations	
0	1511	4	Ribs wavy; 11th and 12th.	
		6	Ribs wavy; 5th and 12th right, 4th and 13th left.	
		8	Ribs wavy; 8th and 12th.	
		10	Ribs wavy; 9th, 10th, and 11th.	
		12	Ribs wavy; 7th right, 8th-12th bilateral, 13th left.	
	1520	8	5th lumbar vertebrae present.	
	1523	5	Ribs wavy; 11th and 12th.	
		14	Ribs wavy; 11th.	
	10	2522	5	Lateral ventricles extremely distended.
	100	3501	8	5th lumbar vertebrae present.
10			5th lumbar vertebrae present.	
12			5th lumbar vertebrae present.	
3505		6	5th lumbar vertebrae present.	
3510		4	Lateral ventricles extremely distended.	
3524		15	Micrognathia, ^b tympanics thickened anteriorly and fused to basisphenoid, pterygoid process of basisphenoid misshapen and positioned anteriorly, orbits reduced in size, and presence of cervical rib.	
350		4511	7	Wavy ribs; 9th, 10th, and 11th.
	4512	2	5th lumbar vertebrae present.	
	4525	10	Wavy ribs; 10th.	
		12	Wavy ribs; 12th.	
700	5504	2	Exencephaly.	
		9	Tail absent, 5th cervical transverse process absent, 4th and 5th cervical transverse processes fused, and 5th lumbar vertebrae present.	
	5506	13	Filamentous tail and slightly tortuous ureter.	
	5517	6	Tail absent, 5th lumbar vertebrae present, and misshapen ossifications posterior to lumbar vertebra.	
		15	Short tail and misshapen ossifications posterior to lumbar vertebra.	
	5519	4	Filamentous tail and imperforated anus.	
	5521	3	Short tail, aortic arch defect (pulmonary artery and ductus arteriosus absent), and lumbar centra fused.	

(continued)

TABLE 5. (continued)

Dose Level (mg/kg/day)	Dam	Fetus	Malformations
700 (cont.)	5525	2	2nd and 3rd lumbar transverse processes fused and thoracic and lumbar vertebra fused.
		4	Tail absent, imperforated anus, edema, renal ureter and bladder agenesis, defect in large intestine, and additional lumbar transverse process present.
		5	7th lumbar vertebrae present.
		14	Ribs fused, several lumbar vertebra absent, sacral and caudal vertebra absent, and additional pelvic ossifications.

^aData were extracted from study No. S-2808, Appendices L, M, and N.

^bListed as agnathia following external examination; however, the diagnosis was changed to micrognathia during skeletal examination.

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TABLE 6. Summary of Total Fetal Malformations and Variations^a

Parameter	Dose Level (mg/kg/day)				
	0	10	100	350	700
<u>External Examination</u>					
No. fetuses (litters) examined	353 (25)	351 (25)	329 (24)	350 (25)	221 (18)
Total malformations					
- fetal (%) incidence	0	0	1 (0.3)	0	8 (3.6)**
- litter (%) incidence	0	0	1 (4.2)	0	6(33.3)*
Total variations					
- fetal (%) incidence	0	0	1 (0.3)	0	0
- litter (%) incidence	0	0	1 (4.2)	0	0
<u>Visceral Examination</u>					
No. fetuses (litters) examined	185 (25)	181 (25)	172 (24)	181 (25)	118 (18)
Total malformations					
- fetal (%) incidence	0	1 (0.6)	1 (0.6)	0	4 (3.4)
- litter (%) incidence	0	1 (4.0)	1 (4.2)	0	3 (16.7)
Total variations					
- fetal (%) incidence	7 (3.8)	8 (4.4)	11 (6.4)	2 (1.1)	2 (1.7)
- litter (%) incidence	4(16.0)	7 (28.0)	9 (37.5)	2 (8.0)	2 (11.1)
<u>Skeletal Examination</u>					
No. fetuses (litters) examined	168 (25)	170 (25)	158 (24)	169 (25)	110 (18)
Total malformations					
- fetal (%) incidence	9 (5.4)	0**	5 (3.2)	4 (2.4)	8 (7.3)
- litter (%) incidence	4(16.0)	0	3 (12.5)	3 (12.0)	4 (22.2)
Total variations					
- fetal (%) incidence	122(72.6)	103 (60.6)	126 (79.7)	150 (88.8)**	106 (96.4)**
- litter (%) incidence	24(96.0)	25(100.0)	24(100.0)	25(100.0)	18(100.0)

^aData were extracted from study No. S-2808, Appendices L, K, and N.

*Significantly different from controls (p <0.05).

**Significantly different from controls (p <0.01).

Skeletal Examinations: The majority of skeletal malformations occurred in fetuses from the 700-mg/kg/day group that had external and visceral malformations. The fetus from the 100-mg/kg/day group having micrognathia also had multiple skeletal defects (see Table 5). In addition, one, zero, four (two litters), one and two (two litters) fetuses from the control, 10-, 100-, 350-, and 700-mg/kg/day groups, respectively, had 5th lumbar vertebrae present. A significant reduction in the incidence of fetuses with total skeletal malformations was observed at 10 mg/kg/day when compared with controls because no skeletal malformations were observed at this dose level (Table 6).

Selected skeletal variations are summarized in Table 7. Significant increases in the fetal incidence of total skeletal variations were observed at 350 and 700 mg/kg/day. The majority of these variations were delays in or absence of ossification.

D. DISCUSSION/CONCLUSION:

1. Maternal Toxicity: Maternal toxicity was observed at 350 and 700 mg/kg/day. At 350 mg/kg/day, significant reductions in body weight gain were observed during the postdosing period and for the entire gestation period. An increased incidence of salivation was also observed at 350 mg/kg/day. At 700 mg/kg/day, excessive mortality (5/25) was observed. These animals also exhibited clinical signs that included excess salivation and lacrimation, anogenital staining and red nasal discharge; reduced body weight gain during the dosing and postdosing periods and for the entire gestation period; reduced food consumption during the dosing period and the entire gestation; and reduced gravid uterine weight. For high-dose animals that died during the study, several findings were recorded at necropsy. However, since moderate to severe autolysis was diagnosed in three of the five animals found dead, the findings noted were probably due to the autolysis and not to administration of the test material.

Based on dose-related increases in the incidence of salivation and reductions in body weight gain, the LOEL for maternal toxicity is 350 mg/kg/day, and the NOEL is 100 mg/kg/day.

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TABLE 7. Summary of Selected Fetal Skeletal Variations^a

Parameter	Dose Level (mg/kg/day):				
	0	10	100	350	700
<u>total fetuses (litters) examined</u>	168 (25)	170 (25)	158 (24)	169 (25)	110 (18) ^b
<u>Thoracic centra</u>					
-Ossification incomplete	16 (11)	21 (14)	22 (13)	50 (20)	64 (18)
-Ossification absent	1 (1)	0	0	0	17 (8)
-Split	1 (1)	4 (3)	0	8 (6)	23 (12)
<u>Lumbar centra</u>					
-Ossification incomplete	0	1 (1)	0	1 (1)	12 (7)
<u>Acral transverse process(es)</u>					
-Ossification incomplete	36 (12)	37 (13)	34 (14)	73 (21)	33 (14)
-Ossification absent	3 (3)	4 (2)	3 (3)	20 (10)	12 (9)
<u>Audal transverse process(es)</u>					
-Ossification absent	14 (8)	11 (5)	18 (10)	53 (17)	35 (12)
<u>Audal centra</u>					
-Ossification absent	1 (1)	0	0	6 (2)	11 (7)
<u>Metatarsals</u>					
-Ossification absent:					
5th	48 (18)	41 (19)	69 (21)	102 (24)	88 (18)
6th	3 (3)	2 (2)	3 (2)	18 (10)	42 (16)
<u>Total skeletal variations</u>	122 (24)	103 (25)	126 (24)	150** (25)	106** (18)

^aData were extracted from study No. S-2808, Appendix W.^bOne dam had 15 resorptions and no viable fetuses.**Significantly different from controls ($p < 0.01$).

2. Developmental Toxicity:

- a. Deaths/Resorptions: An increased number of resorptions was observed at 700 mg/kg/day. The study author attributed this to one high-dose female that had 15 resorptions and only one viable fetus and regarded this animal as an outlier. Another animal that appeared nonpregnant had 15 implantations upon uterine staining, but was not included in the uterine implantation calculations. Since these implantations are early resorptions, the data were included in a recalculation of uterine implantation data by the reviewers, and the following results were obtained:

Dose Level (mg/kg/day)	<u>Resorptions</u>			Mean No. Resorptions per Dam ($\pm$ S.D.)
	Early	Late	Total	
0	20	0	20	0.8 ± 0.8
10	16	0	16	0.6 ± 0.9
100	12	1	13	0.5 ± 0.7
350	14	0	14	0.6 ± 0.6
700	43	6	49	$2.6 \pm 4.5^*$

*Significantly different from controls ($p < 0.05$). Data were analyzed using ANOVA.

The increase in the number of resorptions/dam at 700 mg/kg/day was significantly different from controls and was beyond the range of historical controls (0.2-1.8). Furthermore, since complete or nearly complete resorption of litters (15/litter) occurred in two high-dose females, the reviewers considered this to be a compound-related effect and not a spontaneous event.

- b. Altered Growth: A dose-related reduction in fetal body weight was observed at 350 and 700 mg/kg/day.
- c. Developmental Anomalies: Significant increases in fetal incidences of total skeletal variations were observed at 350 and 700 mg/kg/day. The majority of variations were delayed or absent ossification, and these occurred mostly in lumbar, sacral, or caudal vertebrae.

A significant increase in the fetal and litter incidences of total external malformations was observed at 700 mg/kg/day when compared with controls. The majority of these malformations were tail defects; 40% of high-dose litters (seven fetuses from six litters) had missing, short, or filamentous tails. According to the laboratory's historical control data, tail defects are extremely rare, occurring in 1 to 3/10,000 fetuses, and although these defects were not observed at 350 mg/kg/day, a significant dose-related delay in ossification, particularly in bones of the lower vertebral column, was seen.

Based on these results, the LOEL for developmental toxicity is 350 mg/kg/day, and the NOEL is 100 mg/kg/day.

E. STUDY DEFICIENCIES:

1. Homogeneity of the actual dosing suspensions was not analyzed.
2. The strain of males used for mating was not specified.
3. The historical control data were collected for a period of over 10 years. In EPA Guidelines for Teratology Studies (June 1985), acceptable historical control data generally represent a period of 2 years.

F. CLASSIFICATION: CORE Minimum data.

Maternal NOEL = 100 mg/kg/day.
Maternal LOEL = 350 mg/kg/day.
Developmental Toxicity NOEL = 100 mg/kg/day.
Developmental Toxicity LOEL = 350 mg/kg/day.

G. RISK ASSESSMENT: Not appropriate.

CONFIDENTIAL SOURCE INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-E
TASK No.: 2-45E
May 3, 1990

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DATA EVALUATION RECORD

CLETHODIM IMINE SULFONE

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Hoberman, A.M. Oral teratogenicity and developmental toxicity screen in rats with RE-47719. (Unpublished study No. S-3157, conducted by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated November 8, 1988.) MRID No. 410301-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McFadden for

Date: May 2, 1990

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1. CHEMICAL: 5-[2-(Ethylsulfonyl)propyl]-3-hydroxy-2-(1-imino-propyl)-2-cyclohexen-1-one.
2. TEST MATERIAL: RE-47719, Lot No. SX-1800; 98.6% pure; described as a white solid.
3. STUDY/ACTION TYPE: Developmental toxicity study in rats.
4. STUDY IDENTIFICATION: Hoberman, A.M. Oral teratogenicity and developmental toxicity screen in rats with RE-47719. (Unpublished study No. S-3157, conducted by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Chevron Chemical Company, Richmond, CA; dated November 8, 1988.) MRID No. 410301-17.

5. REVIEWED BY:

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DATA EVALUATION RECORD

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STUDY TYPE: Developmental toxicity. Guideline §83-3.

MRID NUMBER: 410301-17.

TEST MATERIAL: RE-47719.

SYNONYMS: Clethodim imine sulfone, Select imine sulfone.

STUDY NUMBER: S-3157.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham, PA.

TITLE OF REPORT: Oral Teratogenicity and Developmental Toxicity
Screen in Rats with RE-47719.

AUTHOR: Hoberman, A. M.

REPORT ISSUED: November 8, 1988.

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CONCLUSIONS:

In a developmental toxicity study, mated female Sprague-Dawley rats were administered RE-47719 (clethodim imine sulfone) via gavage at dose levels of 0, 10, 100, or 700 mg/kg/day during days 6-15 of gestation. Compound-related maternal effects included significant excess salivation in the highest dose group, and reduced body weight gain and food consumption during compound administration in the 100-mg/kg/day group (not statistically significant) and 700-mg/kg/day group (statistically significant). Based on these results, the maternal NOEL and LOEL are 10 and 100 mg/kg/day, respectively.

Compound-related developmental effects were seen only in the 700-mg/kg/day group and included significant reduction in fetal body weight/litter, significant reduction in the number of fetal sternal ossification sites/fetus/litter, and significantly increased litter and fetal incidences of cervical rib(s). Based on these results, the NOEL and LOEL for developmental toxicity are 100 and 700 mg/kg/day, respectively.

Classification: CORE Supplementary Data.

This study was classified as Supplementary Data because of the use of 9-10 rather than the 20 pregnant rats per dose level recommended by the Guideline.

A. MATERIALS:

Test Compound: Purity: 98.6%.
Description: White solid.
Lot No.: SX-1800.
Contaminants: Not reported.

Vehicles: 0.7% carboxymethyl cellulose and 1% Tween 80 in deionized water.

Test Animals: Species: Rat.
Strain: Sprague-Dawley [CrI:CD(SD)BR].
Source: Charles River Breeding Laboratories, Inc. (No other information was reported.)
Age: 11-12 weeks at mating.
Weight: 232-258 g on gestation day 0.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of RE-47719 in rats when administered by gavage from gestational days (GD) 6 through 15, inclusive.

Mating: Each female was randomly mated 1:1 with a male of the same strain and source for a maximum of 4 days. Day 0 of gestation was designated as the day on which evidence of mating, i.e., spermatozoa in the vaginal smear or a copulatory plug, was observed.

Group Arrangement: Mated females were assigned to dose groups by a computer-generated (weight-ordered) randomization procedure, as follows:

Test Group	Dose Level* (mg/kg/day)	Number Assigned per Group
1 Control	0	10
2 Low dose	10	10
3 Mid dose	100	10
4 High dose	700	10

*No correction was made for purity of test substance.

Dosing: Doses were administered by gavage in a volume of 10 mL/kg with a daily adjustment of individual volume to the actual body weight. Dosing suspensions were prepared weekly and stored refrigerated. Homogeneity analyses were performed on the dosing suspensions prepared on the first day of dosing; after one set of triplicate samples had been collected for each dose level, each suspension was again thoroughly mixed and a second set of triplicate samples was collected. Samples for stability analyses were collected after refrigeration for 1 and 7 days and were immediately frozen. Samples for concentration determinations were collected after 1 day of refrigerated storage of each of the two sets of dosing suspensions and immediately frozen. The rationale for selection of dose levels was not given.

Observations: Viability was checked twice daily throughout the study. The animals were observed once daily on gestation days (GD) 0 and 3 for clinical signs or general appearance. During the dosing period (GD 6-15), the animals were observed immediately prior to dosing and approximately 1 hour after dosing for clinical signs and abortions and/or viability. These observations were also made once daily on GD 16-20. Body weights and food consumption were recorded at least once weekly prior to mating, and once daily on GD 0, 3, and 6-20. Surviving females were sacrificed on GD 20, and litters were delivered by cesarean section. Examination at the time of sacrifice included the following:

- Gross lesions of the abdominal and thoracic cavities;
- Number of corpora lutea;
- Gravid uterine weight;
- Number and location of implantation sites; and
- Number of resorptions (early and late) and fetuses (live and dead).

The uteri of apparently nonpregnant animals were stained with ammonium sulfide to detect early resorptions.

Viable fetuses were examined in the following manner:

- Individual fetuses were weighed and sexed;
- External abnormalities were recorded;
- Visceral alterations were determined by the technique of Staples (1974);
- Heads of approximately half the fetuses in each litter were preserved and examined for soft tissue alterations by the technique of Wilson (1965); and
- The remaining fetuses were examined for skeletal alterations by a modification of the method of Staples and Schnell (1963) after staining with Alizarin Red.

Statistical Analysis: The following statistical analyses were performed:

- Maternal and fetal incidence data--Variance Test for Homogeneity of the Binomial Distribution;
- Maternal body weight, gravid uterine weight, food consumption, litter data for percent male fetuses, fetal body weight, fetal ossification sites, and percent fetal alterations--Bartlett's Test of Homogeneity of Variances followed, as appropriate, by ANOVA and Dunnett's test or the Kruskal-Wallis test, Fisher's Exact test, and/or Dunn's Method of Multiple Comparisons;
- Maternal body weight changes--Analysis of Covariance for some experimental intervals and Bartlett's test of Homogeneity of Variance/ANOVA for other intervals; and
- Cesarean section data--Kruskal-Wallis test followed, if appropriate, by Dunn's Method of Multiple Comparisons.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated November 30, 1988, was provided.
- Signed Statements of Compliance with EPA GLP's, dated November 8, 1988, and November 14, 1988, were provided.
- A signed Quality Assurance Statement, dated November 3, 1988, was provided.

C. RESULTS: The following results were reported by the study author.

1. Dose Analysis: Analysis of dosing suspensions revealed that actual concentrations ranged from a low of 88.1% of target to a high of 106.3% of target. The dosing suspensions were homogeneous; coefficients of variation ranged from 2.1 to 7.4%. The test material was stable in the dosing suspensions; after 7 days of refrigerated storage, dosing suspension concentrations ranged from 92.9 to 105% of initial concentrations.

2. Maternal Toxicity:

Mortality: No animals died during the study.

Abortion: No abortions occurred during the study.

Clinical Observations: Significantly increased excess salivation ($p \leq 0.01$) was observed in 3/9 pregnant rats of the high-dose group on 1-3 days of the dosing period (GD 6-8).

Localized alopecia was observed in 0, 1, 1, and 3 rats of the control, low-dose, mid-dose, and high-dose groups for totals of 0, 8, 1, and 30 days, respectively.

Body Weight: Maternal body weight gain data are presented in Table 1. Compared with control, significantly lower mean body weight gains were seen in high-dose animals on GD 6-9, GD 6-12, and GD 6-16. Body weight gains were also lower in mid-dose animals during these intervals, but differences from control were not statistically significant. No differences were observed between groups for gravid uterine weight. Corrected weight gain was slightly but not statistically significantly lower among high-dose animals.

Food Consumption: Food consumption data are presented in Table 2. Compared with control, significantly lower mean food consumption was seen in high-dose animals on GD 6-9, GD 6-12, and GD 6-16. Mean food consumption was also slightly lower in mid-dose animals during these intervals, but differences from control were not statistically significant.

Gross Pathological Observations: There were no treatment-related gross lesions.

Cesarean Section Observations: A summary of cesarean section observations is presented in Table 3. Pregnancy rates were 100% in the control and low-dose animals and 90% in mid- and high-dose animals. Fetal body weight/litter was significantly reduced in the high-dose group. Early resorptions/dam and total resorptions/dam were significantly increased in the mid-dose group. Postimplantation loss/litter was significantly increased in mid-dose animals.

3. Developmental Toxicity:

Selected fetal alterations are presented in Table 4. Incidences of litters and fetuses with any alteration were significantly increased in the high-dose group; 33 fetuses from 9/9 high-dose litters had one or more alterations, as compared with 4 fetuses from 4/10 control litters.

External Examinations: One fetus from a high-dose litter had a left hind paw rotated inward.

Visceral Examinations: One fetus from a high-dose litter had two left carotids, both of which arose from the aorta.

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TABLE 1. Summary of Maternal Body Weight Gain (mean g $\pm$ S.D.)^a

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period			Post- Dosing Period (GD 16-20)	Entire Gestation Period (GD 0-20)	Corrected bw Gain for Entire Gestation Period ^b (GD 0-20)
		(GD 6-9)	(GD 6-12)	(GD 6-16)			
0 ^c	27.6 $\pm$ 9.6	12.2 $\pm$ 4.3	24.6 $\pm$ 5.9	55.9 $\pm$ 9.1	69.4 $\pm$ 8.5	152.9 $\pm$ 20.3	62.7 $\pm$ 16.0
10 ^c	26.0 $\pm$ 9.6	14.2 $\pm$ 5.5	27.1 $\pm$ 9.0	57.7 $\pm$ 12.7	59.6 $\pm$ 12.1	143.3 $\pm$ 12.5	53.9 10.2
100 ^d	29.2 $\pm$ 9.3	8.3 $\pm$ 5.8	21.8 $\pm$ 7.5	47.8 $\pm$ 8.1	59.9 $\pm$ 8.7	136.9 $\pm$ 12.2	57.6 $\pm$ 11.0
700 ^a	27.9 $\pm$ 9.3	2.8** $\pm$ 4.6	9.1** $\pm$ 8.2	46.1* $\pm$ 12.3	65.3 $\pm$ 9.2	139.3 $\pm$ 14.0	49.8 $\pm$ 14.9

^aData were extracted from study No. S-3157, Table 4.^bCalculated as body weight gain GD 0-20 minus gravid uterine weight.^cN=10.^dN=9.^aN=9 except for corrected body weight gain entire gestation period (N=8).*Significantly different from control ($p \leq 0.05$).**Significantly different from control ($p \leq 0.01$).

TABLE 2. Food Consumption Data (mean g/day $\pm$ S.D.)^a

Dose Group (mg/kg day)	Prior to Dosing Period (GD 0-6) ^b	Dosing Period			Post- Dosing Period (GD 16-20)	Entire Gestation Period (GD 0-20)
		(GD 6-9)	(GD 6-12)	(GD 6-16)		
C ^c	21.0 $\pm$ 2.3	22.0 $\pm$ 3.0	21.6 $\pm$ 2.4	21.9 $\pm$ 2.5	23.9 $\pm$ 2.4	22.0 $\pm$ 2.2
1C ^c	21.3 $\pm$ 1.3	23.0 $\pm$ 2.6	22.3 $\pm$ 2.3	22.5 $\pm$ 2.1	23.6 $\pm$ 1.2	22.4 $\pm$ 1.6
10C	21.4 ^d $\pm$ 2.3	21.1 ^d $\pm$ 2.5	21.2 ^d $\pm$ 2.6	21.0 ^d $\pm$ 2.4	22.7 ^e $\pm$ 1.7	21.8 ^e $\pm$ 2.1
70C ^d	21.4 $\pm$ 2.0	18.6** $\pm$ 1.7	18.4** $\pm$ 1.9	19.4* $\pm$ 1.9	24.4 $\pm$ 3.3	21.1 $\pm$ 1.6

^aData were extracted from study No. S-3157, Table 5.^bCalculated and statistically analyzed by reviewers using ANOVA.^cn=10.^dn=9.^en=7.*Significantly different from control ($p \leq 0.05$).**Significantly different from control ($p \leq 0.01$).

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TABLE 3. Cesarean Section Observations^a

Parameter	Dose Level (mg/kg/day)			
	0	10	30	700
Animals assigned	10	10	10	10
Animals pregnant	10	10	9	9
Pregnancy rate (%)	100	100	90	90
Embryonal wastage				
nonpregnant	0	0	1	1
aborted	0	0	0	0
Corpora lutea ^b	180	174	155	170
Corpora lutea/dam ^c	18.0 ± 1.6	17.4 ± 2.2	17.2 ± 2.4	18.9 ± 2.3
Implantations ^b	163	162	139	159
Implantations/dam ^c	16.3 ± 1.5	16.2 ± 0.9	15.4 ± 1.3	17.7 ± 1.9
Live fetuses	157	158	125	153
Live fetuses/dam ^c	15.7 ± 1.6	15.8 ± 0.8	13.9 ± 1.9	17.0 ± 2.2
Resorptions ^d	6	4	14	6
Resorptions/dam ^c	0.6 ± 0.7	0.3 ± 0.5	1.6 ± 0.9**	0.7 ± 0.5
Dead fetuses/dam ^c	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Resorptions/dam ^c	0.6 ± 0.7	0.4 ± 0.5	1.6 ± 0.9**	0.7 ± 0.5
Dead fetuses	0	0	0	0
Dead fetuses/dam ^c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Weight/litter ^c				
Males	3.43 ± 0.16	3.42 ± 0.22	3.44 ± 0.17	2.98 ± 0.14**
Females	3.29 ± 0.18	3.20 ± 0.30	3.32 ± 0.16	2.87 ± 0.15**
Both sexes	3.37 ± 0.17	3.32 ± 0.21	3.38 ± 0.15	2.92 ± 0.14**
Plantation loss				
Litter (%) ^{b,e,f}	9.0 ± 9.2	5.9 ± 10.2	9.3 ± 10.0	5.8 ± 7.3
Litter (%) ^{b,e,g}	3.7 ± 4.2	2.4 ± 3.1	10.4 ± 6.4**	3.9 ± 3.0
Ratio (% male)	54.8 ± 11.8	47.9 ± 19.1	47.2 ± 15.6	47.8 ± 12.7

^a were extracted from study no. S-3157, Tables 7, 8, and 17.

^b related by reviewers.

^c ± S.D.

^d Historical control incidences of resorptions (66 studies): mean 0.9; range 0.5-1.8.

^e Statistical analysis by reviewers using ANOVA.

^f $\frac{\text{of corpora lutea} - \text{No. of implants}}{\text{No. of corpora lutea}} \times 100$.

^g $\frac{\text{of implants} - \text{No. of live fetuses}}{\text{No. of implants}} \times 100$.

** significantly different from control ($p \leq 0.01$).

TABLE 4. Summary of Selected Fetal Alterations^a

Alterations	Dose Level (mg/kg/day)			
	0	10	100	700
<u>Neurological Alterations</u>				
of fetuses (litters) examined	157 (10)	158 (10)	125 (9)	153 (9)
hind paw rotated inward ^c	0 (0)	0 (0)	0 (0)	1 (1)
<u>Tissue Alterations</u>				
of fetuses (litters) examined	157 (10)	158 (10)	125 (9)	153 (9)
left carotids, both sing directly from aorta ^d	0 (0)	0 (0)	0 (0)	1 (1)
<u>Skeletal Alterations</u>				
of fetuses (litters) examined	81 (10)	82 (10)	65 (9)	78 (9)
abrac, cervical rib present ^e	1 (1)	0 (0)	0 (0)	30** (9**)
nal ossification sites fetus per litter ^f	3.7	3.6	3.7	3.1**
<u>Other Alterations</u>				
of fetuses (litters) examined	157 (10)	158 (10)	125 (9)	153 (9)
alteration present	4 (4)	3 (3)	2 (1)	33** (9**)

were extracted from study No. S-3157, Tables 10, 11, 13, 20, and Appendix E.

than one alteration may be found in one fetus.

orica. control incidences of short and bent fore and hind paws (28 studies):

Litter:

Incidence 0.16% (1/620)
Range of study means 0-4.8%

Fetus:

Incidence 0.02% (1/5444)
Range of study means 0-0.3%

razion not observed among historical control groups.

orica. control incidences of cervical rib present (28 studies):

Litter:

Incidence 3.09% (19/620)
Range of study means 0-30.0%

Fetus:

Incidence 0.39% (21/5,444)
Range of study means 0-2.4%

orica. control incidences of ossification sites in sternal centers per fetus per litter (26 studies):

Incidence 3.63; Range of study means 3.33-3.80

nificantly different from control ($p \leq 0.01$).

Skeletal Examinations: The litter and fetal incidences of cervical rib(s) were significantly increased in the high-dose group; 30 fetuses from 9/9 high-dose litters had one or more cervical ribs present, as compared with 1 fetus from 1/10 control litters. Compared with control, the number of fetal sternal ossification sites/fetus/litter was significantly decreased in high-dose animals.

D. DISCUSSION/CONCLUSIONS:

1. Maternal Toxicity: Maternal toxicity was evidenced by reduced body weight gain during compound administration (evident in 100- and 700-mg/kg/day groups; statistically significant at 700 mg/kg/day) and reduced food consumption during compound administration [evident in 100-mg/kg/day (very slight) and 700-mg/kg/day groups; statistically significant at 700 mg/kg/day]. In addition, excess salivation was observed at 700 mg/kg/day. Although salivation may be indicative of neurotoxicity, the absence of corroborative evidence of neurotoxicity suggests to the reviewers that the excess salivation was likely the result of sensory stimulation by the test compound. The alopecia observed with equal frequency in all dose groups was attributed to rubbing against the feed jars. No maternal effects were noted at the 10-mg/kg/day dose level. On the basis of these results, the maternal NOEL and LOEL are 10 and 100 mg/kg/day, respectively.

2. Developmental Toxicity:

- a. Deaths/Resorptions: No compound-related effects were noted.

Early resorptions/dam and total resorptions/dam were significantly increased in the mid-dose group and were reflected by a significant increase in post-implantation loss/litter. However, the reviewers agree with the study author that this finding is unrelated to test substance administration because the incidence did not exceed the range observed historically and no dose-response relationship was evident.

- b. Altered Growth: A significant reduction in fetal body weight/litter and a significant reduction in the number of fetal sternal ossification sites/fetus/litter were observed at the 700-mg/kg/day dose level. The reviewers agree with the study author that the reduction in sternal ossification sites may represent

a reversible delay in ossification that is related to the decreased fetal weight/litter.

- c. Developmental Alterations: One fetus from a high-dose litter had a hind paw rotated inward. Because historical controls show a low incidence of short and bent forepaws and hind paws (litter incidence 1/620; fetal incidence 1/5444), the reviewers consider that this anomaly may be treatment related.

One fetus from a high-dose litter had two left carotids, both of which arose from the aorta. Because none of the historical controls showed such an anomaly, the reviewers consider that this anomaly may be treatment-related.

The litter and fetal incidences of cervical rib(s) were significantly increased, compared with control, at the 700-mg/kg/day dose level. The study author indicates that cervical rib(s) is a common variation in this strain of rat. However, the reviewers note that the incidence per litter and the incidence per fetus in the high-dose group far exceeded the historical control means and ranges, and thus consider this anomaly to be a clear treatment-related effect.

The litter and fetal incidences of any alteration were significantly increased, compared with controls, at the 700-mg/kg/day dose level. The fact that the numbers of litters and fetuses with any alterations are significant at the high dose is a strong indication of developmental toxicity, while the other single events may or may not be.

Based on the above results, the NOEL and LOEL for developmental toxicity are 100 and 700 mg/kg/day, respectively.

3. Study Deficiencies: This study used 9-10 rather than the 20 pregnant rats per dose level recommended by the 1982 EPA Guideline.

E. CLASSIFICATION: CORE Supplementary Data.

Maternal NOEL = 10 mg/kg/day.
Maternal LOEL = 100 mg/kg/day.
Developmental Toxicity NOEL = 100 mg/kg/day.
Developmental Toxicity LOEL = 700 mg/kg/day.

F. RISK ASSESSMENT: Not applicable.

CONFIDENTIAL BUSINESS INFORMATION
DO NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 245-G
TASK No.: 2-45G
May 1, 1990

007972

DATA EVALUATION RECORD

CLETHODIM TECHNICAL

Two-Generation Reproductive Toxicity Study in Rats

STUDY IDENTIFICATION: Tellone, C.I. Two-generation (one litter) reproduction study in rats with RE-45601 technical. (Unpublished study No. S-2778 conducted by Chevron Environmental Health Center, Inc., Richmond, CA, and submitted by Chevron Chemical Company, Richmond, CA; dated August 27, 1987.) MRID No. 410301-20.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:

William L. McTellan for

Date:

May 1, 1990

007972

1. CHEMICAL: (E,E)-(+/-)-2[1-[[[(3-chloro-2-propenyl)oxy]imino]-propyl]-5-[2(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one.
2. TEST MATERIAL: RE-45601 (clethodim) technical, lot/batch No. SX-1688, 83.2% pure, clear amber liquid.
3. STUDY/ACTION TYPE: Two-generation reproductive toxicity study in rats.
4. STUDY IDENTIFICATION: Tellone, C.I. Two-generation (one litter) reproduction study in rats with RE-45601 technical. (Unpublished study No. S-2778 conducted by Chevron Environmental Health Center, Inc., Richmond, CA, and submitted by Chevron Chemical Company, Richmond, CA; dated August 27, 1987.) MRID No. 410301-20.

5. REVIEWED BY:

Patricia A. Turck, M.S.
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Dynamac Corporation

Signature: Patricia Turck

Date: May 1, 1990

Pia Lindström, DPH
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Signature: Roman Pienta

Date: May 1, 1990

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Date: 5/31/90

DATA EVALUATION RECORD

007972

STUDY TYPE: Reproductive Toxicity. Guideline §83-4.

MRID NUMBER: 410301-20.

TEST MATERIAL: RE-45601 technical.

SYNONYMS: Clethodim technical, Select.

STUDY NUMBER: S-2778.

SPONSOR: Chevron Chemical Company, Richmond, CA.

TESTING FACILITY: Chevron Environmental Health Center, Inc.,
Richmond, CA.

TITLE OF REPORT: Two-Generation (One Litter) Reproduction Study
in Rats with RE-45601 Technical.

AUTHOR: Tellone, C. I.

REPORT ISSUED: August 27, 1987.

CONCLUSIONS:

In a reproductive toxicity study in which groups of rats were fed diets containing 0, 5, 20, 500, or 2500 ppm RE-45601 technical for two consecutive generations, parental toxicity was observed at the highest dietary concentration. The NOEL and LOEL for parental toxicity were 500 and 2500 ppm (approximately 51 and 263 mg/kg/day), respectively, based on reductions in body weight, particularly in males, and food consumption during both generations.

No effects on fertility, length of gestation, or growth and development or survival of offspring were observed. Therefore, the NOEL for reproductive toxicity was 2500 ppm, and the LOEL was not established.

Classification: CORE Minimum Data.

A. MATERIALS:

Test Compound: Purity: 83.2%.
Description: Viscous, clear amber liquid.
Lot/batch No.: SX-1688.
Contaminants: Not reported.

Vehicle: Acetone (carrier); lot No. not reported;
Mallinckrodt Chemical Company, Paris, KY.

Test Animals: Species: Rat.
Strain: Crl:COBS/CD(SD).
Source: Charles River Breeding Laboratories, Inc., Portage, MI.
Age: 6 weeks at initiation of treatment.
Weight: F₀ males, 185-241 g; F₀ females, 131-178 g on study day 1; F₁ males, 50-95 g; F₁ females, 49-89 g on study day 119.

B. STUDY DESIGN:

This study was designed to assess the reproductive toxicity potential of RE-45601 technical when administered continuously in the diet for two consecutive generations.

Mating: After 70 days on the test diets, F₀ males and females from the same group were randomly paired 1:1 for up to 21 days to produce F₁ litters. The day on which mating was confirmed by presence of sperm in the vaginal smear or a copulatory plug was designated as day 0 of gestation. Females showing no evidence of mating 14 days after pairing were paired with a

second male from the same dietary group that had successfully mated. After 21 days of cohabitation, all females showing no evidence of mating were separated from males and observed for 24 days. Any females failing to produce a litter within 24 days were considered nonpregnant and were sacrificed and subjected to a gross necropsy.

$F_{1,}$ parents (designated by the study author as the F_1 parental generation) were paired after 84 days on the test diets. Sibling pairings were avoided. The mating procedure was the same as for the F_0 mating.

Group Arrangement: Parents were randomly assigned to groups by a computerized randomization procedure to minimize intra-group differences in body weight as follows:

Test Group	Dietary Level (ppm)	<u>Number Assigned per Group*</u>	
		Males	Females
I (Control)	0	30	30
II	5	30	30
III	20	30	30
IV	500	30	30
V	2500	30	30

*For both F_0 and $F_{1,}$ parental generations.

Dosing: The test material was administered continuously in the diet for two consecutive generations. The test diets were prepared at least weekly and stored frozen until use. Diets were prepared by dissolving an appropriate quantity of the test material in 7 mL acetone per kg of final diet and adding the solution plus 3 mL acetone per kg as a rinse to half the powdered feed. The feed was then mixed for 5 minutes, the remaining feed was added, and the test diet was mixed for an additional 15 minutes. For the control group, 10 mL acetone per kg was mixed with feed for 15 minutes. Owing to instability problems, feed from the 5- and 20-ppm groups was replaced daily, and feed from all other groups (0, 500, and 2500 ppm) was replaced three times weekly in the animal feeders.

Freshly prepared diets were analyzed for concentration weekly. Concentration and stability analyses were performed on samples collected from feeders twice weekly. In addition, food

remaining (in feeders) from 15 rats per group was consolidated, mixed, and analyzed to determine stability and concentration. Homogeneity of the test diets was determined prior to study initiation.

The dietary levels chosen for this study were based on results of a pilot reproduction study in which groups of eight rats per sex were fed diets containing 0, 500, 2000, or 5000 ppm RE-45601 technical for 1 week and then paired to produce one litter. Mated females received the test diets during mating, gestation, and through day 7 of lactation. Dams and offspring were then sacrificed and grossly examined. Significant reductions ($p < 0.05$) in body weight and food consumption were observed in high-dose parents (5000 ppm). Significant reductions ($p < 0.05$) in pup weight were observed on day 7 of lactation in all test groups. Reproductive parameters, i.e., mating and fertility indices, litter size, offspring survival, and sex ratios, were unaffected.

Observations: Animals were observed twice daily for mortality and once daily for overt signs of toxicity. Body weight of F_2 and $F_{1,2}$ males was measured weekly throughout the study. During the pre-mating and mating periods, body weight of F_0 and $F_{1,2}$ females was measured weekly. During gestation and lactation, maternal body weights were measured on days 0, 7, 14, and 21.

Food consumption for F_0 and $F_{1,2}$ males and females was measured daily for groups II and III and three times weekly for Groups I, IV, and V during the pre-mating periods. Food consumption for males was also measured (as described above) during the gestation and lactation periods. During gestation, maternal food consumption was measured on gestational days (GD) 0, 2, 5, 7, 9, 12, 14, 16, 19, and 21 for Groups I, IV, and V and daily on GD 0-21 for Groups II and III. During lactation, maternal food consumption was measured on days 0, 2, 5, 7, 9, 12, and 14 postpartum for Groups I, IV, and V and daily on days 0-14 postpartum for Groups II and III.

The following data were recorded for each litter:

- Litter size, number of stillborn and live pups, pup body weight, and sex on day of birth;
- Litter size, sex, and pup body weight on days 4, 7, 14, and 21 of lactation; and
- Mortality and overt signs of toxicity (daily).

All litters were culled to eight pups (four per sex, if possible) on day 4 of lactation. Culled pups and surviving pups at weaning (days 21-24 of lactation) were sacrificed and subjected to a gross necropsy. Abnormal tissues were saved for

possible histopathological examination. In addition, any pups stillborn or dying during the study were also necropsied; tissues were discarded.

After weaning of litters, all parental animals were sacrificed and subjected to a gross necropsy.

The following organs from all parental animals were weighed:

- Testes
- Epididymides
- Prostate
- Seminal vesicles
- Uterus with cervix and vagina attached
- Ovaries

The following tissues were collected and preserved for possible histopathological examination:

- Adrenal gland	- Ovaries*
- Bone and marrow (sternum)	- Pituitary
- Brain	- Prostate*
- Cervix and vagina*	- Salivary glands
- Duodenum	(mandibular)
- Epididymides*	- Seminal vesicles*
- Eye and Harderian gland	- Spleen
- Heart and coronary	- Stomach
vessels	- Testes*
- Kidneys	- Thyroid with
- Liver	parathyroids
- Lung with mainstem	- Trachea
bronchi	- Uterus*
- Lymph nodes (mesenteric)	- Gross lesions
- Mammary tissue (inguinal)	

Tissues identified by asterisks (*) were histologically examined for parental animals in Groups I and V. In addition, gross lesions from all animals were histologically examined.

Statistical Analysis: The following statistical analyses were conducted:

- Body weight and body weight gain, food consumption, and organ weights--ANOVA, the F-distribution, and Dunnett's test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated December 28, 1988, was provided.
- A signed Statement of Compliance with EPA GLP's, dated August 31, 1987, was provided.
- A signed Quality Assurance Statement, dated August 31, 1987, was provided.

C. RESULTS:

The study author reported the following results.

1. Test Material Analysis: The author stated that the test diets were unstable at room temperature. The analysis results revealed that actual concentrations in the test diets ranged from 76 to 111% at 5 ppm, from 58 to 105% at 20 ppm, from 79 to 112% at 500 ppm, and from 80 to 111% at 2500 ppm of target concentrations.

Approximately 3 months before study termination, random samples of test diets were analyzed for the major metabolite of the test material, clethodim sulfoxide (RE-45924). A summary of the sulfoxide analyses is presented in Table 1. The addition of the sulfoxide content to that of RE-45601 increased the total equivalent concentration of the target of 20 ppm from 100 to 104% for freshly prepared diet, from 80 to 98% for diets stored frozen for 2 days and put in animal feeders for 1 day, and from 83 to 102% for diets stored frozen for 5 days and put in animal feeders for 1 day.

Analysis of nine samples from each test diet for homogeneity revealed that the samples varied ± 15 , 8.4, 2.8, and 1.9% (coefficient of variation) from target concentrations of 5, 20, 500, and 2500 ppm, respectively.

2. Parental Toxicity:

Mortality: During the F₁ generation, two control females died of accidental causes on study day 19. One female (pregnancy status not determined) from Group II and one male from Group V were sacrificed moribund on study days 72 and 49, respectively. Necropsy revealed that both these animals had broken noses. An additional low-dose male died on study day 124. Necropsy findings included dilated kidneys containing cheesy yellow material, calculi in the urinary bladder, and one flaccid, discolored testis.

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TABLE 1. Summary of Sulfoxide Analysis^a

Dietary Concentrations ^b (ppm)	Sample Type ^c	RE-45601 Present (ppm)	RE-45924 Present (ppm)	Combined Total (ppm)	% Target Concentration ^d
16.6	0	16.6	0.58	17.2	104
	2 + 1	13.3	2.86	16.2	97.6
	5 + 1	13.8	3.08	16.9	102
416	0	434	42.3	476	114
	1 + 2	396	48.1	444	107
	4 + 2	384	60.2	444	107
2080	0	2030	108	2140	103
	1 + 2	1950	116	2070	99.5
	3 + 3	1820	88.2	1910	91.8

^aData were extracted from Study No. S-2778, Appendix II.^bRepresents the actual amount of RE-45601 at each concentration, based on a purity of 83.2%. These correspond to 20, 500, and 2500 ppm of RE-45601 technical, respectively.^c0 refers to freshly prepared diets, 2 + 1 refers to diets stored frozen for 2 days and put in animal feeders for 1 day, 5 + 1 refers to diets stored frozen for 5 days and put in animal feeders for 1 day, etc.^dBased on combined total concentration in ppm.462 A
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During the F_{12} generation, one female from Group II and one male from Group III were sacrificed moribund on study days 135 and 254, respectively. The female exhibited paralysis of the hind limbs prior to sacrifice, but had no abnormalities at necropsy. At necropsy, the F_{12} male had reddened cervical lymph nodes and "digested blood" in the colon.

Clinical Observations: The following clinical observations were noted with similar frequency among control and test groups: nasal and ocular discharge, malocclusion, broken teeth, laceration on roof of mouth, labored respiration, alopecia, swollen and reddened ears, and ear tag irritation.

Body Weight: Body weight data collected during the prematuring intervals (F_0 and F_{12}) are summarized in Table 2, and maternal body weight data for the gestational and lactational periods are summarized in Table 3.

The body weight of Group V males was consistently reduced ($p < 0.05-0.01$) during prematuring periods of both the F_0 and F_{12} generations. This reduction continued throughout the remainder of the study (data not shown). Although the body weight of F_0 females was unaffected by treatment throughout the prematuring, gestation, and lactation periods, consistent, significant reductions ($p < 0.05-0.01$) were observed for Group V F_{12} females throughout the prematuring and gestation periods, and on days 0-14 of lactation.

Food Consumption: Food consumption data collected during the prematuring intervals (F_0 and F_{12}) are summarized in Table 4. Significant reductions ($p < 0.05-0.01$) in food consumption were observed for Group V F_0 males on study days 0-2, 44-47, and 56-58 (data not shown). Similar results were observed for Group V F_0 females; significant reductions were observed on days 0-2 and 5-7 of the prematuring period and on GD 7-9 and days 12-14 of lactation (data not shown). Significantly reduced food consumption was also observed in Group II F_0 females during days 12-14 of lactation. Nominal range of test material intake for males was 0-1, 1-2, 28-60, and 141-295 mg/kg/day for Groups II, III, IV, and V, respectively. For F_0 females, nominal range of test material intake was 0-1, 1-3, 36-61, and 180-282 mg/kg/day for Groups II, III, IV, and V, respectively.

During the F_{12} generation, food consumption was significantly reduced ($p < 0.05-0.01$) for Group V males on study days 121-124, 131-147, 149-163, 184-187, 189-191, 238-240, 252-254, 259-261, and 264-266 of the prematuring period (data not shown). In females, significant reductions ($p < 0.05-0.01$) were observed in Group II on days 119-124, 126-128, 131-133, 159-161, 173-175, 180-182, and 184-187, and in

TABLE 2. Summary of Mean Body Weight (g $\pm$ S.D.) During Premating Periods for Rats Fed RE-45601 Technical for Two Generations^a

Day of Study	Dietary Concentration (ppm):				
	0	5	20	500	2500
<u>Males</u>					
0 ^b	215 $\pm$ 10.1	218 $\pm$ 8.7	213 $\pm$ 9.9	211 $\pm$ 9.1	210 $\pm$ 10.8
21	355 $\pm$ 20.6	352 $\pm$ 19.3	353 $\pm$ 22.9	346 $\pm$ 20.1	336 $\pm$ 19.5**
42	440 $\pm$ 28.5	434 $\pm$ 23.9	440 $\pm$ 31.9	424 $\pm$ 28.0	414 $\pm$ 28.6**
63	495 $\pm$ 34.0	490 $\pm$ 29.9	495 $\pm$ 37.6	474 $\pm$ 43.3	460 $\pm$ 34.7**
<u>Females</u>					
0 ^b	152 $\pm$ 10.4	154 $\pm$ 9.8	152 $\pm$ 8.0	153 $\pm$ 9.0	156 $\pm$ 11.1
21	208 $\pm$ 18.8	211 $\pm$ 17.3	207 $\pm$ 13.0	216 $\pm$ 16.5	211 $\pm$ 18.8
42	242 $\pm$ 24.0	246 $\pm$ 19.8	245 $\pm$ 16.6	246 $\pm$ 18.9	242 $\pm$ 21.8
63	265 $\pm$ 28.6	270 $\pm$ 24.1	265 $\pm$ 16.5	270 $\pm$ 22.6	262 $\pm$ 24.6
<u>1a Males</u>					
119 ^c	77 $\pm$ 10.2	74 $\pm$ 8.8	75 $\pm$ 10.4	74 $\pm$ 8.4	67 $\pm$ 11.1**
140	243 $\pm$ 24.5	240 $\pm$ 27.8	238 $\pm$ 29.3	238 $\pm$ 18.1	203 $\pm$ 43.3**
168	411 $\pm$ 38.6	415 $\pm$ 39.7	408 $\pm$ 29.5	409 $\pm$ 34.8	363 $\pm$ 43.1**
196	492 $\pm$ 51.0	491 $\pm$ 51.8	487 $\pm$ 36.3	489 $\pm$ 45.6	438 $\pm$ 50.8**
<u>1a Females</u>					
119 ^c	70 $\pm$ 8.3	69 $\pm$ 8.1	70 $\pm$ 8.4	70 $\pm$ 7.1	65 $\pm$ 8.1*
140	171 $\pm$ 17.6	168 $\pm$ 20.3	163 $\pm$ 22.4	173 $\pm$ 11.4	157 $\pm$ 23.3*
168	245 $\pm$ 25.4	239 $\pm$ 25.0	237 $\pm$ 24.0	244 $\pm$ 20.6	222 $\pm$ 18.5**
196	279 $\pm$ 29.9	269 $\pm$ 27.2	269 $\pm$ 26.0	279 $\pm$ 25.1	256 $\pm$ 22.7**

^aData were extracted from study No. S-2778, Tables 6 and 16.^bApproximately 6 weeks of age.^cApproximately 4 weeks of age.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

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****Significantly different from controls ($p < 0.01$).**

TABLE 4. Summary of Mean Food Consumption (g/animal $\pm$ S.D.) During the Premating Periods for Rats Fed RE-45601 Technical For Two Generations^a

Interval (days)	Dietary Concentration (ppm)				
	0	5	20	500	2500
<u>F₀ Males</u>					
0-2	50 $\pm$ 3.2	52 $\pm$ 5.7	51 $\pm$ 4.2	49 $\pm$ 2.8	45 $\pm$ 3.7**
21-23	53 $\pm$ 4.4	54 $\pm$ 4.8	56 $\pm$ 4.5	52 $\pm$ 4.6	51 $\pm$ 6.6
42-44	56 $\pm$ 5.3	53 $\pm$ 4.5	54 $\pm$ 5.2	53 $\pm$ 8.3	52 $\pm$ 4.7
61-63	54 $\pm$ 4.5	53 $\pm$ 3.6	53 $\pm$ 4.8	54 $\pm$ 5.4	52 $\pm$ 5.8

<u>F₀ Females</u>					
0-2	36 $\pm$ 4.3	38 $\pm$ 6.9	38 $\pm$ 6.6	36 $\pm$ 3.5	33 $\pm$ 3.8*
21-23	36 $\pm$ 5.0	38 $\pm$ 5.5	36 $\pm$ 4.2	37 $\pm$ 5.4	35 $\pm$ 3.9
42-44	39 $\pm$ 6.6	37 $\pm$ 3.9	36 $\pm$ 4.8	38 $\pm$ 4.4	37 $\pm$ 4.0
61-63	37 $\pm$ 5.0	37 $\pm$ 5.0	35 $\pm$ 5.3	40 $\pm$ 4.6	37 $\pm$ 4.6

<u>F₁ Males</u>					
119-121	33 $\pm$ 10.8	28 $\pm$ 6.1	30 $\pm$ 5.1	33 $\pm$ 8.3	28 $\pm$ 9.3
140-142	52 $\pm$ 4.4	55 $\pm$ 5.3	52 $\pm$ 4.2	53 $\pm$ 3.8	48 $\pm$ 5.8**
168-170	59 $\pm$ 6.8	61 $\pm$ 7.6	59 $\pm$ 5.4	61 $\pm$ 6.2	56 $\pm$ 5.6
189-191	57 $\pm$ 5.9	57 $\pm$ 6.0	56 $\pm$ 5.5	57 $\pm$ 5.2	53 $\pm$ 5.6*

<u>F₁ Females</u>					
119-121	30 $\pm$ 8.0	20 $\pm$ 5.8**	25 $\pm$ 5.9	30 $\pm$ 10.7	25 $\pm$ 7.3
140-142	39 $\pm$ 3.6	40 $\pm$ 5.4	36 $\pm$ 6.9	42 $\pm$ 10.8	37 $\pm$ 6.1
168-170	43 $\pm$ 6.4	43 $\pm$ 4.1	42 $\pm$ 6.1	43 $\pm$ 4.8	43 $\pm$ 6.4
189-191	40 $\pm$ 4.8	40 $\pm$ 4.2	41 $\pm$ 5.1	42 $\pm$ 4.6	43 $\pm$ 7.5

^aData were extracted from study No. S-2778, Tables 7 and 17.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

Group III on study days 126-128, 131-133, 135-140, 152-156, 166-168, 173-177, 180-182, 184-187, and 194-196 (data not shown). During gestation and lactation, significant reductions ($p < 0.05-0.01$) in food consumption were observed in Group V females on GD 0-5 and 9-12, in Group II females on GD 9-14, and in Group III females on GD 9-12 (data not shown). During lactation, food consumption was significantly reduced ($p < 0.05-0.01$) for Group II and Group III females on days 9-12 (data not shown). Nominal range of test material intake for F_1 males was 0-1, 1-5, 30-143, and 156-716 mg/kg/day for Groups II, III, IV, and V, respectively. For F_1 females, nominal range of test material intake was 0-1, 1-5, 13-38, and 207-686 mg/kg/day for Groups II, III, IV, and V, respectively.

Gross Pathological Observations: The following findings were noted at necropsy with similar frequency among control and test groups for both generations: discolored and/or enlarged lymph nodes; dilated renal pelvises; fluid-filled and/or discolored kidneys; renal calculi; small epididymides; enlarged, small, or flaccid testes; and discolored liver.

Male organ weight data (absolute) and terminal body weight data are summarized in Table 5. During the F_0 generation, terminal body weight was significantly reduced ($p < 0.01$) in Group V males. In addition, relative (to body weight), but not absolute, left and right testicles weights were significantly increased ($p < 0.05$) in Group V males when compared with controls (data not shown).

During the F_1 generation, terminal body weight for Group V males was significantly reduced ($p < 0.01$). Significant reductions ($p < 0.05-0.01$) in absolute weight of the prostate and seminal vesicles were observed in Group II and Group V males when compared with controls. Relative epididymis (left) weight was significantly increased ($p < 0.05$) in Group V males (data not shown).

3. Reproductive Toxicity: The effects of dietary administration of the test material on reproductive parameters are summarized in Table 6. Mean gestation length was significantly increased ($p < 0.05$) in Groups IV and V females during the first (F_{1s}) generation, but not during the subsequent generation. The number of litters with stillborn pups was slightly increased, and the total number of stillborn pups was significantly increased ($p < 0.01$) in Group V during the F_{1s} generation when compared with controls (data not shown). Survival indices were significantly increased ($p < 0.05-0.01$) for Group IV during the F_{1s} generation and for Groups II, III, and IV during the F_{2s}

TABLE 5. Summary of Mean Absolute Organ Weight Data (g $\pm$ S.D.) for Male Rats Fed RE-45601 Technical for Two Generations^a

Organ	Dietary Concentration (ppm):									
	F ₀ Generation					F _{1a} Generation				
	0	5	50	580 $\pm$ 62	556 $\pm$ 52	527 $\pm$ 51*	603 $\pm$ 74	590 $\pm$ 74	588 $\pm$ 57	598 $\pm$ 68
Terminal body weight	573 $\pm$ 53	553 $\pm$ 50	580 $\pm$ 62	556 $\pm$ 52	527 $\pm$ 51*	603 $\pm$ 74	590 $\pm$ 74	588 $\pm$ 57	598 $\pm$ 68	537 $\pm$ 65**
<u>Testes</u>										
-left	1.75 $\pm$ 0.12	1.79 $\pm$ 0.17	1.79 $\pm$ 0.15	1.78 $\pm$ 0.24	1.76 $\pm$ 0.14	1.78 $\pm$ 0.20	1.79 $\pm$ 0.47	1.79 $\pm$ 0.37	1.84 $\pm$ 0.17	1.82 $\pm$ 0.33
-right	1.76 $\pm$ 0.14	1.78 $\pm$ 0.15	1.79 $\pm$ 0.14	1.80 $\pm$ 0.16	1.77 $\pm$ 0.16	1.79 $\pm$ 0.19	1.80 $\pm$ 0.26	1.76 $\pm$ 0.32	1.83 $\pm$ 0.20	1.76 $\pm$ 0.38
<u>Epididymides</u>										
-left	0.79 $\pm$ 0.13	0.81 $\pm$ 0.17	0.79 $\pm$ 0.08	0.75 $\pm$ 0.18	0.80 $\pm$ 0.14	0.68 $\pm$ 0.17	0.63 $\pm$ 0.14	0.68 $\pm$ 0.09	0.71 $\pm$ 0.11	0.69 $\pm$ 0.14
-right	0.79 $\pm$ 0.13	0.79 $\pm$ 0.13	0.82 $\pm$ 0.10	0.77 $\pm$ 0.10	0.79 $\pm$ 0.11	0.69 $\pm$ 0.14	0.67 $\pm$ 0.09	0.68 $\pm$ 0.10	0.72 $\pm$ 0.08	0.66 $\pm$
<u>Prostate</u>	1.07 $\pm$ 0.42	0.98 $\pm$ 0.38	1.13 $\pm$ 0.38	1.06 $\pm$ 0.38	0.96 $\pm$ 0.40	0.84 $\pm$ 0.31	0.66 $\pm$ 0.22*	0.78 $\pm$ 0.28	0.71 $\pm$ 0.27	0.63 $\pm$ 0.20*
<u>Seminal Vesicles</u>	2.07 $\pm$ 0.56	1.96 $\pm$ 0.44	2.26 $\pm$ 0.55	2.06 $\pm$ 0.45	1.94 $\pm$ 0.54	2.09 $\pm$ 0.30	1.82 $\pm$ 0.33**	1.94 $\pm$ 0.33	1.92 $\pm$ 0.27	1.86 $\pm$ 0.39*

^aData were extracted from study No. S-2778, Table 30.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

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TABLE 6. Summary of Effects of Dietary Administration of RE-45001 Technical on Reproductive Parameters and Offspring Survival and Body Weight^a

	Dietary Concentration (ppm)											
	0				5				20			
	f _{1a}	f _{2a}	f _{1a}	f _{2a}	f _{1a}	f _{2a}	f _{1a}	f _{2a}	f _{1a}	f _{2a}	f _{1a}	f _{2a}
No. matings	28	30	30	29	30	30	30	30	30	30	30	30
No. pregnancies	22	21	25	23	23	29	24	27	28	28	25	25
Female fertility index (%)	78.6	72.4	83.3	79.3	76.7	96.7	82.8	93.1	96.6	96.6	83.3	83.3
Gestation index (%)	100	100	100	100	100	100	100	100	100	100	100	100
Mean gestation length (days)	21.7	22.0	21.9	21.9	22.0	21.9	22.0*	21.9	22.0*	21.9	22.0*	21.9
Total No. pups born	298	264	308	254	286	371	285	344	367	367	367	367
Mean litter size at birth	13.5	12.6	12.3	11.0	12.4	12.8	11.9	12.7	13.1	13.1	11.8	11.8
Total No. pups alive, Day 0	296	257	303	247	281	363	278	332	353	353	311	311
Mean No. live pups/litter	13.5	12.2	12.1	10.7	12.2	12.5	11.6	12.3	12.6	12.6	12.1	12.1
Live birth index (%) ^b	99.7	99.7	100.0	99.7	100.0	99.8	100.0	98.8	100.0	100.0	98.7	98.7
Total No. pups alive, Day 4 ^c	289	231	289	240	275	350	278	321	346	346	283	283
Mean No. live pups/litter ^c	13.1	11.0	11.6	10.4	12.0	12.1	11.6	11.8	12.4	12.4	11.3	11.3
Viability index (%) ^d	98.0	87.0	93.5	97.5	97.8	93.5	100.0	94.7	98.2	98.2	98.0	98.0
Total No. pups alive, Day 21	171	147	187	174	180	218	179	207	219	219	186	186
Mean No. live pups/litter	7.8	7.7	7.8	7.6	7.8	7.8	7.5	8.0	7.8	7.8	7.0	7.0
Survival index (%) ^e	95.0	85.5	93.5	95.6**	96.8	93.2*	99.4*	94.5**	96.9	96.9	84.1	84.1
Mean pup weight, Day 0 (g)	6.0	5.9	6.0	6.1	6.1	6.1	6.3	6.1	6.1	6.1	5.7	5.7
Mean pup weight, Day 7 (g)	14.9	14.6	15.4	14.6	15.9	14.9	15.9	14.7	15.0	15.0	13.6	13.6
Mean pup weight, Day 21 (g)	50.0	47.1	49.5	47.6	51.6	49.5	50.5	47.9	47.7	47.7	44.3	44.3

^aData were extracted from study No. S-2778, Tables 26, 27, and Appendix 27.^bLive Birth Index = $\frac{\text{No. pups alive per litter on Day 1}}{\text{No. pups alive per litter at birth}} \times 100$; calculated by the reviewers and analyzed using ANOVA and Dunnett's T test.^cPrior to culling; calculated by the reviewers using individual animal data and analyzed using ANOVA and Dunnett's T test.^dViability Index = $\frac{\text{No. pups alive per litter on Day 4 before culling}}{\text{No. pups alive per litter at birth}} \times 100$; calculated by the reviewers and analyzed using ANOVA and Dunnett's T test.^eSurvival Index = $\frac{\text{No. pups alive on Day 21}}{\text{No. pups alive on Day 4 after culling}} \times 100$.

*Significantly different from controls (p < 0.05).

**Significantly different from controls (p < 0.01).

generation. An increase in the number of $F_{1\alpha}$ pups with dilated renal pelves was observed in all the test groups when compared with controls; a significant increase ($p < 0.05$) was observed in Group IV. In Groups I, II, III, IV, and V, 0, 5, 3, 5, and 5 pups, respectively, were affected. In the subsequent generation, however, the frequency of dilated renal pelves was similar among control and test groups. The total incidence of necropsy findings in Groups III and IV $F_{2\alpha}$ pups was significantly reduced ($p < 0.05-0.01$) when compared with controls. Total incidences were similar between Groups I and V.

D. REVIEWERS' DISCUSSION/CONCLUSIONS:

1. Test Material Analysis: The test material was unstable at room temperature, readily forming the sulfoxide metabolite. However, even though the study author realized this, and fresh (or frozen) test diet was offered daily (Groups II and III) or every 3 days (Groups IV and V), analysis revealed that concentrations were highly variable. Analysis of the 5-ppm test diet (Group II) was discontinued after 3 weeks because of problems with reproducibility of the results; excessive variation of duplicates occurred. Toward the end of the study, the study author began analyzing sulfoxide concentration in diets containing 20, 500, or 2500 ppm and found that combining sulfoxide content with that of the test material improved the analysis results, increasing levels at 20 ppm as much as 22% after 1 day at room temperature. The study author also found that the sulfoxide content increased at 20 and 500 ppm as length of storage increased, as would be expected since the test material is readily oxidized.

Because the reviewers were unable to determine the amount of test material ingested by rats fed 5 ppm, the results of this group (Group II) were disregarded in the evaluation of this study. We assessed the concentrations of the test material in the other test diets to be adequate when the amount of the sulfoxide produced with time was taken into consideration. However, the possible toxic effects associated with the combination of parent compound and metabolite were not taken into consideration.

2. Parental Toxicity: Compound-related, significant reductions in the body weight of F_0 and $F_{1\alpha}$ parental animals, particularly males, were observed throughout the study. These reductions were supported by sporadic, significant decreases in food consumption in Group V F_0 and $F_{1\alpha}$ males and females. Sporadic, significant reductions in food consumption were also observed in Groups II and III, particularly in females. However, these reductions were

not accompanied by decreases in body weight, and therefore, were not considered to be compound related. Significant changes in absolute and/or relative organ weights were observed for Group V males. These were attributed to significant reductions in terminal body weight and not to the test material. This was supported by the lack of effect on reproductive performance among Group V males.

Based on these results, the NOEL and LOEL for parental toxicity are 500 and 2500 ppm, respectively.

3. Reproductive Toxicity: No compound-related adverse effects on reproductive parameters were observed. The length of gestation was significantly increased in F₀ females from Groups IV and V when compared with concurrent controls. However, similar increases were not observed during the second generation, and this value (22.0 days) was well within the expected range for this species. Therefore, this was not considered to be compound related. A slight, but nonsignificant, reduction (3-12%) in the number of live fetuses/litter at birth was observed in all test groups when compared with controls, but particularly during the F₁ generation. Unfortunately, no historical control data were presented, and we were unable to compare these values with historical control ranges for this laboratory. However, the live birth, viability, and survival indices were comparable among control and test groups. Therefore, the reviewers considered these differences to be due to biological variation rather than to the test material.

A slight, but nonsignificant, reduction in pup weight was observed in Group V F₂ pups, beginning on day 7 of lactation. However, since the decrease was <10%, the reviewers considered the change to be due to biological variation.

The NOEL for reproductive toxicity was 2500 ppm. A LOEL was not established.

4. Study Deficiencies: The following deficiencies were noted:
 - a. No historical control data were presented.
 - b. Analysis of the 5-ppm test diet was discontinued 3 weeks after study initiation, and therefore, the results for this group were discounted in the evaluation of the study.
 - c. Statistical methods used for analyzing the fetal parameters were not reported.

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E. CLASSIFICATION: CORE Minimum Data.

Parental Toxicity NOEL = 500 ppm (approximately 51 mg/kg/day).

Parental Toxicity LOEL = 2500 ppm (approximately 263 mg/kg/day).

Reproductive Toxicity NOEL = 2500 ppm.

Reproductive Toxicity LOEL = not established.

F. RISK ASSESSMENT: Not applicable.

Data Evaluation Report

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Compound Select, RE-45601, clethodimCitation

The percutaneous absorption of ^{14}C -SELECT 2.0 EC (RE-45601) in male rats. G.A. Mulholland & L.C. Griffis, Chevron Environmental Health Center Inc, Richmond CA, S-2961, Dec 18, 1987, MRID 410302-02

Reviewed by Robert P. Zendzian Ph.D.
Senior Pharmacologist

Core classification AcceptableConclusions

ation to a 10 cm^2 area of the rat's back resulted in the following percent absorption;

ure hrs)	0.05 mg/rat			0.5 mg/rat			5.0 mg/rat		
	On/In Skin	Skin Wash	Absorbed	On/In Skin	Skin Wash	Absorbed	On/In Skin	Skin Wash	Absorbed
	8.1	74.4	8.5	11.2	70.7	4.4	4.7	79.7	2.0
	13.6	50.6	28.4	14.5	59.3	14.4	8.0	69.8	6.7
	13.4	40.5	42.9	6.6	47.0	34.1	4.2	65.2	29.7

Materials

Select, [Cyclohexenone-4,6- ^{14}C]-clethodim, RE-45601, 98.7% radiochemical pure, specific activity 356.436 dpm/ug from Wuzards laboratories, Davi w Calif

Select 2EC (25.85% clethodim) Chevron

Sprague-Dawley® Crl:CD® (SD)BR male rats from Bantin and Kingman Inc.

Experimental design

Group	Identy	Dose mg/rat	No of Rats*	Exposure Times Hr After Application
I	Low dose	0.05	12	2, 10, 24
II	Mid dose	0.50	12	2, 10, 24
III	High dose	5.0	12	2, 10, 24

*four rats per exposure time

"An appropriate amount of SELECT 2EC was weighed out and immediatly mixed with ^{14}C -clethodim to approximately 40 uCi/mg, 4 uCi/mg and 0.4 uCi/mg. The final dosing solution was adjusted by adding dionized water to give clethodim concentrations of approximatley 1, 10 and 100 mg/ml." Specific activity of the test material in the dosing solutions was 88526, 8282 and 891 dpm/ug for the respective solutions.

"The application site was clipped on the day prior to dosing. Approximately one hour prior to dosing, the application site was shaved free of hair with a disposable razor and washed with mild soap and water to remove sebaceous gland secretions. Each animal was patted dry."

"A neoprene rubber template, that defined a 2.5 x 4 cm application site, was glued to the back of each animal with cyanoacrylate glue."

"The test material was vortexed before dosing each animal. An aliquot (0.05 ml) of the test material suspension was taken before dosing the first animal and after dosing the last animal-- for analysis--". "Approximately 0.05 ml of the dosing solution was applied to the application site of each animals back with an VWR Digital Microdispenser. The microdispenser was weighed before and after dosing to determine the exact dose administered to each animal."

"Each animal was placed individually into a Metrap® --- animal restrainer which was equipped with separate collectors for urine and feces." Volatiles were collected.

"After a 2-, 10-, or 24-hour exposure, the animals were anesthetized with sodium pentobarbital, removed from the restrainer and placed ventral side down in the necropsy tray. The application site was scrubbed vigorously in situ with a two-inch square, 12-ply gauze pad which was immersed in soapy water (5 ml Dove dishwashing liquid in 1 liter of deionized water). The pad was squeezed before scrubbing until no water dripped from the pad. This procedure was repeated three times with soapy water followed by one scrubbing with deionized water alone." The scrubs, skin at the application site and the template were collected for analysis. The excised skin site was rinsed with acetone and a 1-cm strip around the site taken for analysis.

Samples collected for analysis were, the wash solutions, blood, carcass, urine from bladder and collector, feces from colon and collector, activated charcoal from the volatiles trap and a methanol cage rinse.

Results

Table 1 summarizes the data obtained from groups I, II and III.

Exposure Time (Hrs)	Skin Wash	Template Wash	On/In Skin	Blood	Urine	Feces	Carcass	Cage Wash	Total Unabsorbed ²	Absorbed ³	Recovered
<u>0.05 mg/rat</u>											
2	74.4	3.8	8.1	0.3	0.9	0	7.3	0	78.2	8.5	94.9
10	50.6	1.8	13.6	0.5	9.5	0.7	16.3	1.4	52.4	28.4	94.4
24	40.5	1.6	13.4	0.2	23.6	2.2	14.3	2.5	42.1	42.8	98.1
<u>0.5 mg/rat</u>											
2	70.7	2.0	11.2	0.2	0.4	0	2.7	1.1	72.7	4.4	88.3
10	59.3	2.2	14.5	0.2	4.8	0.1	9.3	0.5	61.5	14.5	90.9
24	47.0	1.3	6.6	0.3	19.9	2.1	10.4	1.4	48.3	34.1	88.7
<u>5.0 mg/rat</u>											
2	79.7	2.6	4.7	<0.1	0.2	0	1.7	0.1	82.3	2.0	89.0
10	69.8	2.6	8.0	0.1	2.3	0.3	3.6	0.4	72.4	6.7	87.0
24	65.2	1.9	4.2	0.1	8.7	2.1	5.0	0.8	67.1	29.7	87.9

1. Total of skin residue and acetone skin wash.

2. Total of skin and template washes.

3. Total of blood, urine, feces, carcass and cage wash.

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